

Nitrous oxide emission and ammonia volatilization induced by vinasse and N fertilizer application in a sugarcane crop at Rio de Janeiro, Brazil

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Abstract In Brazilian sugarcane plantations, fertilization with vinasse, supplemented or not with mineral fertilizer, is a common practice. But little is known about the effects of this application on N losses, especially those forms of N which give rise to greenhouse gas emissions. The aim of this study was to quantify N_2O and NH_3 emissions from soil after vinasse application and urea fertilizer addition and to examine the possible impact adding vinasse before or after urea. Two experiments were conducted under greenhouse conditions and one in the field with treatments of vinasse and urea fertilizer, either alone, or in sequence. The highest proportions of N emitted as N_2O were registered in the vinasse treatment, which amounted to 15 % of the N applied in the first greenhouse experiment, and 2.5 % in the field experiment. With respect to the losses by NH_3 volatilization, urea was the only treatment where this process was significant. N_2O emission from vinasse was 2.5 %, somewhat above the default emission factor of 1 % of the IPCC. N_2O emissions from urea were

also variable, but emission factors registered were still well below the default IPCC factor for organic residues. The order of addition of urea and vinasse had little effect on NH_3 volatilization in the field, but there were evidences it was important for N_2O .

Keywords Greenhouse gas emissions · N fertilizer · Nitrous oxide · Sugarcane · Vinasse

Introduction

The Brazilian “Proálcool” program to produce ethanol from sugarcane (*Saccharum officinarum* L.) on a large scale to fuel Otto cycle engines for light vehicles started in the early 1970s. In 1975, the area under sugarcane was 1.9 Mha of which 63 % was used to produce approximately 600 million litres of ethanol (MME 2007). In the first years of this century the launch of vehicles powered by Flexfuel engines capable of running any mixture of hydrated ethanol (95, 5 % water) and gasoline gave a boost to the program such that the harvested area in 2011 was 8.6 Mha of which 54 % was used to produce 27.7 billion litres of ethanol (CONAB 2011).

The Proálcool program was financed initially to reduce Brazil's dependency on imported oil, but today is regarded as part of the effort to reduce greenhouse gas (GHG) emissions from the transport sector. In the

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production of any biofuel there are inputs of fossil energy in the agricultural, manufacturing and distribution phases. With improved agronomic practices, plant breeding for higher yields and sugar content, and increased efficiency of fermentation and distillation in the factories, today national yield is close to 80 Mg cane ha⁻¹ and produces approximately 85 L ethanol Mg cane⁻¹, or 6,800 L ha⁻¹ (CONAB 2011; IBGE 2012). Under such a scenario, recent estimates of the energy balance are very positive, between 8 and 9 (Macedo 1998; Boddey et al. 2008; Macedo et al. 2008). The energy balance is considerably more favourable than for other sources (e.g. maize grain, Hill et al. 2006) mainly because all factory processing is fuelled by bagasse, while for other crops fossil energy is generally used.

However, to evaluate the potential of bioethanol to mitigate GHG emissions, other important GHGs arising from the production system must be taken into account. Methane (CH₄) and nitrous oxide (N₂O) are the most important GHGs in sugarcane cropping system. As trash burning is no longer a common practice in sugarcane production, N₂O is produced principally from nitrogen fertilizers added to the crop. N₂O and CH₄ can be also produced from vinasse. Vinasse is the liquid waste remaining after ethanol has been distilled from the “wine”. For every litre of ethanol approximately 12 L of vinasse are produced (Macedo 1998). The practice adopted on nearly all plantations today is to transport this material to the field to irrigate the crop, as it is a valuable source of potassium and to a lesser extent nitrogen. To return the nutrients removed at harvest it is necessary to apply approximately 80 m³ ha⁻¹ of vinasse and normal practice is to add between 80 and 100 m³ ha⁻¹ distributed by overhead aspersion.

Such large additions of vinasse are likely to produce significant emissions of CH₄ and N₂O in the field. IPCC tier 1 emission factor has been used to estimate N₂O emissions from N fertilizer and vinasse applied in the field (Macedo et al. 2008; Boddey et al. 2008; Galdos et al. 2010), however it is not well accepted to represent real emissions (Smith et al. 2012). Lisboa et al. (2011) estimated N₂O emissions on one sugar cane field in Brazil using the eddy-covariance technique over 5 months, the period from harvest of a ratoon crop through the renovation of the plantation by ploughing, application of filter cake (25 Mg ha⁻¹) and replanting of the crop. The

location and soil type were not described but the total emission during this period was 2.1 kg N₂O-N ha⁻¹. They also calculated a N₂O emission factor for N fertilizer application to sugarcane of 3.87 % (i.e. 3.87 kg of N₂O-N are emitted for each 100 kg of fertilizer N applied) but the estimate was a mean based on studies in Australia and Hawaii, and none on Ferralsols, the soil type that predominates in cane fields in Brazil. Ferralsols are strongly weathered soils commonly found in Brazilian cropping areas and in view of their particular structure are very well drained, which is considered unfavourable for N₂O emissions (Jantalia et al. 2008).

More recently, Carmo et al. (2013) published the first results of N₂O and CH₄ emissions from soils treated with vinasse and N fertilizer in typical sugarcane areas of São Paulo State in Brazil. Methane emissions were not detected but results suggested the CH₄ oxidation as the dominant process. On the other hand, N₂O emissions were close to 3 % of the N added as vinasse and <1 % of the N added as fertilizer, but emissions were increased when vinasse and N fertilizer were applied together and also with the quantity of trash left after harvesting crop. Such high N₂O emission factor was not confirmed by Oliveira et al. (2013), who found roughly 0.5 % of the N in vinasse emitted as N₂O.

Most N fertilizer used in sugarcane production is urea, and as soon as this form of N fertilizer is distributed on the surface of the trash or soil, a large proportion is rapidly transformed into ammonia by the action of urease. Hence, large losses of N via ammonia volatilization may occur. This has two major consequences: firstly it reduces the amount of fertilizer N available for nitrification and subsequent reduction in anoxic zones do N₂O and N₂, thereby reducing direct N₂O emissions, and, secondly the ammonia volatilized will finally be deposited elsewhere and some proportion of this will eventually be nitrified and subsequently contribute to off-site “indirect” N₂O emissions. The IPCC (2006) Tier 1 default factor for direct emissions is 1 % of the applied N, which should be increased to 1,325 % when indirect N₂O emissions are also to be accounted for. In sugarcane areas, N-urea losses by NH₃ volatilization were quantified and found to vary from 0 to 40 % (Freney et al. 1992; Costa et al. 2003; Cantarella et al. 2008; Mariano et al. 2012), the highest values associated with placement on top of the trash left on soil after plant harvest.

The composition of vinasse may vary a lot depending on several factors and C contents are generally between about 1 and 2 %. Apart from the time the vinasse remains stored, its composition depends on the cane variety used in the preparation of the “must” (cane juice), the soil type, climatic conditions during crop growth and the process used in the manufacture of ethanol. Nitrogen ranges from 90 and 885 mg N L⁻¹ with an average content of 357 mg N L⁻¹ (Elia-Neto and Nakahodo 1995). Depending on the management of fertilizer and the high biological oxygen demand of vinasse large emissions of N₂O can be expected (Carmo et al. 2013).

