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ORIGINAL ARTICLE

Increases in nitrogen use efficiency decrease nitrous oxide emissions but can penalize yield in sugarcane

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Abstract Nitrogen (N) fertilization strategies focused on increasing nitrogen use efficiency (NUE) and decreasing nitrous oxide (N_2O) emissions are important for sustainable crop production. In sugarcane, however, a joint assessment of NUE, N_2O emissions and yield is still required. We aimed to establish, in a subtropical sugarcane cropping system, if variations in NUE (by decreasing rates or changing formulations of N fertilization) allow decreasing N_2O emissions and, to what extent, yield is penalized. Four fertilization treatments were used: without fertilizer, with low and high urea fertilization (55 and 110 kg N ha^{-1}) and with ammonium nitrate fertilization (110 kg N ha^{-1}). There was a significant negative relationship between N_2O emissions and NUE. At high N rates (110 kgN ha^{-1}) ammonium nitrate produced 37% higher cumulative N_2O emissions and 13 and 12% lower NUE and cane yield, respectively, than urea. The highest N_2O emissions of the ammonium nitrate treatment occurred within 48 hs after N fertilization and were mainly associated with the direct addition of nitrate (NO_3^-–N). Results showed that, for the environmental conditions of Tucuman (Argentina), NUE above 160 kg of cane per kg of N available in soil penalized cane yield, whereas NUE below 140 kg of cane per kg of N available in soil penalized N_2O emission abatement.

Keywords Greenhouse gases · N fertilization · N_2O emission intensity · Nitrogen uptake efficiency

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Introduction

Nitrogen (N) is a fundamental element for plant growth and crop production (Vitousek et al. 1997). In the atmosphere, N forms diatomic nitrogen (N_2) (the most abundant gas; 80%) and traces of oxides as nitrous oxide (N_2O, the most powerful greenhouse gas; 330 ppb) (Galloway et al. 2004; Thompson et al.

2019). Nitrous oxide is mainly released to the atmosphere from soils by microbial nitrification and denitrification (Mosier and Hutchinson 1981; Bremner 1997) and can naturally return to the biosphere (Fowler et al. 2009; Schreiber et al. 2012; Hu et al. 2015). In the last decades, increased bio-products demand leading to increased production and high fertilization rates in cropping have altered natural N_2O fluxes, increasing global atmospheric N_2O concentrations at a rate of 0.95 ppb y^{-1} (World Meteorological Organization and Global Atmosphere Watch 2019) and intensifying global warming (Bouwman 1989). Thus, it is important to develop management strategies for reducing N fertilizer use and mitigate N_2O emissions, maintaining or increasing crop yields (promoting sustainable development and food security). Therefore, it is important to understand how N fertilization influences both the N_2O emissions of the crop-soil system and crop N use efficiencies (Zhang et al. 2015).

The use of fossil fuels and agricultural emissions are the most important contributors to greenhouse gases (GHG) and global warming (Maslin 2009; IPCC 2018; Reichle 2020). To mitigate emissions, the demand for clean energy (sun-, wind- and biomass-based energy, among others) is expected to increase. Sugarcane (*Saccharum spp.*) is a high biomass crop used worldwide as a feedstock to produce sugar and bioethanol, which represents a source of energy of low carbon (C) emissions (IPCC 2014). In addition, sugarcane harvest generates large amounts of by-products that can be potentially used to cogenerate electricity. The positive effect of sugarcane-based bioenergy in reducing GHG emissions could be counterbalanced by high N fertilizer rates (Crutzen et al. 2007; Lisboa et al. 2011). In sugarcane, research has been carried out to investigate the effects of N regimes on yield, NUE and N_2O emissions, separately. For instance, increases in N fertilization rates increased crop yields (Wiedenfeld 1995; Franco et al. 2011; Degaspari et al. 2020) but also generated low nitrogen use efficiencies (Robinson et al. 2007; Otto et al. 2016; Acreche 2017; Yang et al. 2019), high N_2O emissions (Allen et al. 2010; Wang et al. 2016; Chalco Vera et al. 2017) and high N_2O emissions intensity (mg of N_2O–N emitted per kg of sugarcane produced; Degaspari et al. 2020). Few studies have attempted to demonstrate, in the same experiment, relationships among sugarcane yields, N_2O emissions and nitrogen use efficiencies under different N fertilization treatments.

