GLOBAL CHANGE BIOLOGY
BIOENERGY

GCB Bioenergy (2016) 8, 269–280, doi: 10.1111/gcbb.12251

Direct N_2O emission factors for synthetic N-fertilizer and organic residues applied on sugarcane for bioethanol production in Central-Southern Brazil

MARCOS SIQUEIRA NETO[1], MARCELO V. GALDOS[2], BRIGITTE J. FEIGL[1], CARLOS E. P. CERRI[3] and CARLOS C. CERRI[1]

[1]Centro de Energia Nuclear na Agricultura, Universidade de São Paulo (CENA/USP), Av. Centenário, 303, P.O. Box. 96, 13400-970 Piracicaba, SP, Brazil, [2]Laboratório Nacional de Ciência e Tecnologia do Bioetanol (CTBE), R. Giuseppe Máximo Scalfaro, 10.000, P.O. Box. 6170, 13083-970 Campinas, SP, Brazil, [3]Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo (ESALQ/USP), Av. Pádua Dias, 11, 13400-970 Piracicaba, SP, Brazil

Abstract

The production and use of biofuels have increased rapidly in recent decades. Bioethanol derived from sugarcane has become a promising alternative to fossil fuel for use in automotive vehicles. The 'savings' calculated from the carbon footprint of this energy source still generates many questions related to nitrous oxide (N_2O) emissions from sugarcane cultivation. We quantified N_2O emissions from soil covered with different amounts of sugarcane straw and determined the direct N_2O emission factors of nitrogen fertilizers (applied at the planting furrows and in the topdressing) and the by-products of sugarcane processing (filter cake and vinasse) applied to sugarcane fields. The results showed that the presence of different amounts of sugarcane straw did not change N_2O emissions relative to bare soil (control). N-fertilizer increased N_2O emissions from the soil, especially when urea was used, both at the planting furrow (plant cane) and during the regrowth process (ratoon cane) in relation to ammonium nitrate. The emission factor for N-fertilizer was 0.46 ± 0.33%. The field application of filter cake and vinasse favored N_2O emissions from the soil, the emission factor for vinasse was 0.65 ± 0.29%, while filter cake had a lower emission factor of 0.13 ± 0.04%. The experimentally obtained N_2O emission factors associated with sugarcane cultivation, specific to the major sugarcane production region of the Brazil, were lower than those considered by the IPCC. Thus, the results of this study should contribute to bioethanol carbon footprint calculations.

Keywords: bioethanol, carbon footprint, filter cake, N-fertilizer, *Saccharum officinarum* L., vinasse

Received 31 July 2014; accepted 16 December 2014

Introduction

N_2O is a long-lived trace gas that is naturally present in the atmosphere. This gas is able to absorb infrared radiation and relay it in the form of thermic energy. The heating potential of this gas is 298 times higher than CO_2 (IPCC, 2001), and it participates directly in stratospheric ozone depletion (Ravishankara *et al.*, 2009). The atmospheric N_2O concentration has steadily remained at 270 ppbv since the last glacial period (Flückiger *et al.*, 1999). However, by 2013, the concentration increased to 325.9 ppbv, with an average absolute increase during the last 10 years of 0.82 ppbv yr^{-1} (WMO, 2014). The major N_2O producers are the soil microorganisms that are responsible for N transformation (Bouwman, 1998). Agricultural activity with large N inputs from the Haber–Bosch synthesis is the most significant N_2O source to the atmosphere (Butterbach-Bahl *et al.*, 2013).

Brazil's 2nd National Communication to the Framework Convention of the United Nations on Climate Change estimated that 84% (456.8 Gg) of the total N_2O emissions were from agricultural soils, of which 17% (77.8 Gg) were from the use of synthetic fertilizers and crop residues (Brazil, 2010).

Currently, Brazilian biofuel production is based on sugarcane. In 2013, bioethanol production was 21 million m^3 (UNICA, 2014) from a planted area of 9.1 million ha, of which 2.1 million ha were from new areas and reform/planting (CANASAT, 2014). The success of bioethanol production is due to the hardiness of the sugarcane crop, enabling regrowth after harvesting, high tillering production and positive energy balance (Macedo *et al.*, 2008). The expansion of sugarcane production in the central-southern region of Brazil is due to the increase in the domestic fleet of flex fuel vehicles

Correspondence: Dr Marcos Siqueira Neto, tel./fax +55 19 3429 4726, e-mail: msiqueir@usp.br

and the export demand for bioethanol (Rudorff *et al.*, 2010).

The uncertainty associated with the emissions of non-CO_2 gases affects the carbon footprint calculation of the bioethanol derived from sugarcane. N_2O emissions from the use of different N-sources (fertilizer, by-products and crop residues) are considered to be major negative contributions to the 'savings' of this biofuel compared with fossil fuels (Smeets *et al.*, 2009). This consideration is because the estimate of N_2O emissions uses a single default emission factor based only on the amount of applied N, which ignores the complex interactions between the microorganisms responsible for N_2O production and environmental factors (IPCC, 2006).

The most common fertilization management practices on sugarcane plantations in the central-southern region of Brazil are N applied to planting furrows for reform and topdressing for sugarcane ratoons. Synthetic N-sources, urea and ammonium nitrate, account for over 85% of the fertilizer used in sugarcane cultivation. In addition to synthetic fertilizers, by-products of the sugarcane agribusiness production phase (filter cake and vinasse) can cause considerable N input. The application of these byproducts to the crop is common and has been extensively studied as a means of nutrient cycling for plant growth, potentially reducing input costs (Prado *et al.*, 2013).

The filter cake is obtained during the sugar manufacture, with every ton of sugarcane processed generating close to 30 kg (Veiga *et al.*, 2006) from the clarification of the juice obtained during milling. This composite is rich in phosphorus, in addition to calcium, magnesium, sulfur and micronutrients (Fravet *et al.*, 2010). The filter cake is applied to the planting furrow in doses between 15 and 40 ton ha^{-1}. The vinasse is considered to be the major residue of the ethanol production; every liter of ethanol produced generates between 10 and 18 L of vinasse (Freire & Cortez, 2000). Vinasse is rich in organic matter and stands out as a potassium source, in addition to other minerals (Silva *et al.*, 2014). The vinasse is commonly applied as fertirrigation directly onto sugarcane straws in the ratoons at a dose of 150 m^3 ha^{-1}.

The different sources (synthetic or organic), amounts applied, the application forms (furrow or topdressing, single or combined) and edaphoclimatic factors influence the resulting N_2O emissions and thereby necessitate different emission factors for each N-fertilizer management process on a sugarcane plantation (Choudhard *et al.*, 2001; Bouwman *et al.*, 2002; Khalil *et al.*, 2004; Stehfest & Bouwman, 2006; Denmead *et al.*, 2010; Aguilera *et al.*, 2013; Gu *et al.*, 2013).

To increase the knowledge of the contribution of N_2O emissions of sugarcane production to bioethanol carbon footprint calculations, we quantified N_2O emissions from soil covered with different amounts of sugarcane straw and determined the direct N_2O emission factors of N-fertilizers (applied at planting furrows and topdressing) and by-products of sugarcane agribusiness (filter cake and vinasse) applied to sugarcane fields in the south central region of Brazil.

Materials and methods

Site description

The experiments were conducted in an area of 4.83 ha (22°36′35.7″S 47°36′06.5″W) that has been cultivated continuously with sugarcane since 1971. This area is located in the southwest region of Brazil, municipality of Piracicaba, São Paulo State. The regional climate is classified as Köppen's Cwa – mesothermal humid subtropical, with a dry winter and a hot and wet summer. The mean annual precipitation is 1400 mm yr^{-1}, and the mean annual temperature is 22.5°C. The soil is classified as clayey Oxisol, a Typic Acrustox (Soil Survey Staff, 1999). Soil samples were taken to characterize the physical and chemical properties, shown in Table 1.

The experimental area was cultivated with sugarcane variety RB 86-7515. At the time of the experiments, the crop was in the third growth cycle, or second ratoon. Sugarcane has been harvested mechanically without straw burning in this area since 2009.

Three experiments were sequentially installed: the first evaluated N_2O emissions from different quantities of straw deposited on the ground, the second experiment evaluated emissions due to the application of N-fertilizer and vinasse to ratoon sugarcane, and the third trial measured N_2O emissions from the application of N-fertilizer and filter cake in the planting furrow during the reform of the sugarcane plantation.