Obviously, more studies are required to better understand the interaction of vinasse and N fertilizer as there is no specific recommendation on timing for the use of both products. In addition, possible losses of N by ammonia volatilization need to be considered as this could explain differences in N₂O losses. For this reason, the objective of this study was to evaluate NH₃ volatilization and N₂O emissions from applications of vinasse and N fertilizer to soils of sugarcane areas with the focus on timing of the applications.

Materials and methods

Three experiments were carried out at the experimental area of Embrapa Agrobiologia at Seropédica, Rio de Janeiro State (22°49'S, 43°39'W), two under controlled conditions to investigate the potential of vinasse as soil N₂O inducers and the other under field conditions to measure the proportion of the N from vinasse and N fertilizer emitted as N₂O and lost as NH₃ with reference to timing of the applications.

Pot experiments

The first pot experiment was initiated on 05 November 2009. Pots presented a surface area of 530 cm² and contained 5 kg of the 0–10 cm layer of a soil classified as an Haplic Acrisol (FAO) or Typic Hapludult (USDA, Soil Taxonomy), with 41 % clay and 49 % sand, pH 5.8, 12 g C kg⁻¹ and 1.0 g N kg⁻¹.

Five treatments were imposed with additions of: A—control (no N added); B—urea fertilizer; C—old vinasse (collected from a channel about 1 km from the main source); D—fresh vinasse (collected close to the pipe that issues hot vinasse from the distillery); and

E—filter cake. Each treatment was replicated 5 times and pairs of pots (one for the evaluation of NH₃ volatilization and the other for N₂O emissions) were laid out in a complete randomized design. The two types of vinasse and the filter cake were obtained from a mill in the north of the State of Rio de Janeiro (COAGRO, Campos dos Goytacazes).

The vinasse was added at 795 ml pot⁻¹ (equivalent to 150 m³ ha⁻¹) and the filter cake at 530 g pot⁻¹ (10 Mg ha⁻¹). Urea was added in solution at 12 g urea pot⁻¹ (250 kg N ha⁻¹). Every 4 days 23 mm of distilled water were added to each pot to simulate rainfall. Fresh and old vinasse applications were equivalent to 13.2 and 9.4 kg N ha⁻¹ on an area basis (0.09–0.06 g N L⁻¹ vinasse, respectively) whilst the filter cake represented 79 kg N ha⁻¹.

The pots destined for the evaluation of N₂O were equipped with plastic lids which were perforated and fitted externally with a three-way “LuerLock” valve. The pots were only sealed with the lids for the period of the sampling the enclosed head space. Gas sampling was performed at some time between 08.30 and 10.30 h (Alves et al. 2012) every day for the first 15 days and three times a week thereafter. Sampling time was 30 min, one sample being taken immediately after closure and another at the end of incubation time. The gas accumulated in the head space of each chamber was transferred to 20 ml chromatographic vials using a hand vacuum pump adapted to be connected to the chamber and the vial simultaneously.

Analyses of N₂O concentrations were performed using a Perkin Elmer Auto System XL gas chromatograph equipped with an electron capture detector and a back-flush system with a Porapak Q GC column (Jantalia et al. 2008). Fluxes of N₂O were calculated as described by Jantalia et al. (2008).

Loss of N from the pots via NH₃ volatilization was also evaluated, using the method described in Jantalia et al. (2012). For this technique the pots were covered by a 2 litre plastic (PET) soft-drink bottle, the base of which had been cut off. From the mouth of the bottle a strip (wick) of polyurethane foam (3 × 25 × 250 mm) is suspended to which was added 10 ml of 1.0 M sulphuric acid amended with 20 g L⁻¹ glycerine. Volatilized ammonia is retained on the wick.

The wicks were changed every 3 days for the first 15 days of the study. The wicks were transferred to a 125 ml Erlenmeyer flask and agitated with 40 ml of distilled water for 20 min and a sample of 5 or 10 mL

was removed and the ammonium content determined by distillation followed by titration against dilute sulphuric acid using the standard technique of Keeney and Nelson (1982). Soil temperature was monitored using digital thermometers at 5 cm depth in the soil every 3 days over the 36-day period of the experiment. At the same time soil samples from 0 to 10 cm depth were taken to evaluate soil moisture and the concentrations of mineral N (NH_4^+ and NO_3^-). To determine soil moisture samples were weighed immediately after sampling and then again after 48 h in an oven at 105 °C. Soil bulk density and particle density were determined for each treatment, allowing water filled pore space (%WFPS) to be calculated (Morais et al. 2013).

Samples of 20 g of moist soil were shaken with 60 mL of 1 M KCl at 220 rpm for 30 min. The soil suspensions were filtered and the extracts stored in a refrigerator. Subsequently, the extracts were analysed for NH_4^+ using the colorimetric salicylate technique of Kempers and Zweers (1986), and for NO_3^- using ultraviolet spectrophotometry as described by Miyazawa et al. (1985).

The second pot experiment, also conducted in the greenhouse, was performed with the same soil type and pots as in experiment 1, being installed on 18 May 2010 with just three treatments: A—Control treatment with no addition of urea or vinasse; B—Fresh vinasse added at 795 ml pot⁻¹ (equivalent to 150 m³ ha⁻¹) followed 6 days later by 12 g of urea (equivalent to 225 kg N ha⁻¹); and C—Urea fertilizer added at 12 g pot⁻¹ followed 6 days later by 795 ml of fresh vinasse (equivalent to 150 m³ ha⁻¹).

Each treatment was replicated 4 times and the pots were laid out in a complete randomised design.

For the first 4 days emissions of N₂O were evaluated every 3 h from 07.00 to 17.00 h. Subsequently, emissions were evaluated once a day between 08.00 and 10.00 h. To estimate the flux of N₂O the pots were sealed with lids and the gas sampled through the three-way valve as described for Experiment 1. Only N₂O fluxes were monitored in this phase.

Field experiment

The study was initiated on 22 September 2010 at the field station of Embrapa Agrobiologia (Seropédica) on the same soil type (Acrisol) as for the previous pot experiments. The area was planted with sugarcane cv

RB 72454 (1.20 m interrow space) and at the time of the study the cane was into the first ratoon at 1 month regrowth. Plot size was 2 × 2 m. Plots were side by side along a sugarcane row. Each plot comprised a section of 2 m length with the plant row in the centre. The organisation of the treatments allowed the simultaneous estimation of emissions of N₂O from the plots when urea or vinasse was applied to unamended soil or to prior additions of vinasse or urea, respectively. The plot width comprised a full interrow space where the band of N-urea was applied. In this experiment two phases are defined, the first comprising 2 days following the initial N addition (T1) (only urea and vinasse of treatments 1 and 2, respectively) and the second, comprising the remaining period after the second N addition (T2) (all treatments, except in the control). The experimental plots were arranged in randomized complete blocks and consisted of five treatments with five replicates: A—Control treatment with no additions of urea or vinasse; B—Vinasse added at the start of the experiment (T1) followed 2 days later (T2) by an addition of urea; C—Urea added at T1 followed 2 days later by an addition of vinasse; D—Vinasse (150 m³ ha⁻¹) added at T2, 2 days after the start of the experiment; E—Urea (80 kg ha⁻¹) added at T2, 2 days after the start of the experiment.