Controversial results arise from studies evaluating N_2O emissions associated with different N sources (mainly as urea and/or ammonium nitrate) in sugarcane (Weier 1999; Carmo et al. 2013; Signor et al. 2013; Degaspari et al. 2020). Therefore, N fertilizer type may alter the observed pattern of high N dose on increasing N_2O emissions and decreasing nitrogen use efficiencies. Understanding the relationship between nitrogen use efficiency (NUE), N_2O emissions and crop yield, jointly, is important for moving N management practices toward sustainable sugarcane production that mitigate GHG emissions whilst maintaining or increasing crop yields (Acreche and Valeiro 2013; Chalco Vera and Acreche 2018). To the best of our knowledge, this sort of experiment has not been conducted in sugarcane. Our study aimed to determine, in a subtropical sugarcane agroecosystem, (1) if variations in NUE (by decreasing rates of urea or changing formulations of N fertilization) allow decreasing N_2O emissions and (2) to what extent, N_2O emissions mitigation generates yield penalization. We hypothesized that the increases of NUE due to our fertilization treatments strongly decrease N_2O emissions but generates yield penalization.

Materials and methods

Study site and field experiment

The experimental fields were located at the Famaillá Experimental Station (27°030 S, 65°250 W, 363 m a.s.l.) of the National Institute of Agricultural Technology in the Tucuman province (Argentina). This area, traditionally cultivated with sugarcane under rainfed conditions, has mean temperatures of 26.6 and 9.5 (°C) for the warmest (January) and the coldest (July) months, respectively. The annual average potential evapotranspiration is 1348 mm, while the annual average rainfall is 1324 mm (occurring mainly between November and April). Chalco Vera et al. (2020) present a detailed description of the climate and soil type.

Two field experiments were carried out with the first ratoon of sugarcane under rainfed conditions. They were conducted on soils classified as Aquic Argiudoll (USDA 1975) or Luvic Oxiaquic Phaeozem

(IUSS Working Group WRB 2015). The experimental area has had sugarcane monoculture for over 50 years. Harvest without burning was adopted since 2005. Sugarcane is renewed every 5 years and it is traditionally fertilized with urea at 110 kgN ha^{-1} year^{-1} rates except for plant cane. Also in both experiments, four treatments of N fertilization were arranged in a strip plot design with three replicates. Treatments were applied on dates traditionally used by growers (Table 1): without fertilizer (control), with low and high urea fertilization (55 and 110 kg N ha^{-1}, respectively) and with ammonium nitrate fertilization (110 kg N ha^{-1}; AN). High urea treatment represents the traditional N fertilizer dose used by growers in Tucuman. Each fertilization treatment was applied in six 100 m rows, 1.6 m apart from each other. Fertilizers used were granular in solid form and incorporated in the row band at 10 cm soil depth. In order to reproduce possible application effects in the control treatment, the fertilization machinery was used without fertilizer.

The first experiment was repeated in two sites (site 1 and 2, located 1.6 km apart from each other) and included monthly gas samplings during the whole crop cycle (2015–2016 growing season) in order to show the magnitude of N_2O emission peaks and calculate cumulative N_2O emissions for each treatment. The second experiment (2018–2019 growing season) was performed in a third site (site 3, which differed from the other sites in the physicochemical soil properties; see Table 1) to intensively measure N_2O emissions immediately after applying N fertilization treatments. In this experiment gas samplings were mostly concentrated around the N fertilization period: before N fertilization and then, at 1, 2, 5, 7, 11 and 15 days after N fertilization, respectively.

Soil physicochemical properties were determined at the beginning of each experiment, from 0 to 20 cm depth (Table1) as the average values of three replicates. Soil pH was determined on a mixture of soil and distilled water (1: 2.5) using a digital pH-meter (Instituto Argentino de Normalización y Certificación 2009). Soil organic carbon (SOC) was determined by dichromate oxidation method (Walkley and Black 1934). Soil organic nitrogen (SON) was determined by Kjeldahl principle (Bremner 1965a). Exchangeable potassium (K) was estimated by the ammonium acetate method (Jackson 1958). Available phosphorus (P) was measured by ammonium fluoride extraction (Bray and Kurtz 1945). The particle size classification of soils was determined by the pipette method (Soil Conservation Service 1972).

Environmental conditions

Meteorological data (daily rainfalls and mean air temperatures) was obtained from a meteorological station located near the experimental sites. Cumulative rainfall of both growing seasons (1650 for the 2015–2016 growing season and 1626 mm for the 2018–2019 growing season) were higher than the mean cumulative rainfall for the same period of the historical series 1968–2015 (1324 mm) (Fig. 1a). Total rainfall during the rapid initial growth period of sugarcane (when fertilization was performed; December to March) was 23.0 and 16.9% higher than the mean value of the historical series for the 2015–2016 and 2018–2019 growing seasons, respectively.