Table 1 Soil physic-chemical characteristics in the experimental area with sugarcane cultivation in the Central-southern part of the Brazil

	Soil layer (m)	
Soil parameter	0.0–0.1	0.1–0.2
Texture (g kg^{-1})		
Clay	676	684
Silt	101	93
Sand	223	223
Bulk density (g dm^{-3})	1.18	1.17
pH (CaCl2)	4.2	4.2
Total C (g kg^{-1})	15.1	14.5
Total N (g kg^{-1})	1.2	1.1
Avail. P (mg dm^{-3})	8	16
K^+ ($mmol_c$ dm^{-3})	2.7	2.1
Ca^{++} ($mmol_c$ dm^{-3})	12	12
Mg^{++} ($mmol_c$ dm^{-3})	4	4
CEC ($mmol_c$ dm^{-3})	70.3	76.0
BS (%)	26	24

CEC, cation exchanged capacity; BS, base saturation.

Experimental design and treatments

The first experiment started with the quantification of the newly deposited plant residues on the soil surface by the sugarcane harvested on July 6, 2012. To that end, we collected all the straw in ten 1 m^2 quadrants randomly distributed in the area. All of the collected materials were dried at 60°C until a constant weight was reached. Total C and N were determined using an elemental analyzer (LECO© CN 2000®, St. Joseph, Michigan). The amount of straw produced was 15 Mg ha^{-1}, with a total C content of 41.2% and 0.82% N. The field experiment was installed on August 8 and 9, 2012. We used a completely randomized design with four treatments (quantities of straw) and five replications. The quantities of straw were equivalent to (i) a full dose, 15 Mg ha^{-1} dry mass straw; (ii) a 66% dose, 10 Mg ha^{-1} dry mass straw; (iii) a 33% dose, 5 Mg ha^{-1} dry mass straw; and (iv) no dose (control). The sample chambers were installed in the inter-row, in an area over 450 m^2 (an useful area of twelve 32-m-long rows of sugarcane in 1.2-m intervals). Gas sampling to determine N_2O emissions began the following day and was performed weekly between August 2012 and March 2013, totaling 176 days.

The second experiment, designed to determine N_2O emissions from N-fertilizer and vinasse application to sugarcane ratoons, was installed on November 12 and 13, 2012. We delimited a plot of 0.25 ha of the total area (an useful area of twenty five 32-m-long rows of sugarcane in 1.2-m intervals), and the experiment was allocated using a completely randomized design consisting of eight situations – six treatment (mineral and organic N-sources in two doses), an interaction between sources (mineral and organic) and a control, each with five replicates. N_2O emissions from fertilizer were evaluated from two N-sources (ammonium nitrate at 35% of N and urea at 46% N) in doses of 80 and 120 kg N ha^{-1}. The vinasse treatments were equivalent to doses of 150 and 300 m^3 ha^{-1} of vinasse (6.45 g C L^{-1} and 0.57 g N L^{-1}) applied directly on the straw, and there was additionally a control treatment (without N-fertilizer or vinasse). In addition, to verify the interaction between sources (N-fertilizer plus vinasse) that usually occurs along the regrowth process, we evaluated a treatment of an 80 kg N ha^{-1} dose of urea followed by 150 m^3 ha^{-1} of vinasse. To apply the exact N-fertilizer quantity of each source, the doses were weighed on a precision balance and surface-applied in a band about 0.1 m from the plant row directly inside the chambers on the straw. The vinasse application was made using a garden watering can to an area of 1 m^2 bordering the row where N-fertilizer was applied. Gas sampling to determine N_2O emissions began the day after the experiment installation and was performed from November to December 2012, first daily for 15 days, and then every 2–3 days following this period, for a total of 30 days.

The third experiment, designed to determine the N_2O emissions from N-fertilizer and filter cake application in the sugarcane planting furrow, began with the conventional preparation of the soil for sugarcane planting on a 0.25-ha plot (an useful area of ten 40-m-long planting furrows of 1.2-m intervals). This preparation consisted of manually harvesting the sugarcane without burning the straw followed by soil tillage operations for the physical destruction of the stumps, plowing to 30 cm and making the planting furrows. These three operations were performed three days prior to the experiment installation, which occurred on April 29, 2013. The experimental design was completely randomized consisting of six situations with five replications. To evaluate N_2O emissions from N-fertilizer, two N-sources (ammonium nitrate and urea) were tested at a single dose of 60 kg N ha^{-1}. To determine N_2O emissions from the application of filter cake, doses equivalent to 25 and 40 Mg ha^{-1} (wet mass) of filter cake (29.9% C and 1.67% N in dry weight) were applied to the planting furrow, in addition to a control without N-fertilizer or filter cake. In addition to these treatment conditions, another treatment was performed to evaluate the interaction between the mineral and organic sources of nitrogen that usually occurs in the planting furrow. Therefore, in the bottom furrow were applied an equivalent to 25 Mg ha^{-1} of filter cake plus an equivalent to 60 kg N ha^{-1} urea. To apply the exact N-fertilizer or filter cake (wet mass) quantity, the doses were weighed on a precision balance and applied directly to 1 m of a planting furrow. Sugarcane stalks were placed on top of the different N-sources and the furrows were then covered with soil, as is usually done during the planting process. After the furrow was covered, the chambers were installed on the row. Gas sampling to determine N_2O emissions began the day after experiment installation and was performed from April to June 2013, daily for 15 days, every 2–3 days for the next 30 days, and every 5–10 days after this period, for a total of 60 days.

N_2O sampling and analysis

The static chambers used to collect N_2O emitted by the soil and added materials consisted of two parts, a base and a lid. The dimensions of the base were 45 (width) × 70 (length) × 30 (height) cm; it was buried in the soil between 5 and 7 cm deep. Air samples of the chamber's headspace were collected with a nylon syringe of 20 mL (Becton Dickinson Ind. Surgical Inc.) at four fixed time intervals (0, 10, 20 and 30 minutes) to determine the N_2O concentration. The measurement of N_2O concentration in the syringes was performed using a Shimadzu© GC-2014® (Kyoto, Japan) gas chromatograph with a packed Porapak™ Q column (80–100 mesh) maintained at 82°C to separate molecular gases. N_2O was quantified using an electron capture detector (ECD) operating at 325°C. The N_2O fluxes were calculated by the linear change in the amount of N_2O in the chambers as a function of sampled time. During the sampling period, we also monitored environmental temperature and precipitation as well as ambient N_2O concentration to check the order of magnitude of the N_2O concentration in the chambers.

Result analysis

In the N_2O concentrations obtained for each sampling time, critical limits (lower and upper) were calculated by the box chart type (quartile). Thus, the 'outlier' values were discarded (missing subplots). We performed classical statistical analyses of the soil N_2O emission results for each treatment to verify the frequency and distribution of the data. The cumulative

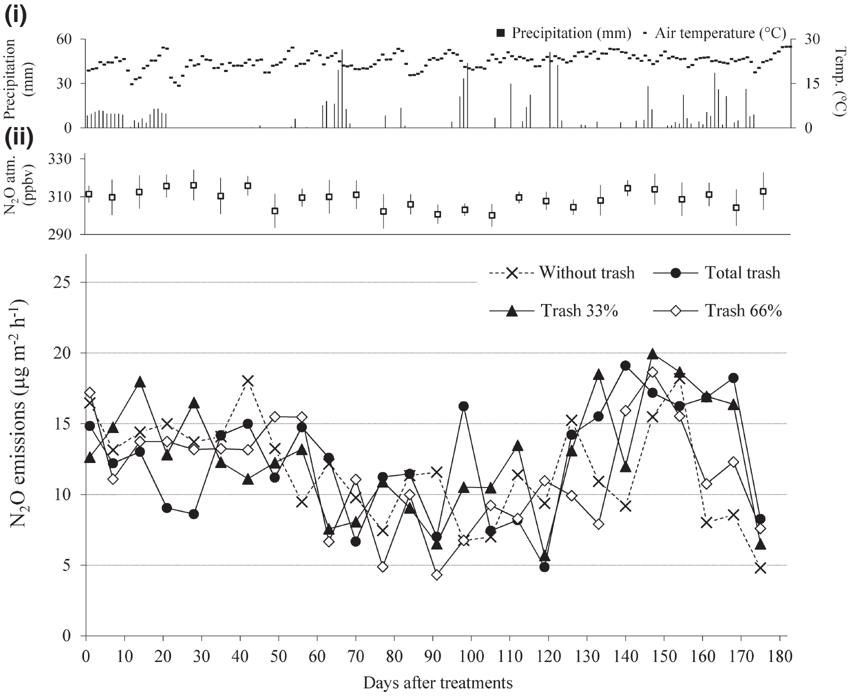

Fig. 1 N_2O emissions ($\mu g\ m^{-2}\ h^{-1}$) from different levels of sugarcane trash added to the soil surface. In the top figures (i and ii) represented environmental information during the experiment: (i) Precipitation (mm) and air temperature (°C); (ii) N_2O atmospheric concentration (ppmv).