Where no vinasse was added the equivalent volume of water was added.

The fresh vinasse was obtained from the same sugar mill as for the pot experiments at 0.1 kg N m⁻³. The vinasse was spread evenly over the whole plot to the equivalent of approximately 15 mm of rainfall. In treatments where vinasse was not added, the same volume of water was added. Urea was added in a band approximately 10 cm wide close to the line of cane plants. The entire experimental area was irrigated with approximately 33 mm of water 2 days before the start of the experiment.

Nitrous oxide emissions were evaluated every day between 08.00 and 10.00 h for from 18 September (6 days before the first addition of urea or vinasse) until 04 November using closed static chambers as described by Alves et al. (2012). Each was composed of a rectangular hollow metal frame, 38 cm wide × 58 cm long × 6 cm in height which was inserted 5 cm into the soil and left for the whole experimental period. A trough was made around the top of the frame, and filled with soft rubber to ensure

the system could be sealed after coupling the top portion of the chamber. This was a polyethylene tray of the same width and length as the base, 9 cm high, and was only coupled to the base during the periods of gas sampling. The top of each chamber was fitted with a three-way valve as used for the pot experiments. Sampling and analysis were performed as described for the first pot experiment.

Losses of N via ammonia volatilization were quantified using the same apparatus and procedures as those described for the first pot experiment. In the treatments where urea fertilizer was added the chamber (modified plastic bottle) was placed over the fertilizer band.

Soil samples were taken from 0 to 10 and 10–20 depth for the evaluation of soil moisture content (and %WFPS) and mineral N (NH_4^+ and NO_3^-) as described for the first pot experiment.

Statistical analysis

The data generated were subjected to tests for normality and homogeneity of variance of errors with the Lilliefors and Cochran and Bartley tests, respectively, using the statistical package Sisvar 4.6 (Federal University of Lavras, MG, Brazil). The required conditions were met in all cases. Means were separated by *t* test (LSD), using the same statistical package, Sisvar 4.6.

Results

Pot experiments

In the first pot experiment, the application of both types of vinasse gave rise to steep increases in soil N_2O fluxes which were, however, short lived (Fig. 1). For the fresh vinasse a N_2O peak of $8.9 \text{ mg N}_2\text{O-N m}^{-2} \text{ h}^{-1}$ was observed and for the old vinasse it was $3.6 \text{ mg N}_2\text{O-N m}^{-2} \text{ h}^{-1}$. On the other hand, N_2O emissions from applied urea were slow to start and long-lived, with N_2O fluxes increasing from the fourth day onwards. The addition of filter cake did not induce significant soil N_2O fluxes compared to those registered in the control area where no N addition was realized (Fig. 1). High fluxes are to be expected because of the high soil temperature, ranging from 30 to 49 °C (Fig. 2a), and the generous irrigation regime

which kept the soil moist enough to bring about WFPS levels of between 60 and 90 % (Fig. 2b). The drier period corresponded to decreased N_2O fluxes (Fig. 1). Where urea was applied soil available N as NH_4^+ was 4–5 times higher compared with the other treatments (Fig. 2c), but decreased gradually after the first week accompanied by an increase in soil NO_3^- concentration (Fig. 2d). This trend was only observed for urea-treated soil as no NO_3^- increase was observed in the remaining treatments.

Nitrous oxide emissions accounted for 11.5 and 14.9 % of the applied N as old and fresh vinasse, respectively, and a non-significant emission of the N applied as filter cake, whilst for urea-treated soil $\text{N}_2\text{O-N}$ emissions accounted for 0.8 % of the applied N (Table 1). The high N_2O fluxes observed in the day after vinasse applications represented, respectively, 88 and 61 % of the whole $\text{N}_2\text{O-N}$ emitted during the entire evaluation period.

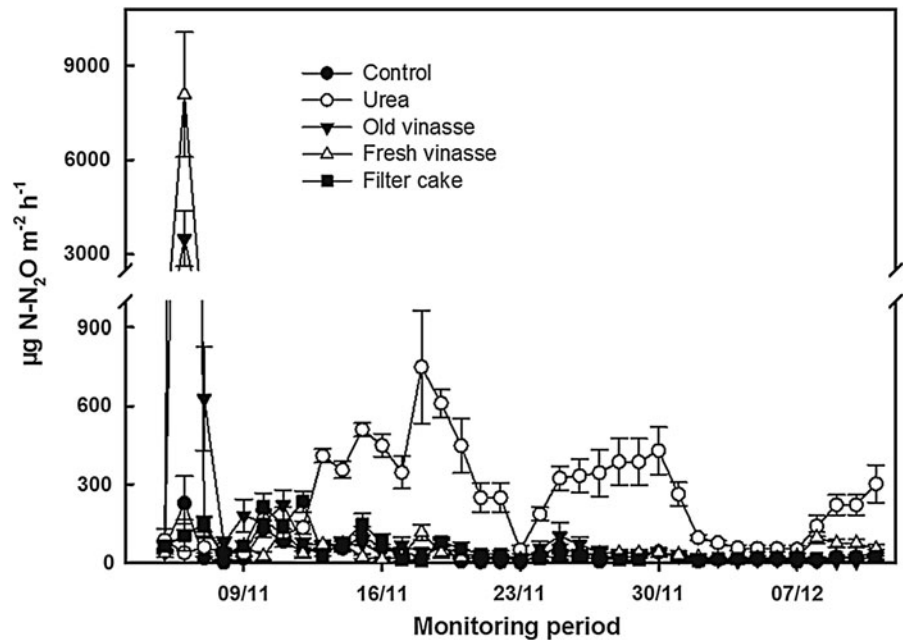
Except in the case of urea-treated soil, losses of N due to NH_3 volatilization were under $1.5 \text{ mg NH}_3\text{-N chamber}^{-1}$ for all treatments, the highest figure being registered in the control treatment, which would imply negative proportions of $\text{NH}_3\text{-N}$ lost by volatilization of each N source or an inhibition of NH_3 volatilization. About 31 % of the added N as urea was lost from the soil.

In the second pot experiment, an increase in N_2O fluxes was already registered in the first three to 9 h after the application of fresh vinasse, but no significant induction of N_2O emission was observed after urea application (Fig. 3). The N_2O fluxes from the vinasse-treated soil decreased until the fourth day of monitoring when N_2O fluxes of all treatments were close to background (control). There was a one-day delay before N_2O emissions were detected in the treatment where vinasse was added 6 days after the urea, or where urea was added 6 days after the vinasse. After this period the treatment where vinasse was added after urea presented the highest N_2O flux, whereas the application of vinasse before urea did not promote an N_2O flux significantly higher than the control (Fig. 3).

Field experiment

The highest soil N_2O fluxes were prominent for the first 20 days of the experiment (Fig. 4) subsequently decreasing to the background levels observed in the control treatment. In the day after the initial

Fig. 1 Emission of N_2O from a soil taken from sugarcane area treated with urea, old vinasse, fresh vinasse and filter cake together with a control with no product addition. First pot experiment carried out in greenhouse. Error bars represent standard errors of the means



application of vinasse and urea (T1 in Fig 4a) only the soil treated with vinasse gave rise to a significant N_2O flux, which decreased to background levels on the following day when a new application of N was made (T2 in Fig 4a). In this second application, vinasse was applied on soil where no urea had been added and also on the area that received urea in the first application. The areas receiving vinasse in this second application presented the highest N_2O fluxes, but the area where vinasse was applied after urea, the N_2O flux almost doubled compared to the N_2O flux from the area that received only vinasse ($p = 0.026$, l.s.d. test). Only treatments receiving urea irrespective of the presence of vinasse presented persistently higher N_2O fluxes (Fig 4b). The experimental conditions were not potentially as favourable as those registered in the pot experiment, but precipitation during the evaluation period amounted to 69 mm, which was distributed over the whole study period. Air and soil (0–5 cm) temperatures were tightly coupled, oscillating between 20 and 35 °C (Fig 5a). The high soil moisture at the start of the experiment due to previous rainfall and also due to rainfall occurring in the first days of monitoring provided relatively high WFPS levels (Fig 5b) for about 2 weeks, which were similar among all treatments.

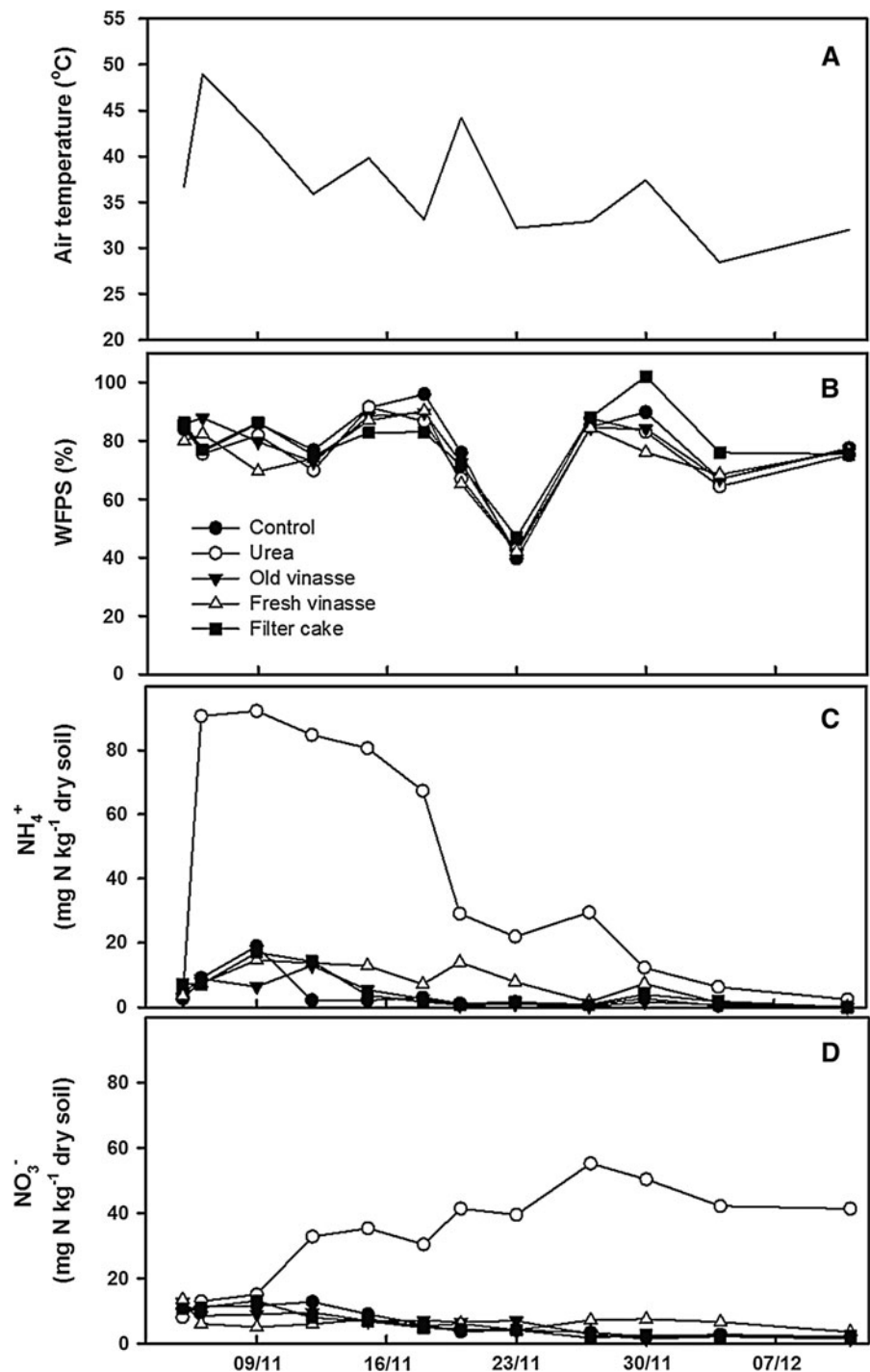
For the treatments where urea was applied, increased concentrations of NH_4^+ (0–10 cm) were

observed (Fig 5c). The application of vinasse alone did not change soil NH_4^+ above the control. A decreasing trend in soil NH_4^+ was accompanied by an increase in soil NO_3^- , but again only where urea were present (Fig 5d).

The top 10 cm of the soil showed the highest soil mineral N concentrations in the treatments where urea was applied (Fig 6a). The soil treated only with vinasse either in the first (T1) or second application (T2) showed no change in mineral N concentration. The effect of N sources applied on soil was only perceived in the layer of 10–20 cm about 1 month later, when mineral N levels increased in the area treated with urea (Fig. 6b).

The soil treated only with vinasse at T1 showed very similar NH_3 volatilization behaviour to the control (Fig 7a, b). This same trend was observed where vinasse was applied at T2 but without previous application of urea N (Fig 7d), with NH_3 -N volatilization being close to zero throughout the monitoring period. The major N losses by NH_3 volatilization occurred in treatments with urea application, with the greatest losses occurring in the first 6 days after N addition (Fig. 7b, c, e). Even though the same N rate as urea was applied on the area previously treated with vinasse the volatilized NH_3 was similar to that in the area that received urea at T2 without any N addition at T1 (Fig 7b, e). The application of vinasse on soil

Fig. 2 Air temperature (a), water-filled pore space (WFPS) of the soil (b) and soil available ammonium (c) and nitrate (d) during the whole experimental period of the first pot experiment carried out in greenhouse where the soil was treated with urea, old vinasse, fresh vinasse and filter cake and a control with no product addition



previously treated with urea did not change the decreasing trend of N loss from the urea (Fig 7c).

The total quantities of N lost as N_2O were not proportional to the amounts of N applied in the different N sources (Table 2). The proportion of urea

N lost as N_2O varied from 0.2 to 0.7 % of added N, being similar irrespective of being added without vinasse or after, or before, vinasse addition. When only vinasse was applied onto soil the proportion of added N lost as N_2O was 2.5.