(integral) N_2O emissions were calculated considering the period (days after application of nitrogen source) at which N_2O emission of N treatments no longer presented a difference significantly higher ($P < 0.05$) than the control treatment. Analysis of variance was performed using the Kruskal–Wallis test for the values of the cumulative N-N_2O emissions for each treatment compared with their respective controls. Means of the cumulative N_2O and emissions factors in different doses (same source – mineral or organic) were statistically separated using the Student–Newman–Keuls test ($P < 0.05$).

Calculating the N_2O emission factor

The emission factor for N_2O was calculated only when the treatments with N-sources showed significant differences from their respective controls. In other words, the difference in the integral ($\Sigma_{N_2O} - \Sigma_{Co}$) must always be positive.

To calculate the N_2O emission factor (*EF*) due to the application of N-fertilizer or by-products, we used the methodology described in Guidelines for National Inventories of Greenhouse Gases (IPCC, 2006), according to Eqn (1):

$$\mathrm{EF} = \left(\frac{\Sigma_{N_2O} - \Sigma_{Co}}{\mathrm{Napll.}}\right) \tag{1}$$

Where Σ_{N_2O} is the total emission of N_2O in the chamber from the treatment when a source containing N was applied, Σ_{Co} is the total N_2O emission in the chamber from the control area, and Napll. is the amount of nitrogen applied as fertilizer or by-product in the chamber.

Results

N_2O emissions from soil amended with different amounts of sugarcane straw

During the sampling period, the average environmental temperature was 22.8 ± 2.5°C, and 86 days with precipitation events were recorded, totaling 1025.7 mm (Fig. 1i). The atmospheric concentration of N_2O was 309.8 ± 5.1 ppbv (Fig. 1ii).

The presence of different amounts of straw and even the absence of crop residues did not alter N_2O emissions (Fig. 1). On average, treatments emitted ~ 12 μg N_2O $m^{-2}\ h^{-1}$ (minimum = 0.0 to maximum = 40.6 μg N_2O $m^{-2}\ h^{-1}$) with an accumulated value of ~ 30 mg N-N_2O m^{-2} in 176 days. No significant differences were found among treatments (Table 2).

In this experiment, emission factors were not determined because the results showed that straw should not be considered a N_2O source, in that treatments (quantities of straw) showed no significant cumulative emissions compared with the control (without straw).

Soil N_2O emissions derived from the sugarcane planting process

During the period of the experiment, the average temperature was 20.5 ± 2.0°C and 29 days with precipitation

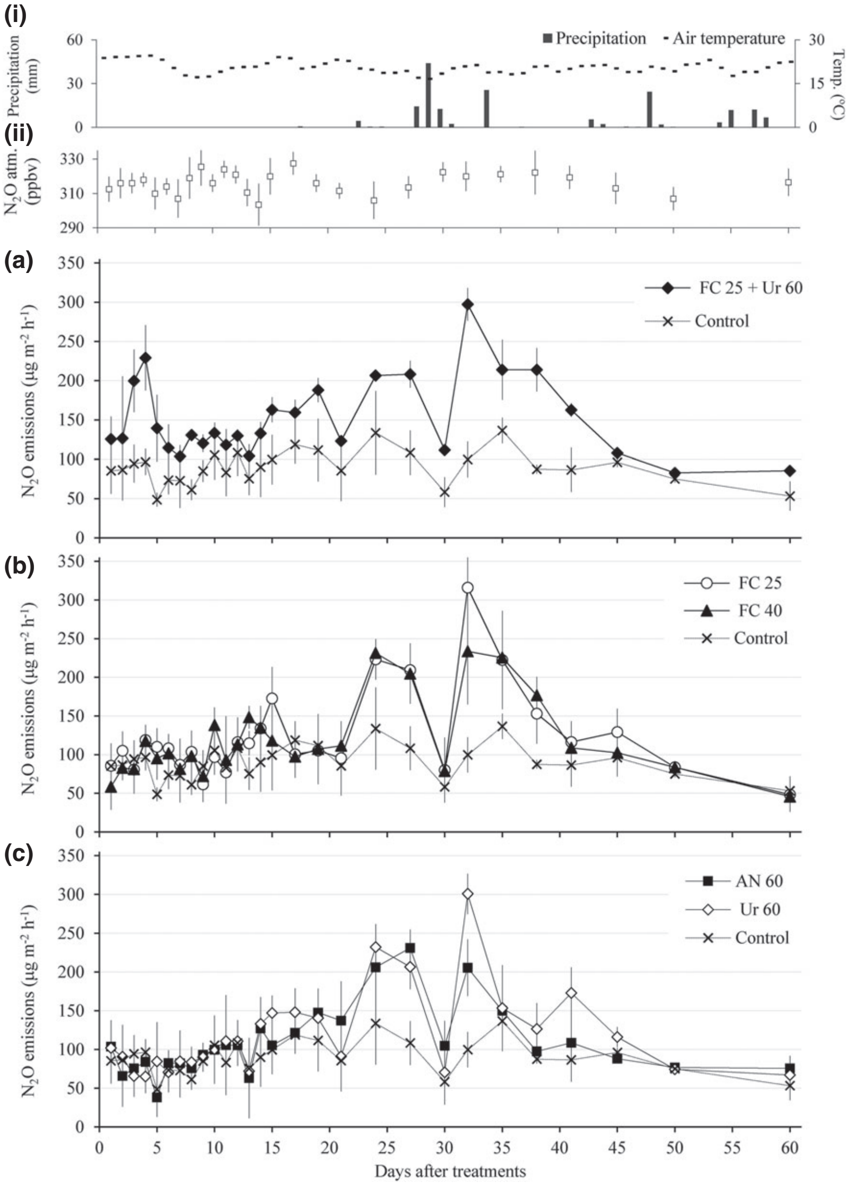

Fig. 2 N_2O emissions ($\mu g\ m^{-2}\ h^{-1}$) from filter cake (FC 25 = 25 Mg ha^{-1} ≈ 195 kg N ha^{-1}) and N-urea fertilizer (Ur 60 = 130 kg ha^{-1} ≈ 60 kg N ha^{-1}) applied in the furrow to the sugarcane plantation (a). From different doses of the Filter cake: FC 25 and FC 40 (40 Mg ha^{-1} ≈ 310 kg N ha^{-1}) (b). From different synthetic N-sources Ammonium nitrate – AN 60 (170 kg ha^{-1} ≈ 60 kg N ha^{-1}) and Urea – Ur 60 (c). In the top figures (i and ii) represented environmental information during the experiment: (i) Precipitation (mm) and air temperature (°C); (ii) N_2O atmospheric concentration (ppmv).

events were recorded, for a total of 173.6 mm (Fig. 2i). The mean atmospheric N_2O concentration was 316.0 ± 16.1 ppbv (Fig. 2ii).

Figure 2a shows the daily average N_2O emissions resulting from the treatment combining filter cake (FC 25 Mg ha^{-1}) with N-fertilizer urea (Ur 60 kg N ha^{-1}), compared with the control. N_2O emissions from the organic and synthetic nitrogen sources (FC + Ur) were higher than the control from the start and throughout the sampling period, maintaining an average emission level of 130 μg $N_2O\ m^{-2}\ h^{-1}$, higher than the 90 μg $N_2O\ m^{-2}\ h^{-1}$ observed for the control.

On the 3rd day after treatment (DAT), N_2O emissions increased in treatment FC + Ur and reached an emission three times greater than the control by the 4th DAT. This increase in N_2O emissions was observed exclusively in the treatment with the combined of organic and synthetic sources. Other treatments in which synthetic (urea and ammonium nitrate) or organic (filter cake) sources were applied alone did not increase N_2O emissions before the

Table 2 N_2O emissions (mean ± standard deviation and range), cumulative $N\text{-}N_2O$ emissions from different levels to the sugarcane trash deposited on the soil surface

	Mean ± SD*	Range†	Cumulative $N\text{-}N_2O$‡
Treatments	$\mu g\ N_2O\ m^{-2}\ h^{-1}$		$mg\ N\text{-}N_2O\ m^{-2}$
Without trash	11.7 ± 7.0	1.1–34.1	28.7 ± 4.3 ^{ns}
Trash 33%	12.6 ± 7.5	1.2–33.8	30.6 ± 3.0
Trash 66%	11.6 ± 10.0	0.6–39.7	28.0 ± 3.0
Total trash	12.5 ± 11.1	0.5–40.6	29.9 ± 3.7

*Mean ± SD = standard deviation.