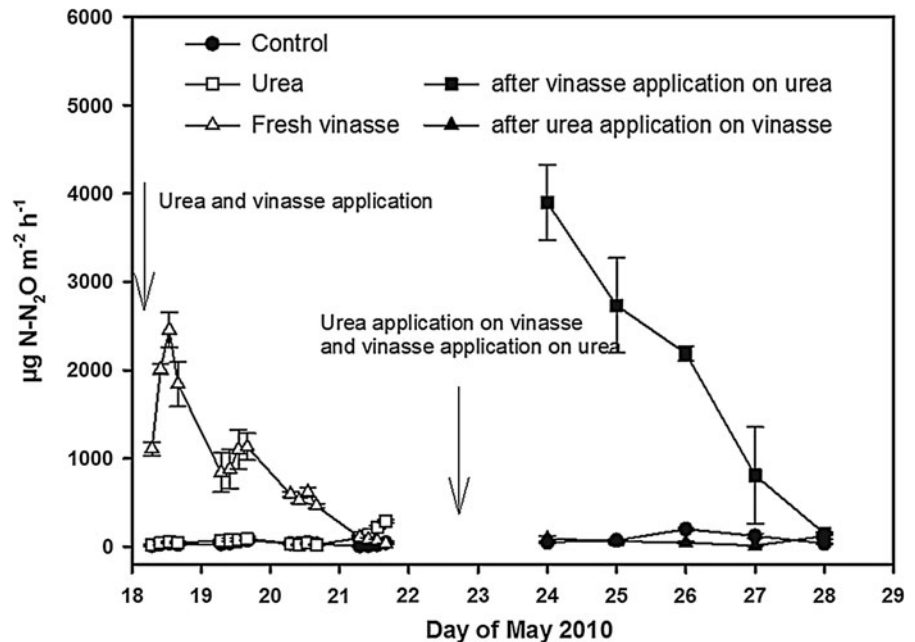
Table 1 Volatilized N and N₂O emission after the application of different N sources in the first pot experiment containing surface soil (*Acrisol* 0–10 cm) from a sugarcane field

Nitrogen source applied to soil	Applied N (g m ⁻²)	Volatilized N-NH ₃ (mg chamber ⁻¹)	Volatilized N-NH ₃ (%)	N ₂ O emission (g N m ⁻²)	N-N ₂ O emitted from each source ^b (%)
Control	–	1.5	–	0.032	–
Urea	22.5	86.2	31.3	0.218	0.8 b
Old vinasse	0.9	0.8	–	0.140	11.5 a
Fresh vinasse	1.3	0.6	–	0.229	14.9 a
Filter cake	7.9	1.4	–	0.044	0.2 b

Data followed by different letters in the same column show statistical difference based on I.s.d. test at $p < 0.05$

^a $100 \times (\text{Net volatilized N-NH}_3 / \text{Applied N})$; Net volatilized N-NH₃ is the difference between volatilized N from treated soil and volatilized N from the control

^b $100 \times (\text{Net emission of N-N}_2\text{O} / \text{Applied N})$; Net emission of N-N₂O is the difference between N-N₂O emission from treated soil and N-N₂O emission from the control

Fig. 3 Emission of N₂O from a soil taken from sugarcane area treated initially with urea and vinasse, and 5 days later with vinasse in the area treated with urea and urea in the area treated with vinasse, together with a control with no product addition. Second pot experiment carried out in a greenhouse. Error bars represent standard errors of the means

In the case of volatilized NH₃ the influence of urea was large. Losses of N by NH₃ volatilization were low for all N sources even though a significantly greater value was registered when vinasse was applied on urea.

Discussion

Results obtained in the first pot experiment under greenhouse conditions showed the potential production of N₂O from urea and vinasse after their addition

to the soil. The experimental conditions favoured N₂O production as the frequent irrigation led to WFPS levels varying from 60 to 90 % for most of the time (Fig 2b) and this is considered an optimum range to induce N₂O emissions from soils (Smith et al. 2003). Air temperature was relatively high (30–40 °C) which also contributed to enhance N₂O fluxes (Alves et al. 2012). Hence, as expected, the application of urea resulted in high N₂O fluxes. The total N₂O-N emitted during the monitoring period was 0.8 % of the N applied as urea, close to the direct N₂O emission factor of 0.01 (1 %) adopted in the IPCC guidelines for

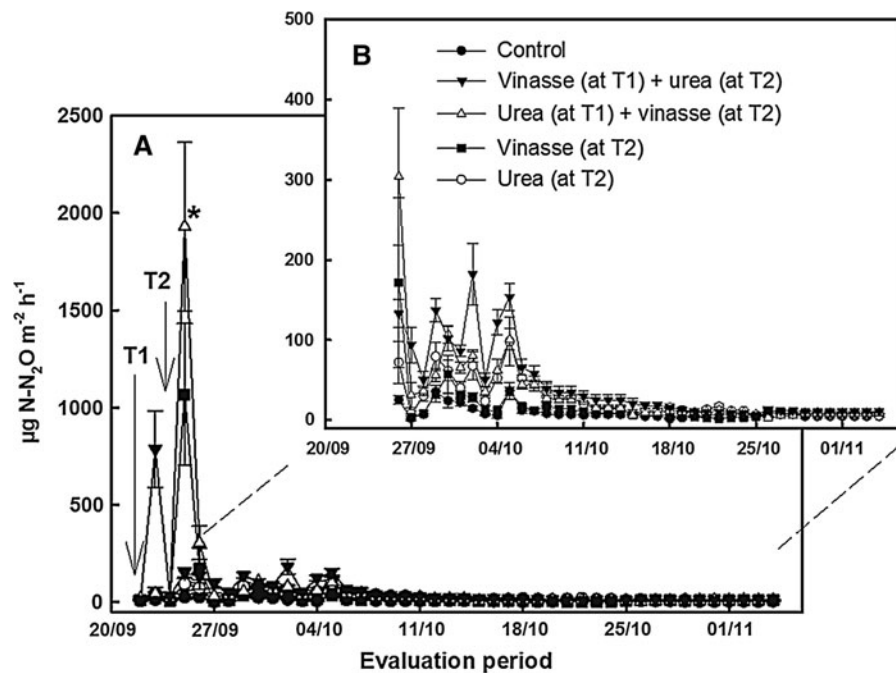


Fig. 4 Soil N_2O emission from a sugarcane field experiment initially treated with urea and vinasse (T1), and 2 days later (T2) with vinasse in the area treated with urea and urea in the area treated with vinasse. Another two areas that did not receive N additions were treated with vinasse and urea 2 days later (T2) to serve as controls, together with another control without any

product addition. A—the entire evaluation period; B—the period excluded the first 6 days after product application. The asterisk indicates a significant difference between N_2O fluxes of day 25 September 2010 ($p < 0.05$, l.s.d. = 749). Error bars represent standard errors of the means