†Range = Minimum and maximum

‡Cumulative $N\text{-}N_2O$ = sampled time = 175 days

ns = Cumulative means without significant differ in the Student–Newman–Keuls test ($P < 0.05$).

20th DAT, when the first precipitation occurred since the beginning of the experiment (Fig. 2b and c).

At the 20th DAT, the first rainfall of the period occurred (0.5 mm – 18th DAT) and emissions began to respond to rainfall. The lower N_2O emissions at the 30th DAT can be attributed to consecutive high rainfall events (~70 mm) that occurred the day before sampling. After the effect of the heavy rainfall ceased, the highest average emission of N_2O was recorded at the 32th DAT (~300 $\mu g\ N_2O\ m^{-2}\ h^{-1}$), approximately three times the emission observed for the control. After the 40th DAT, emission values of the treatment with N application decreased until they were similar to the control.

Cumulative N_2O emissions from the filter cake application were significantly higher than the control (40%), but the two doses (25 and 40 Mg ha^{-1}) did not show a significant difference in N_2O emissions (Fig. 2b).

In general, the pattern of N_2O emissions from soils treated with ammonium nitrate and urea fertilizers were similar (Table 3). However, more pronounced peaks were observed in the treatment with the urea application (Fig. 2c).

The accumulated values showed no significant difference between the N-sources, but they were 36 and 40% (ammonium nitrate and urea, respectively) higher than the control (Table 3).

Table 3 shows that not only were the average N_2O emissions similar, but the ranges of presented values were also similar. The accumulation for the period from the different doses of filter cake was on average 40% higher than the control.

The use of synthetic N-fertilizer during sugarcane planting generated an emission factor of 0.48%, while the filter cake application showed an average value three times lower (0.13%). The combination of organic and synthetic sources (FC + Ur), which is the most common condition, showed an emission factor of 0.21% (Table 3).

N_2O emissions from soil for ratoon sugarcane

During this experimental period, the average temperature was 24.7 ± 1.2°C, and 12 days with precipitation events were recorded, totaling 172.3 mm (Fig. 3i). The

Table 3 N_2O emissions (mean ± standard deviation and range), cumulative $N\text{-}N_2O$ emissions and N_2O emissions factor from N-sources (mineral and organic) applied in the sugarcane planting

	N_2O emissions			N_2O emission factor	
	Mean ± SD*	Range†	Cumulative $N\text{-}N_2O$‡	N-source/level§	N-source¶
Treatments (sources/levels)	$\mu g\ N_2O\ m^{-2}\ h^{-1}$		$mg\ N\text{-}N_2O\ m^{-2}$	% N-applied	
Control	89.3 ± 31.0	12.9–190.8	80.3 ± 9.7^{B}		
Mineral source					
Ammonium nitrate (60 kg N ha^{-1})	110.9 ± 53.7	10.8–268.6	108.1 ± 4.9^{A}	0.44 ± 0.08^{ns}	0.48 ± 0.12^{a}
Urea (60 kg N ha^{-1})	119.3 ± 60.8	15.5–335.4	113.0 ± 9.5^{A}	0.52 ± 0.15	
Organic source					
Filter cake (25 Mg ha^{-1})	123.3 ± 60.3	12.2–332.6	112.0 ± 8.8^{A}	0.17 ± 0.02^{ns}	0.13 ± 0.04^{b}
Filter cake (40 Mg ha^{-1})	123.5 ± 57.8	14.0–326.5	114.6 ± 4.7^{A}	0.10 ± 0.03	
Filter cake (25 Mg ha^{-1}) + urea (60 kg N ha^{-1})	156.7 ± 75.3	10.4–463.7	132.5 ± 14.7^{A}	0.21 ± 0.05	

*Mean ± SD = standard deviation.

†Range = Minimum and maximum.

‡Cumulative N_2O = sampled time in the sugarcane planting = 60 days.

§N_2O emissions factor from each N-sources and level applied in the sugarcane planting.

¶N_2O emissions factor from N-source applied in the sugarcane planting. Different capital letter to the Cumulative $N\text{-}N_2O$ (N-source and dose × control) and small letter to the Emission Factor (N-source/level and N-source) with significant differ in Student–Newman–Keuls test ($P < 0.05$); ns, not significant.

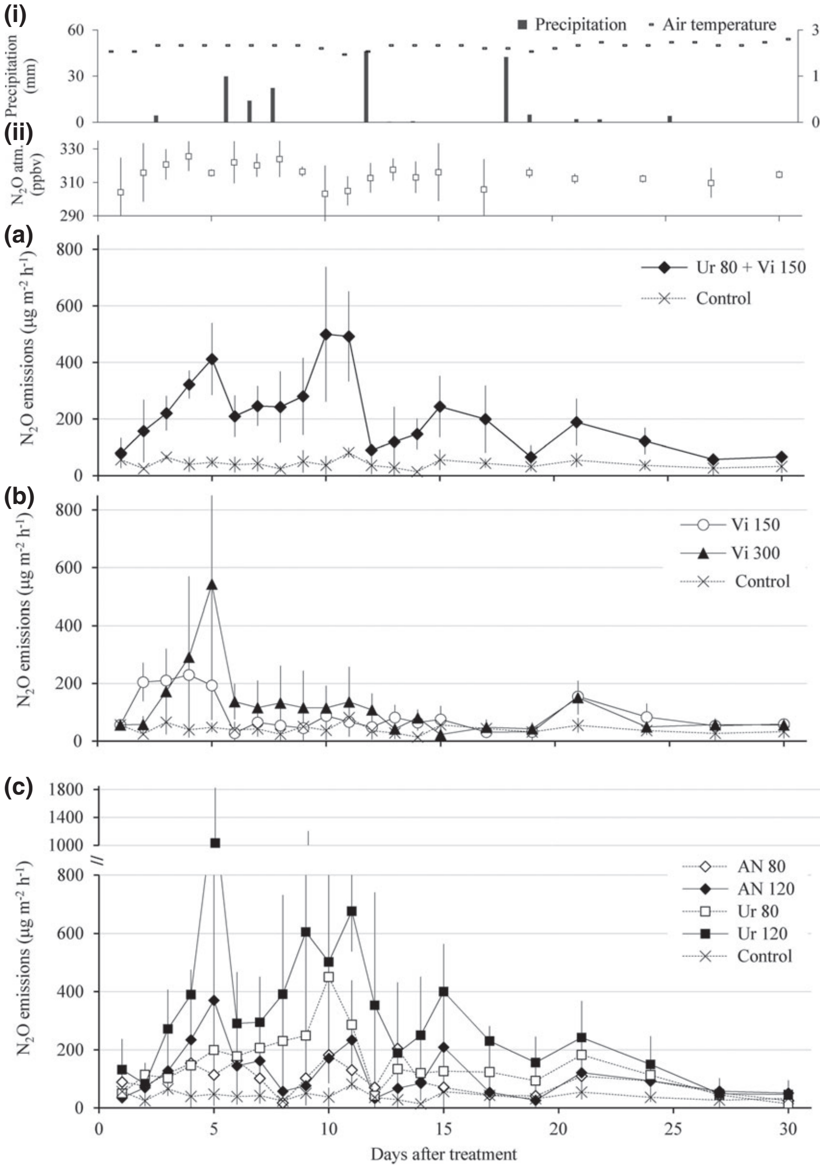

Fig. 3 N_2O emissions ($\mu g\ m^{-2}\ h^{-1}$) from Urea – Ur 80 (174 kg $ha^{-1} \approx$ 80 kg N ha^{-1}) and vinasse – Vi 150 (150 $m^3\ ha^{-1} \approx$ 195 kg N ha^{-1}) applied in the sugarcane ratoon (a). From different doses of the vinasse – Vi 150 and Vi 300 (300 $m^3\ ha^{-1} \approx$ 390 kg N ha^{-1}) (b). From different synthetic N-sources and doses: Ammonium nitrate – AN 80 (225 kg $ha^{-1} \approx$ 80 kg N ha^{-1}) and AN 120 (340 kg $ha^{-1} \approx$ 120 kg N ha^{-1}); and Urea – Ur 80 and Ur 120 (260 kg $ha^{-1} \approx$ 120 kg N ha^{-1}) (c). In the top figures (i and ii) represented environmental information during the experiment: (i) Precipitation (mm) and air temperature (°C); (ii) N_2O atmospheric concentration (ppmv).

atmospheric N_2O concentration was 314.5 ± 18.1 ppbv (Fig. 3ii).