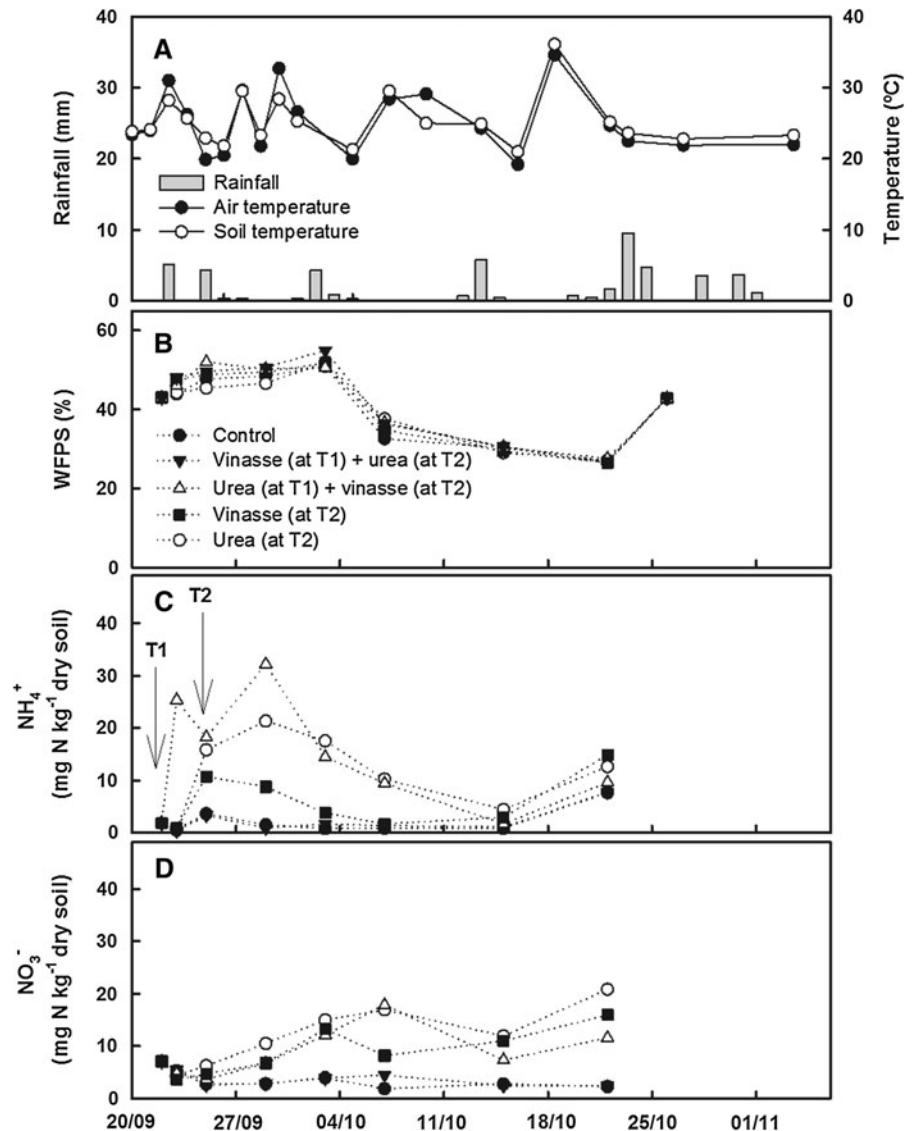
fertilizer as N source (IPCC 2006). Filter cake was the N source that resulted in the lowest N_2O fluxes even though the N applied was 6–8 times higher than that of old or fresh vinasse (Table 1). The C to N ratio of filter cake is normally in the range of 15–60, which varies depending on how it is managed and how long it has been stored before being applied in the field. However, even when the C to N ratio is low, immobilization of soil N for long periods after its application has been reported (Rasul et al. 2008), which would explain the low induction of soil N_2O emission in our study. On the other hand, the particular environmental conditions in the pots probably explain the extremely high N_2O fluxes arising after the vinasse application (Fig. 1). Even though the N_2O fluxes from vinasse-treated soil did not last for more than a couple of days, they were equivalent on average to 13 % of the total N in the vinasse-N added onto soil.

Losses of N by ammonia volatilization were stimulated in the pot experiment in the case of urea, representing 31 % of the applied N. Humid and warm

soil conditions favour rapid urea dissolution and hydrolysis and the soil moisture fluctuation between the 3-day periods between irrigations increases NH_3 volatilization (Sommer et al. 2004). However, where vinasse and filter cake were applied, NH_3 volatilization was low and similar to the NH_3 volatilized from the control pot where no N was added. The suppression could be attributed to vinasse in view of its low pH (Elia-Neto and Nakahodo 1995), the low N availability being the explanation for the very low emissions for filter cake.

The second pot experiment, of shorter duration, confirmed the rapid promotion of N_2O fluxes after vinasse application within a few hours of its application on the soil (Fig. 3). This second pot experiment also presented evidence that vinasse as an organic source would increase N_2O emission from soils treated with fertilizer N, as observed by Carmo et al. (2013). However, in the pot experiment the application of urea on previously treated soil with vinasse did not increase N_2O emissions.

Fig. 5 Rainfall, air and soil temperatures (a), water-filled pore space (WFPS) of the soil (b) and soil available ammonium (c) and nitrate (d) during the whole experimental period of the field experiment where the soil was treated initially with urea and vinasse (T1) and 2 days later (T2) with vinasse in the area treated with urea and urea in the area treated with vinasse together with two areas that did not receive N additions initially but were treated with vinasse and urea 2 days later (T2) to serve as controls apart from a control without any product addition



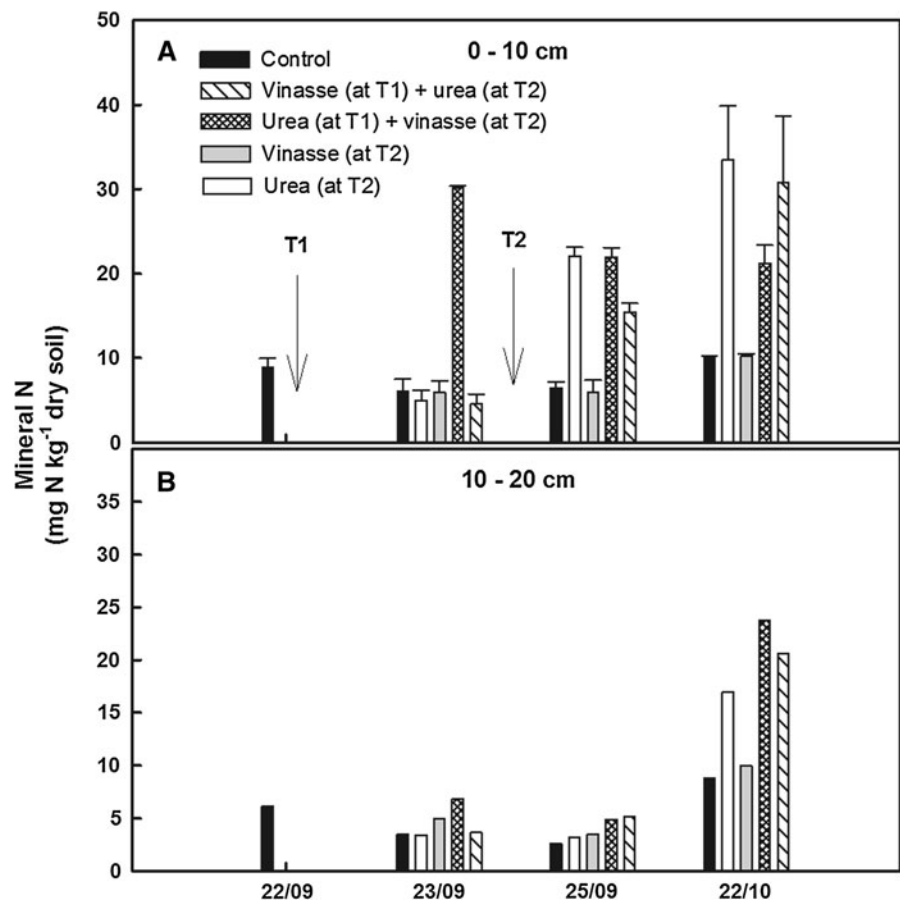
The field study was designed to also investigate this possible effect of sequence of addition of vinasse and N fertilizer. As reported by Carmo et al. (2013) the application of both N sources is usual practice in sugarcane areas, but there is no recommendation on which operation should be carried out first. This is especially important for the ratoon crops as fertilizer is usually broadcast and vinasse is distributed by asperion although not in any defined sequence.