Figure 3a presents the daily N_2O emissions from the treatment with the application of N-fertilizer urea (Ur 80 kg N ha^{-1}) with vinasse (Vi 150 $m^3\ ha^{-1}$) compared with the control. N_2O emissions from the combination of synthetic and organic N-sources (Ur + Vi) were higher than the control starting from the 2nd DAT and maintained a median level of 188 $\mu g\ N_2O\ m^{-2}\ h^{-1}$, while the control emission was 41 $\mu g\ N_2O\ m^{-2}\ h^{-1}$. This means that the combination of organic and synthetic sources was 4.5 times higher than the control.

On the 5th DAT, N_2O emissions from the Ur + Vi treatment were nine times higher than the control value. This high value of N_2O emissions early in the experimental period was observed in all treatments in which synthetic (urea and ammonium nitrate) and organic (vinasse) sources were applied alone (Fig. 3b and c). The maximum values in the range of results clearly expresses the magnitude of N_2O emissions using differ-

Table 4 N_2O emissions (mean ± standard deviation and range), cumulative $N\text{-}N_2O$ emissions and N_2O emissions factor from N-sources (mineral and organic) applied in the sugarcane ratoon

	N_2O emissions			N_2O emission factor	
	Mean ± SD*	Range†	Cumulative $N\text{-}N_2O$‡	N-source/level§	N-source¶
Treatments (sources/levels)	μg N_2O m^{-2} h^{-1}		mg $N\text{-}N_2O$ m^{-2}	% N-applied	
Control	41.9 ± 24.6	5.2–103.2	17.9 ± 1.0^{B}		
Mineral source					
Ammonium nitrate (80 kg N ha^{-1})	97.5 ± 77.5	5.0–478.3	39.1 ± 5.8^{A}	0.25 ± 0.07^{ns}	0.24 ± 0.06^{b}
Ammonium nitrate (120 kg N ha^{-1})	119.2 ± 112.8	4.9–530.2	47.3 ± 8.6^{A}	0.23 ± 0.07	
Urea (80 kg ha^{-1})	153.4 ± 145.0	2.5–998.4	61.8 ± 12.9^{A}	0.52 ± 0.15^{ns}	0.68 ± 0.24^{a}
Urea (120 kg ha^{-1})	323.9 ± 329.7	3.8–2188.4	122.0 ± 28.1^{A}	0.83 ± 0.22	
Organic source					
Vinasse (150 m^3 ha^{-1})	97.3 ± 77.7	3.8–312.1	38.6 ± 4.4^{A}	0.77 ± 0.16^{ns}	0.65 ± 0.29^{a}
Vinasse (300 m^3 ha^{-1})	125.5 ± 170.8	3.5–1117.9	46.9 ± 19.9^{A}	0.54 ± 0.37	
Urea (80 kg N ha^{-1}) + vinasse (150 m^3 ha^{-1})	212.6 ± 156.5	3.6–831.3	83.6 ± 21.3^{A}	0.59 ± 0.19	

*Mean ± SD = standard deviation.

†Range = Minimum and maximum.

‡Cumulative N_2O = sampled time in the sugarcane ratoon = 30 days.

§N_2O emissions factor from each N-sources and level applied in the sugarcane ratoon.

¶N_2O emissions factor from N-source applied in the sugarcane ratoon. Different capital letter to the Cumulative $N\text{-}N_2O$ (N-source and dose × control) and small letter to the Emission Factor (N-source/level and N-source) with significant differ in Student–Newman–Keuls test ($P < 0.05$); ns, not significant.

ent N-sources (synthetic and organic) applied on the surface (Table 4). The reduction in N_2O emissions between the 6th and 9th DAT can be related to consecutive high rainfall events (~ 66 mm) that occurred during the sampling period. The same occurred at 12 (46 mm) and 19 DAT (42 mm), when precipitation events occurred the day before sampling. At the 10th DAT, a second increase in N_2O emissions was observed in the treatment with N application at a magnitude thirteen times higher than the control. Two other increases in emissions were observed: one at the 15th DAT, four times higher than the control, and another at the 21th DAT, three times higher than the control. After the 24th DAT, N_2O emissions from the treatment with N application dropped to levels similar to the control.

The vinasse applied to the ratoon produced cumulative emissions 110% and 160% (for 150 and 300 m^3 ha^{-1}, respectively) higher than the control. Although N_2O emissions were higher as a result of the applied dose, there was no significant difference between them (Fig. 3b, Table 4).

The patterns of N_2O emissions from N-fertilizers (ammonium nitrate and urea) were quite different (Fig. 3c, Table 4). Ammonium nitrate had lower emissions than urea. The cumulative emissions for the different doses of ammonium nitrate showed differences of more than 115 and 160% (80 and 120 kg N ha^{-1}, respectively) compared with the control. However, the increase in the applied dose did not cause a proportional increase in N_2O emissions, and no showed significant difference.

N_2O emissions derived from urea were high in both applied doses. The highest dose had an accumulated value about twice the lowest dose. Thus, the source urea emitted 240 and 570% (80 and 120 kg N ha^{-1}, respectively) more than the control.

By the sugarcane ratoon, the use of ammonium nitrate presented an emission factor of 0.24%, while the source urea generated an emission factor almost three times higher (0.68%). The vinasse application presented an emission factor close to that observed for urea (0.65%). The combination of synthetic and organic sources (Ur + Vi), which is the most normal field condition, presented an emission factor of 0.59% (Table 4).

Discussion

N_2O emissions from soil and sugarcane straws

N_2O emissions from different quantities of sugarcane straw did not cause measurable changes. For the conditions of this study, our goal was to determine whether during the course of decomposition of sugarcane straw, it presents itself as a source for soil N, which would contribute significantly to N_2O emissions, because from the first cut, the straw covering the soil remains throughout the conduction period after the ratoon regrowth, in a cycle of deposition and decomposition.

Carmo *et al.* (2012) found increased N_2O emissions with an increasing amount of sugarcane residues deposited on the ground; however, this study included application of N-fertilizer and vinasse. Malhi & Lemke (2007) found no significant differences in N_2O emissions due to the maintenance or removal of different crop residues. The authors also commented that due to the high variability in the results, it is difficult to determine the interaction between factors (e.g., soil water content and temperature) that may influence the N_2O emissions, even with the application of the N-fertilizer. Wang *et al.* (2008) reported a reduction between 24 and 30% in N_2O emissions due to removal of the sugarcane straw and attributed this finding to the importance of organic compounds in the regulation of N_2O fluxes.

Our study evaluated only sugarcane straw as a variable, without introducing other N-sources. Sugarcane straw is a plant residue with a high C:N ratio (approximately 50–80). Moreover, sugarcane straw has a large amount of lignin and polyphenols that reduce the decomposition rate (Abiven *et al.*, 2005) and the availability of the minimal N (0.8%) is slow (Fortes *et al.*, 2012; Leal *et al.*, 2013). Huang *et al.* (2004) evaluated the influence of crop residue application with different C:N ratios on N_2O emissions, including sugarcane straw. This study showed that increasing the C:N ratio of the plant residues incorporated into the soil promoted reduction of N_2O emissions.

Currently, it is known that the amount of straw added by sugarcane is approximately 14.1 (7.4 to 24.3) Mg ha^{-1} yr^{-1}; however, there are different methods of managing the straw in the field (Leal *et al.*, 2013). In the central-southern region of Brazil, it is common to bunch the excess straw between the rows of crops because the producers claim that the high amount deposited along the cycles of regrowth promotes a shallower root system, which reduces the length of the crop cycle. The growth in the production of second generation ethanol can increase an industrial demand for excess straw remaining in field (Goldemberg, 2008).

It is worth mentioning that the N_2O emission was approximately 12 μg N_2O m^{-2} h^{-1}, a low value for agricultural soils. However, one must consider that the experiment was conducted at the beginning of a regrowth cycle, after exportation of nutrients by the crop (stalks) with no contribution of N-sources. In an experiment to assess N_2O emissions from elephant grass cultivation for biomass production, Morais *et al.* (2013) found N_2O emissions ranging from 0.1 to 10 μg m^{-2} h^{-1}, which increased due to soil management and N-fertilizer application.