The application of 80 kg N ha⁻¹ of urea stimulated N₂O emissions but not as much as observed in the pot experiment. The proportion of the added N emitted as N₂O was 0.2 %, well below the IPCC emission factor

(IPCC 2006). Ammonia volatilization was concentrated in the first days following fertilizer application (Fig. 7b, c, e) as was observed by Morais et al. (2013). However, the proportion of the added N volatilized as NH₃ was 2.5 %, which is negligible compared to the figures over 40 % reported by Morais et al. (2013) for this same soil cropped to elephant grass and fertilized with the same N rate as urea. However, such large variations in NH₃ volatilization are common in view of the many driving factors involved (Sommer et al. 2004).

At the start of the field experiment (T1), high and short-lived N₂O fluxes were detected in the first 2 days

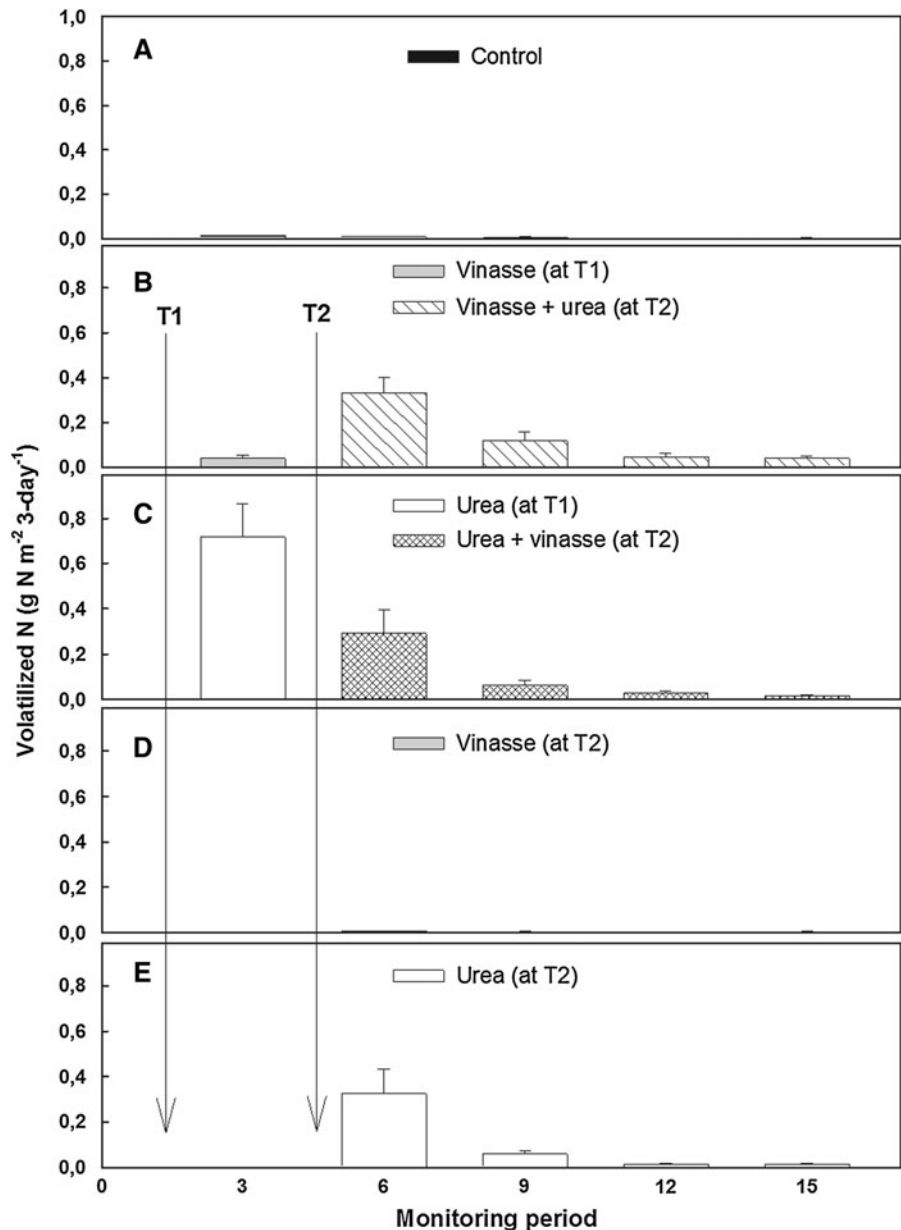
Fig. 6 Soil available N at soil depths of 0–10 cm (a) and 10–20 cm (b) during the experimental period of the field experiment where the soil was treated initially with urea and vinasse (T1) and 2 days later (T2) with vinasse in the area treated with urea and urea in the area treated with vinasse together with two areas that did not receive N additions initially but were treated with vinasse and urea 2 days later (T2) to serve as controls apart from a control without any product addition. Error bars represent standard errors of the means



after vinasse application (Fig. 4a—between T1 and T2). However, where urea was added at T1, N_2O emissions were minimal until vinasse was added at T2 (Treatment C) even though the same volume of water had been added in the soil receiving urea to equalize the soil pore saturation. Approximately 2.5 % of the added N as vinasse was lost as N_2O . In a field study, Oliveira et al. (2013) also found a similar behaviour after vinasse application, but the magnitude of the initial N_2O flux was much smaller, and the whole N_2O loss represented only 0.44–0.68 % of the N applied. An even lower emission factor of 0.09 % was found by Zuchello (2010) after applying 48 kg N ha^{-1} as vinasse at a rate of $150 \text{ m}^{-3} \text{ ha}^{-1}$ in a sugarcane area in the north Rio de Janeiro state. Another field study conducted by Carmo et al. (2013) reported $N-N_2O$ emissions corresponding to 2.99 % of the N in vinasse added onto the sugarcane interrow space free of fertilizer N. Differences in vinasse composition and in environmental conditions, especially if the study was

carried out during wet or dry seasons, could explain the differences between the results. Rainfall was evenly distributed during the experiment and the high temperature resulted in a warm and humid soil environment during the first days of the monitoring period when vinasse was applied (Fig. 5). It is interesting that the application of vinasse alone did not increase mineral N significantly (Fig. 5c, d). Furthermore, it was unlikely that vinasse N was leached down the profile as only urea treated areas showed an increase of mineral N below the first 10 cm soil depth after a month (Fig. 6). This indicates that either vinasse is not an important N source for N_2O production in the soil or its N is very promptly to be lost by denitrification. The very large N_2O fluxes that were observed in the space of only one or 2 days after vinasse application led us to the hypothesis that this very short term N_2O production could be principally attributed to the stimulation of reducing conditions in the soil induced by the application of labile C present

Fig. 7 Soil NH_3 volatilization amounted every 3 days for 15 days from a sugarcane soil without any product addition (control) (**a**), and from areas treated initially with vinasse (T1) and 2 days later (T2) with urea (**b**), and treated with urea (T1) and 2 days later (T2) with vinasse (**c**), and also from two areas that did not receive N additions initially (T1) but were treated with vinasse (**d**) and urea (**e**) 2 days later (T2) to serve also as controls. *Error bars* represent standard errors of the means



in the vinasse (Azam et al. 2002). Hence, the mineral soil N can be an important driver of N_2O emissions where vinasse is applied, probably of greater importance than N derived from vinasse, in contrast to the conclusion of Carmo et al. (2013).

In the second phase of the field study (after T2), the areas receiving vinasse presented high and short-lived N_2O fluxes, but the highest flux was observed in the area treated with urea followed by vinasse. Considering the N_2O fluxes of the control areas that

received vinasse or urea in the second phase, it seems clear that the vinasse application on the soil initially treated with urea brought about a synergistic effect as reported by Carmo et al. (2013). The application of urea in the area previously treated with vinasse did not promote the same effect. However, when integrating N_2O fluxes for the whole period no significant differences were observed in the proportions of the added N (urea + vinasse) lost as N_2O between the different managements of the N sources (Table 2).

Table 2 Volatilized N and N₂O emission from the field experiment set up on an Acrisol cropped to sugarcane and amended with different N sources at the start of the experiment (T1) or 2 days later (T2)

Nitrogen source applied to soil	Applied N (g m ⁻²)	Volatilized N-NH ₃ (mg chamber ⁻¹)	Volatilized N-NH ₃ ^a (%)	N ₂ O emission (g N m ⁻²)	N-N ₂ O emitted from each source ^b (%)
Control	–	0.79	–	0.011	–
Vinasse (at T1) + urea (at T2)	9.2	13.88	3.2 b	0.064	0.6 b
Urea (at T1) + vinasse (at T2)	9.2	26.69	6.2 a	0.079	0.7 b
Vinasse (at T2)	1.2	0.85	0.6 c	0.042	2.5 a
Urea (at T2)	8.0	10.97	2.5 bc	0.027	0.2 b

Data followed by different letters in the same column show statistical difference based on l.s.d. test at $p < 0.05$

^a $100 \times (\text{net volatilized N-NH}_3 / \text{Applied N})$; Net volatilized N-NH₃ is the difference between volatilized N from treated soil and volatilized N from the control

^b $100 \times (\text{net emission of N-N}_2\text{O} / \text{Applied N})$; Net emission of N-N₂O is the difference between N-N₂O emission from treated soil and N-N₂O emission from the control

Table 3 Literature data on N₂O emission factors for vinasse applied to sugarcane crops at different locations and cropping phases on different soils and trash management

Location/crop phase ^a	Soil type	Trash level on soil (Mg ha ⁻¹)	Vinasse rate (m ³ ha ⁻¹)	Vinasse N applied (kg ha ⁻¹)	Emission factor (%)	Reference
Jaú—SP/plant cane	Haplic Lixisol (10 % clay at 0–20 cm)	~0	100	21	2.99	Carmo et al. (2013)
Piracicaba—SP/plant cane	Haplic Ferralsol	~0	200	46	0.68	Oliveira et al. (2013)
	(51–57 % clay at 0–20 cm)	>0 (n.i.) ^b	200	46	0.44	
Campos dos Goytacazes—RJ (plant cane)	Fulvic Cambisol (43 % clay at 0–20 cm)	~0	150	48	0.09	Zuchello (2010)
Seropédica—RJ/1st ratoon	Haplic Acrisol (17 % clay at 0–20 cm)	~0	150	12	2.50	This study
Average emission factor (confidence interval) ^c					1.94 (0.30–12.47)	

^a Plant cane is the sugarcane crop phase that follows planting until harvest; the following cycles (on average 5 consecutive cycles) of regrowth and harvest are called ratoon crops

^b Not informed. It was only reported that the straw produced after cane harvest was left on the ground

^c Average and confidence interval calculated from a log-normal distribution (Olsson 2005) after rejecting Normal distribution (Lilliefors test; $p = 0.05$)

This appeared to be due to lower emissions of N₂O over a more extended period when urea followed vinasse than when the order of application was reversed.

Gathering the existing data in Brazil (Table 3) a direct N₂O emission factor for vinasse of 1.94 % could be suggested, irrespective of the concentration of N and C in the vinasse. There are too few studies available to assess how the rate of vinasse application,

its N content or if it is applied on bare or trash-covered soil, among other factors, would affect the value of this factor. It is evident that more studies are required to reduce the very large uncertainties (Table 3) associated with this factor.

Losses of N via NH₃ volatilization were low as was observed for the area receiving only urea, and there is no evidence vinasse can contribute positively to this process.

Conclusion

Nitrogen is a key nutrient for sugarcane production irrespective of the source used. In Brazil, fertilizer N is commonly applied to the crop at low rates, which may be explained by a complementary contribution from biological N₂ fixation (Urquiaga et al. 2012), but other N sources are also applied such as vinasse and filter cake. Owing to the large area (almost 10 Mha) dedicated to sugarcane which annually receives a total of almost 600,000 Mg of N fertilizer, 500 million m³ of pure vinasse (~0.35 g N L⁻¹) and 18,000,000 Mg of filter cake (10 g N kg⁻¹) the potential impact of this crop on GHG emissions is extremely large.

The results of this study indicated that N₂O emissions from vinasse and urea should not be estimated from a single emission factor as proposed by the IPCC, vinasse being a strong inducer of soil N₂O emission. For the field study, 0.2 % of the added N as urea was emitted as N₂O, which is agreement to what is expected for aerated soils (Rochette 2008) and close to 0.57 % quantified by Carmo et al. (2013) in an area of sugarcane with no trash cover. When vinasse was applied 2.5 % of the added N was emitted as N₂O, which when considered together with literature data give rise to an emission factor of 1.94 % with a very high uncertainty (0.30–12.47 %).

This present study suggests that if vinasse is added soon after N fertilizer, emissions per kg of added N are increased to above those from urea alone, but the opposite sequence of addition is of minor importance. More studies are required to confirm this trend but it is advisable the N management in sugarcane areas take it into account when the decision does not lead to negative practical or economic consequences. Also, probably because of its low pH, vinasse application is not an important source of volatilized ammonia, which means indirect emissions calculated from volatilized N should be better evaluated.

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