Throughout the experimental period (~180 days), interventions related to sugarcane management in the area were made only to meet the goals of the experiment (i.e., herbicide application), without the normal crop management practices in the region (i.e., topdressing N-fertilizer or vinasse application), so as to prioritize only the N_2O emissions from sugarcane straw. Additionally, one must note that the determinations were made between crop rows (Cai *et al.*, 2012). The ground in this area has a high clay content (> 60%), and the third regrowth is sugarcane, which means that it has high soil bulk density (maximum 1.52 g cm^{-3}) as a result of machinery traffic and consequently lower pore space for N_2O production and gas exchange.

N_2O emissions from organic and synthetic N-sources in sugarcane cultivation

The N_2O emissions in the control plots at sugarcane planting (~ 90 μg N_2O m^{-2} h^{-1}) were considered to be high for agricultural soils. This result is most likely due to soil disruption in the reform area cultivation due to tillage operations (physical destruction of stumps, plowing, and opening and closing of the furrows) causing the incorporation of crop residues and aeration into the soil surface layer, which favors the priming effect and soil organic matter mineralization (La Scala *et al.*, 2006; Silva-Olaya *et al.*, 2013). These processes accelerate the labile-C availability in the soil, and thus, the set of microbiological processes enabled by the formation of anaerobic sites with high CO_2 concentrations together with the mineralization-N increase and N_2O emissions from the soil (Rochette, 2008; Morais *et al.*, 2013). The same effect was observed by Morais *et al.* (2013), who observed higher N_2O emissions after soil plowing of between 30 and 100 μg m^{-2} h^{-1} (maximum 450 μg m^{-2} h^{-1}) for elephant grass management. In the same period, the authors also found higher CO_2 and inorganic N concentrations.

The increase in N_2O emissions at the beginning of the measurements in the experiment involving sugarcane planting that occurred for the trial with a combination of organic and synthetic sources (filter cake + urea) shows the effect of the interaction between the source; however, it has not been possible to establish with certainty whether this synergistic effect was due to a reaction between the filter cake and urea, or, more simplistically, as soil moisture was low, the filter cake moisture would have initiated the urea transformation, favoring N_2O emissions.

In the regrowth crop, the N_2O emissions control had a value (~ 40 μg N_2O m^{-2} h^{-1}) that is considered normal for cultivated agricultural soils in tropical regions (Gomes *et al.*, 2009; Kachenchart *et al.*, 2012; Morais *et al.*, 2013). The increase in N_2O emissions during the initial period of sampling is most likely associated with transformations in soil N because the application of the fertilizer+vinasse (Vi + Ur) promoted the same effect on the soil in sugarcane planting as filter cake plus urea. The urea and vinasse provided N-reactive and a faster increase in soil labile-C and humidity by vinasse.

Ball *et al.* (1999) showed that high doses of N-fertilizer promoted changes in N_2O emissions for a period of up to six weeks. Morais *et al.* (2013) showed that 80% of the N_2O emissions due to N-fertilizer occurred in the 60 days after the N-fertilizer was applied.

N_2O emissions from vinasse were high when compared to the control or to other sources containing nitrogen (e.g. ammonium nitrate). This was most likely due to the available N form in this by-product in addition to the high organic content readily available to microorganisms in the soil, in which the higher microbial activity produced anaerobic sites with high N_2O production.

During the experiments, the N_2O emissions were largely influenced by precipitation, both the lack and excess thereof. There was no irrigation during planting; thus, the lack of moisture hindered the availability of N-fertilizer. In low soil moisture conditions, there was little development of the microbiota responsible for the N transformation (immobilization, mineralization and denitrification). The onset of precipitation and the resulting increased soil moisture promoted the development of microorganisms, increasing the availability of the reactive forms of soil N.

Regardless of the situation (planting or regrowth), the entry of water into the porous soil system promoted the 'expulsion' of gases contained in the pore spaces (physical movement) generated by soil disturbances at planting and in the macro- and mesopores formed between the particles and aggregates in the soil of regrowth.

Excessive rainfall on consecutive days, even in tropical soils with good drainage, causes most of the pore space to temporarily fill with water (~100% saturation of WFPS), thereby decreasing microbial activity and reducing greenhouse gas emissions (Bouwman, 1998). Hence, at higher WFPS (> 80%), the major product of the denitrifying community is N_2 (complete denitrification) (Davidson *et al.*, 2000). Leaching may also occur because N_2O has a high solubility in water (Heincke & Kaupenjohann, 1999).

Emissions factor from N applied in sugarcane cultivation

For the synthetic N-fertilizer applied in the sugarcane, the overall EF was 0.46 ± 0.33%. The ammonium nitrate has a lower emission factor than the urea. This behavior was most evident during the sugarcane regrowth and surface N application. This difference in N_2O emissions between sources is related to changes occurring in the soil related to the transformation of each source especially changes in soil pH. When applied, the breakdown of urea by the enzyme urease leads to a rapid pH increase (Lara-Cabeza & Souza, 2008); this brief increase in the soil pH is sufficient to promote the conversion of ammonium to nitrate (Wickramasinghe *et al.*, 1985) and, consequently, start the denitrification. However, the conversion of ammonium to nitrate promotes soil acidification, which hinders nitrification (Page *et al.*, 2002), also affecting the denitrifying community (Daum & Schenk, 1998).

Carmo *et al.* (2012) calculated EF for N-fertilizer in sugarcane as 1.1% for N applied at planting as urea at the same dose as in this study (60 kg N ha^{-1}) and, for the N applied to the topdressing, the EF was 0.76% for a dose of 120 kg N ha^{-1}, a factor very similar to that determined in our experiment (0.83%). Lisboa *et al.* (2011) presented an emission factor of 3.8% for N-fertilizer in calculating the carbon footprint of Brazilian ethanol, based mostly on literature review. Morais *et al.* (2013) evaluated urea application in elephant grass, which has a similar management as sugarcane involving harvest and regrowth cycles. In this situation, the authors found an EF of 0.51% after three cycles.

The EF for filter cake and vinasse did not showed the expected behavior, or, the increasing the dose did not increase N_2O emissions linearly. Thus, the highest dose showed a smaller EF than the lowest dose, this occurs because the EF calculation is based on the amount of N applied.

The filter cake applied at sugarcane planting had a lower EF relative to other synthetic and organic sources, even with similar cumulative emissions to synthetic sources used in planting (0.13%). This occurred because even with a low N content (~ 2%), the amount of filter cake applied at planting (25 and 40 Mg ha-1) was high. However, the amount of N applied was not available to denitrifying organisms in the soil, and when the EFs were calculated, the values were low.

The vinasse applied generated a high emission factor (0.65%) analogous to that observed for urea application. N contained in the vinasse, together with the application form of fertirrigation, favored N_2O production in the soil. In a study of short duration with application of 200 m^{-3} ha^{-1} of vinasse, Oliveira *et al.* (2013) calculated an EF of 0.44%.

The EF calculated from the sources combination filter cake + urea at planting and urea + vinasse in covering regrowth did not produce values representing a summation of sources when individually evaluated, but rather demonstrated an interaction between sources leading to a specific emissions factor.

Carmo *et al.* (2012) evaluated emissions from mixing organic and synthetic sources for the supply of N to the crop of sugarcane planting and regrowth. The specific EF were much higher for the combination planting (filter cake + urea); EF was 1.1% vs. the 0.21% observed in our study. In the regrowth crop period (urea + vinasse), the difference was even greater, with an EF of the 3.0%, vs. 0.59% in our study.

Schils *et al.* (2008) studied the effect of the combination of fertilizer with an organic source (liquid slurry) in ryegrass in the Netherlands and found an EF between 0.13 and 0.17% in the application of calcium ammonium nitrate, an EF of 0.12% in the application of liquid slurry, and values of 0.35 to 0.39% when a mix of fertilizer and slurry was applied. Dambreville *et al.* (2008), in a maize crop in the Brittany region (France), found an EF between 0.01 and 0.23% from liquid pig slurry application alone and of 0.01% for ammonium nitrate.

In the literature, N_2O emission factors from N-fertilizer application in sugarcane cultivation mostly come from studies conducted in Australia, with values ranging from 1.0 to 21% (Weier, 1999; Galbally *et al.*, 2005; Wang *et al.*, 2008; Allen *et al.*, 2010; Denmead *et al.*, 2010). In terms of commercial sugarcane cultivation in Australia, the nitrogen cycle peculiarities, which reflect on the direct and indirect N_2O emissions and consequently on the EFs, are related especially to the use of irrigation, drainage and presence of the seasonal fluctuations in the groundwater levels that directly influence soil oxygenation (and gas diffusion) against water-filled pore space (WFPS%). Abbasi & Adams (2000) found that for the same amount of N-fertilizer applied, the EF responded differently to increased WFPS%, with EFs of 0.15, 0.40 and 2.8%, respectively, for 63, 71 and 84% WFPS% values.

In the central-southern region of Brazil, where more than 85% of the Brazilian sugarcane is cultivated (UNICA, 2014), the management does not include the irrigation, or the soil does not report the occurrence of this specific phenomenon of poor drainage or seasonal fluctuations in the groundwater.

As shown by Smeets *et al.* (2009), our results indicate that the use of the default value of IPCC (2006) for the conditions of sugarcane cultivation in the central-southern region of Brazil overestimates the contribution of direct N_2O emissions from N-fertilizer (synthetic or organic). Our results not only supply measured values for EFs from N-applied but also help to reduce the variance of the values. Combining these results with other values for this region (Carmo *et al.*, 2012; Morais *et al.*, 2013; Oliveira *et al.*, 2013) should facilitate eliminating the uncertainties associated with these emission sources.

Other studies showed that sugarcane usually does not respond to increasing rates of N (Reis Jr *et al.*, 2000; Franco *et al.*, 2011). This is due to the contribution of biological nitrogen fixation (BNF) by groups of diazotrophic endophytics responsible for 25–60% of the N supply to the crop (Reis Junior *et al.*, 2000; Boddey *et al.*, 2001; Resende *et al.*, 2003). Thus, the N management in sugarcane varies considerably with respect to sources and doses. Evaluating different variety behavior to reduce the external N supply (synthetic and/or organic) and the possibility of finding the best economic level and not 'standardized' quantities of fertilizers based on expected yields can effectively reduce N_2O emissions.

In the case of greenhouse gas inventories from biofuels, we suggest a top-down approach using EFs evaluated for specific situations (Tier 2 – IPCC, 2006) in addition to extrapolating N_2O emission values to regional scales to include more sophisticated mathematical models with a bottom-up approach (Del Grosso *et al.*, 2008) that seek a better understanding of the N cycle, including the influence of other factors (e.g., water, temperature, aeration, labile-C availability and plant N demand), rather than only the amount of N-applied and amount of N_2O emitted.

Acknowledgements

We would like to thank Usina Capuava, Piracicaba, SP, for allowing us to conduct our experiments in their sugarcane areas. We also thank Admilson Margato and Ralf Araújo for assistance in the field and laboratory analysis. Support for this research was provided by the São Paulo Research Foundation (FAPESP) through the Regular Research Program (Process Number 2011/07276-6) and, to the first author, through the Post-doctoral Program (Process Number 2010/20065-1).

References

Abbasi MK, Adams WA (2000) Gaseous N emission during simultaneous nitrification-denitrification associated with mineral N fertilization to a grassland soil under field conditions. *Soil Biology & Biochemistry*, **32**, 1251–1259.

Abiven S, Recous S, Reyes V, Oliver R (2005) Mineralisation of C and N from root, stem and leaf residues in soil and role of their biochemical quality. *Biology and Fertility of Soils*, **42**, 119–128.

Aguilera E, Lassaletta L, Sanz-Cobena A, Garnniere J, Vallejo A (2013) The potential of organic fertilizers and water management to reduce N_2O emissions in Mediterranean climate cropping systems. A review. *Agriculture, Ecosystems & Environment*, **164**, 32–52.

Allen DE, Kingston G, Rennenberg H, Dalal RC, Schmidt S (2010) Effect of nitrogen fertilizer management and waterlogging on nitrous oxide emission from subtropical sugarcane soil. *Agriculture, Ecosystems & Environment*, **136**, 209–217.

Ball BC, Parker JP, Scott A (1999) Soil and residue management effects on cropping conditions and nitrous oxide fluxes under controlled traffic in Scotland 2. Nitrous oxide, soil N status and weather. *Soil & Tillage Research*, **52**, 191–201.

Boddey RM, Polidoro JC, Resende AS, Alves BJR, Urquiaga S (2001) Use of the ^{15}N natural abundance technique for the quantification of the contribution of N_2 fixation to sugarcane and other grasses. *Australian Journal of Plant Physiology*, **28**, 889–895.

Bouwman AF (1998) Nitrogen oxides and tropical agriculture. *Nature*, **392**, 866–867.

Bouwman AF, Bouwman LJM, Batjes NH (2002) Modeling global annual N_2O and NO emissions from fertilized fields. *Global Biogeochemistry Cycles*, **16**, 1080–1088.

Brazil. (2010) *Brazil's 2nd National Communication to the Framework Convention of the United Nations on Climate Changes*. Ministério de Ciência & Tecnologia, Brasília, DF.

Butterbach-Bahl K, Baggs EM, Dannenmann M, Kiese R, Zechmeister-Boltenstern S (2013) Nitrous oxide emissions from soils: how well do we understand the processes and their controls? *Philosophical Transaction of The Royal Society B*, **368**, 20130122.

Cai Y, Ding W, Luo J (2012) Spatial variation of nitrous oxide emission between interrow soil and interrow plus row soil in a long-term maize cultivated sandy loam soil. *Geoderma*, **181–182**, 2–10.

CANASAT (2014) Sugarcane crop monitoring in Brazil by Earth observing satellite images. Available online: http://www.dsr.inpe.br/laf/canasat/ (accessed on 14 May 2014).

Carmo JB, Filoso S, Zotelli LC *et al.* (2012) Infield greenhouse gas emissions from sugarcane soils in Brazil: effects from synthetic and organic fertilizer application and crop trash accumulation. *Global Change Biology Bioenergy*, **5**, 267–280.

Choudhard MA, Akramkhanov A, Saggar S (2001) Nitrous oxide emissions in soils cropped with maize under long-term tillage and under permanent pasture in New Zeland. *Soil & Tillage Research*, **62**, 61–71.

Dambreville C, Morvan T, Germon JC (2008) N_2O emission in maize-crops fertilized with pig slurry, matured pig manure or ammonium nitrate in Brittany. *Agriculture, Ecosystems & Environment*, **123**, 201–210.

Daum D, Schenk MK (1998) Influence of nutrient solution pH on N_2O and N_2 emissions from a soil less culture system. *Plant and Soil*, **209**, 279–287.

Davidson EA, Keller M, Erickson HE, Verchot LV, Veldkamp E (2000) Testing a conceptual model of soil emissions of nitrous and nitric oxides. *BioScience*, **50**, 667–680.

Del Grosso SJ, Wirth T, Ogle SM, Parton WJ (2008) Estimating agricultural nitrous oxide emissions. *Eos*, **89**, 259–540.

Denmead OT, Macdonald BCT, Bryant G *et al.* (2010) Emissions of methane and nitrous oxide from Australian sugarcane soils. *Agricultural and Forest Meteorology*, **150**, 748–756.

Flückiger J, Dällenbach A, Blunier T, Stauffer B, Stocker TF, Raynaud D, Barnola J-M (1999) Variations in atmospheric N_2O concentration during abrupt climatic changes. *Science*, **285**, 227–230.

Fortes C, Trivelin PCO, Vitti AC (2012) Long-term decomposition of sugarcane harvest residues in Sao Paulo state, Brazil. *Biomass and Bioenergy*, **42**, 189–198.

Franco HCJ, Otto R, Faroni CE, Vitti AC, Oliveira ECA, Trivelin PCO (2011) Nitrogen in sugarcane derived from fertilizer under Brazilian field conditions. *Field Crops Research*, **121**, 29–41.

Fravet PRF, Soares RAB, Lana RMQ, Lana AMQ, Korndörfer GH (2010) Effect of filter cake doses and methol of application on yield and technologycal quality of sugar cane ratoon. *Ciência e Agrotecnologia*, **34**, 618–624.

Freire WJ, Cortez LAB (2000) *Vinhaça de Cana-de-Açúcar*. Agropecuária, Guaíba.

Galbally I, Meyer M, Bentley S *et al.* (2005) A study of environmental and management drivers of nitrous oxide emissions in Australian agro-ecosystems. *Environmental Sciences*, **123**, 225–237.

Goldemberg J (2008) The Brazilian biofuels industry. *Biotechnology for Biofuels*, **1**, 6.

Gomes J, Bayer C, Costa FS, Piccolo MC, Zanatta JA, Vieira FCB, Six J (2009) Soil nitrous oxide emissions in long-term cover crops-based rotations under subtropical climate. *Soil & Tillage Research*, **106**, 36–44.

Gu J, Nicoullaud B, Rochette P, Grossel A, Hénault C, Cellier P, Richard G (2013) A regional experiment suggests that soil texture is a major control of N_2O emissions from tile-drained winter wheat fields during the fertilization period. *Soil Biology & Biochemistry*, **60**, 134–141.

Heincke M, Kaupenjohann M (1999) Effects of soil solution on the dynamic of N_2O emissions: a review. *Nutrient Cycling in Agroecosystems*, **55**, 133–157.

Huang Y, Zou J, Zheng X, Wang Y, Xu Y (2004) Nitrous oxide emissions as influenced by amendment of plant residues with different C: N ratios. *Soil Biology & Biochemistry*, **36**, 973–981.

IPCC (2001) Technical summary. In: *Climate Change 2001. The Scientific Basis. Contributions of Working Group I of the Third Assessment Report of the Intergovernmental Panel on Climate Change* (eds Houghton JT, Ding Y, Griggs DJ, Noguer M, van der Linden PJ, Dai X, Maskell K, Johnson CA), pp. 239–288. Cambridge University Press, Cambridge.

IPCC (2006) Guidelines for National Greenhouse Gas Inventories. Vol. 4. *Agriculture, Forestry and Other Land Use.* Prepared by the National Greenhouse Gas Inventories Program (eds Eggleston HS, Buendia L, Miwa K, Ngara T, Tanabe K), pp. 5.1–5.50. IGES, Hayama, Kanagawa.

Kachenchart B, Jones DL, Gajaseni N, Edwards-Jones G, Limsakul A (2012) Seasonal nitrous oxide emissions from different land uses and their controlling factors in a tropical riparian ecosystem. *Agriculture, Ecosystems & Environment*, **158**, 15–30.

Khalil K, Mary B, Renault P (2004) Nitrous oxide production by nitrification and denitrification in soil aggregates as affected by O_2 concentration. *Soil Biology & Biochemistry*, **36**, 687–699.

La Scala NJr, Bolonhezi D, Pereira GT (2006) Short-term soil CO_2 emission after conventional and reduced tillage of a no-till sugar cane area in southern Brazil. *Soil & Tillage Research*, **91**, 244–248.

Lara-Cabeza WAR, Souza MA (2008) Ammonia volatilization, leaching of nitrogen and corn yield in response to the application of mix of urea and ammonium sulphate or gypsum. *Brazilian Journal of Soil Science*, **32**, 2331–2342.

Leal MRLV, Galdos MV, Scarpare FV, Seabra JEA, Walter A, Oliveira COF (2013) Sugarcane straw availability, quality, recovery and energy use: a literature review. *Biomass and Bioenergy*, **53**, 11–19.

Lisboa CC, Butterbach-Bahl K, Mauder M, Kiese R (2011) Bioethanol production from sugarcane and emissions of greenhouse gases. Know and unknowns. *Global Change Biology Bioenergy*, **3**, 1–16.

Macedo IC, Seabra JEA, Silva JEAR (2008) Green house gases emissions in the production and use of ethanol from sugarcane in Brazil: The 2005/2006 averages and a prediction for 2020. *Biomass and Bioenegy*, **32**, 582–595.

Malhi SS, Lemke R (2007) Tillage, crop residue and N fertilizer effects on crop yield, nutrient uptake, soil quality and nitrous oxide gas emissions in a second 4-yr rotation cycle. *Soil & Tillage Research*, **96**, 269–283.

Morais RF, Boddey RM, Urquiaga S, Jantalia CP, Alves BJR (2013) Ammonia volatilization and nitrous oxide emissions during soil preparation and N fertilization of elephant grass (*Pennisetum purpureum* Schum.). *Soil Biology & Biochemistry*, **64**, 80–88.

Oliveira BG, Carvalho JLN, Cerri CEP, Cerri CC, Feigl BJ (2013) Soil greenhouse gas fluxes from vinasse application in Brazilian sugarcane areas. *Geoderma*, **200**, 77–84.

Page KL, Dalal RC, Menzies NW, Strong WM (2002) Nitrification in a Vertisol subsoil and its relationship to the accumulation of ammonium-nitrogen at depth. *Australian Journal of Soil Research*, **40**, 727–735.

Prado RM, Caione G, Campos CNS (2013) Filter cake and vinasse as fertilizers contributing to conservation agriculture. *Applied and Environmental Soil Science*, **1**, 1–9.

Ravishankara AR, John SD, Robert WP (2009) Nitrous oxide (N_2O): the dominant ozone-depleting substance emitted in 21st century. *Science*, **326**, 123–125.

Reis Junior FB, Reis VM, Urquiaga S, Döbereiner J (2000) Influence of nitrogen fertilisation on the population of diazotrophic bacteria *Herbaspirillum* spp. and *Acetobacter diazotrophicus* in sugar cane (*Saccharum* spp.). *Plant and Soil*, **219**, 153–159.

Resende AS, Xavier RP, Quesada DM, Urquiaga S, Alves BJR, Boddey RM (2003) Use of green manures in increasing inputs of biologically fixed nitrogen to sugarcane. *Biology and Fertility of Soils*, **37**, 215–220.

Rochette P (2008) No-till only increases N_2O emissions in poorly-aerated soils. *Soil & Tillage Research*, **101**, 97–100.

Rudorff BFT, Aguiar DA, Silva WF, Sugawara LM, Adami M, Moreira MA (2010) Studies on the rapid expansion of sugarcane for ethanol production in São Paulo State (Brazil) Using landsat data. *Remote Sensing*, **2**, 1057–1076.

Schils RLM, Groenigen JW, Velthof GL, Kuikman PJ (2008) Nitrous oxide emissions from multiple combined applications of fertilizer and cattle slurry to grassland. *Plant and Soil*, **310**, 89–101.

Silva APM, Bono JAM, Pereira FAR (2014) Fertigation with vinasse in sugarcane crop: effect on the soil and on productivity. *Revista Brasileira de Engenharia Agrícola e Ambiental*, **18**, 38–43.

Silva-Olaya AM, Cerri CEP, La Scala Jr N, Dias CTS, Cerri CC (2013) Carbon dioxide emissions under different soil tillage systems in mechanically harvested sugarcane. *Environmental Research Letters*, **8**, 1–9.

Smeets EMW, Bouwman LF, Stehfest E, van Vuuren DP, Posthuma A (2009) Contribution of N_2O to the greenhouse gas balance of first-generation biofuels. *Global Change Biology*, **15**, 1–23.

Soil Survey Staff (1999) *Soil Taxonomy: A Basic System of Soil Classification for Making and Interpreting Soil Surveys*, 2nd edn. Natural Resources Conservation Service. U.S. Department of Agriculture Handbook, Washington, DC, USA.

Stehfest E, Bouwman L (2006) N_2O and NO emission from agricultural fields and soils under natural vegetation, summarizing available measurement data and modeling of global annual emissions. *Nutrient Cycling in Agroecosystems*, **74**, 207–228.

UNICA (2014) Union of the Sugarcane industries. Available online: http://www.unicadata.com.br/. (Accessed on 14 May 2014).

Veiga CFM, Vieira JR, Morgado IF (2006) *Diagnóstico da Cadeia Produtiva da Cana-de-Açúcar do Estado do Rio de Janeiro: Relatório de Pesquisa*. FAERJ:SEBRAE, Rio de Janeiro.

Wang WJ, Moody PW, Reeves SH, Salter B, Dalal RC (2008) Nitrous oxide emissions from sugarcane soils: effects of urea forms and application rate. *Proceedings of the Australian Society of Sugar Cane Technologists*, **30**, 87–94.

Weier KL (1999) N_2O and CH_4 consumption in a sugarcane soil after variation in nitrogen and water application. *Soil Biology & Biochemistry*, **31**, 1931–1941.

Wickramasinghe KN, Rodgers GA, Jenkinson DS (1985) Transformations of nitrogen fertilizers in soil. *Soil Biology & Biochemistry*, **17**, 625–630.

World Meteorological Organization. 2014WMO Greenhouse gas bulletin: the state of greenhouse gases in the atmosphere based on observations through 2013. Available online: http://www.wmo.int/pages/prog/arep/gaw/gaw_home_en.html. (Accessed on 21 Oct 2014).