Monthly mean temperature also showed differences between the historical 1968–2015 series and the 2015–2016 growing season (Fig. 1b). This season had a similar mean temperature during the summer months (December–March), whereas it was 1.4 and 0.8 °C lower than the 1968–2014 series for the spring months (September–November) and autumn–winter period (April–August), respectively. The 2018–2019 growing season showed similar temperatures to the average values of the 1968–2015 series.

Nitrous oxide measurements

Nitrous oxide emissions were measured using the static chamber method (Hutchinson and Livingston 2001). Each chamber consisted of a rectangular non-reactive (polyvinyl chloride; PVC) head of 32.5 cm long × 22 cm wide × 15 cm high, covered by a light-reflecting aluminium foil (to avoid direct sunlight effect within the chamber and limit air temperature changes) and vented with a 10-cm-long stainless steel tube (Parkin and Venterea 2010; De Klein and Harvey 2012). This head was coupled to an iron anchor/frame inserted into the soil at 8 cm depth. We used six chambers per treatment equally distributed at row, inter-row and band space of the crop. Gas samplings were always performed when mean daily temperature occurred (between 9:00 AM and 12:30 PM). From each chamber, three samples were

Table 1 Physicochemical soil properties, last harvest dates and N-fertilization dates for the two experiments conducted in Tucuman, Argentina

Experiment	Site	Physicochemical soil properties (0–20 cm depth; n = 3)								N-fertilization date	Last Harvest date
		pH (1:2.5)	SOC (%)	SON (mg/g)	P (ppm)	K (meq/100 g)	Sand (%)	Silt (%)	Clay (%)		
2015–2016	1	6.45 ± 0.16	2.35 ± 0.15	1.67 ± 0.17	31.33 ± 5.16	1.08 ± 0.17	17 ± 2.83	58 ± 5.66	25 ± 8.49	1 Dec 2015	1 Oct 2015
	2	5.9 ± 0.39	1.52 ± 0.1	1.08 ± 0.29	15.5 ± 2.17	0.65 ± 0.12	34.5 ± 4.6	43 ± 2.95	22.5 ± 3.01	29 Nov 2015	7 Aug 2015
2018–2019	3	6.38 ± 0.25	1.24 ± 0.13	0.88 ± 0.14	17.41 ± 3.26	ND	ND	ND	ND	15 Nov 2018	11 Sep 2018

SOC, soil organic carbon; SON, soil organic nitrogen; ND, not determined

collected at 0, 15 and 30 min and sealed in 10 ml vials (previously emptied with a vacuum pump) for laboratory analysis. Chambers were moved between successive samplings. N_2O fluxes were calculated from the concentration rate change in the chamber by linear regression between N_2O concentration and sampling time (0, 15 and 30 min) (Parkin et al. 2003). Concentrations of N_2O were determined by gas chromatography (GC 7890 A with auto-sampler 7697 A, Agilent Technologies, USA). For the first experiment, cumulative N_2O emissions were also calculated by integrating mean monthly fluxes over time (expressed as kg N_2O–N ha^{-1} $year^{-1}$) as described by Chalco Vera et al. (2017).

Plant measurements

At ripening, the number of stalks per lineal meter was determined from 3 lineal meters in the central rows of each experimental unit and all aboveground plant biomass from 1.6 m^2 was sampled and separated into its components. A sub-sample was oven-dried at 62 °C to constant weight to determine, on a dry basis per hectare, total biomass, cane (stalk) yield, harvest residues (tops and senescent leaves) and sugar yield.

Laboratory analysis

From each sample, a sub-sample of 10 representative fresh canes was crushed by using an experimental mill (50% of juice extraction in the first crushing at 10.5 kg cm^{-2} of pressure). After filtering and mixing the juice, two 250 ml samples were analysed to determine soluble solids (°Brix) using a refractometer (Smart-1, ATAGO Co. LTD; Japan) and pol in juice (% juice sucrose concentration) after clarifying the juice with lead subacetate using a digital polarimeter (Polatronic NCE-Germany). Sugar content, on a fresh weight basis, was determined as follows:

$$\begin{aligned} Sugar\ \ content =& Winter's\ \ factor \times Pol\ \ in\ \ juice \\ & \times Java's\ \ factor\ \ (0.8) \\ & \times Sucrose\ \ extraction\ \ efficiency\ \ (0.978) \\ & \times Industrial\ \ efficiency\ \ (0.82) \end{aligned}$$

where Winter's factor = 1.4 × 41.51/Purity in first extraction juice; Sucrose extraction efficiency = Purity in mix juice/Purity in first extraction juice;

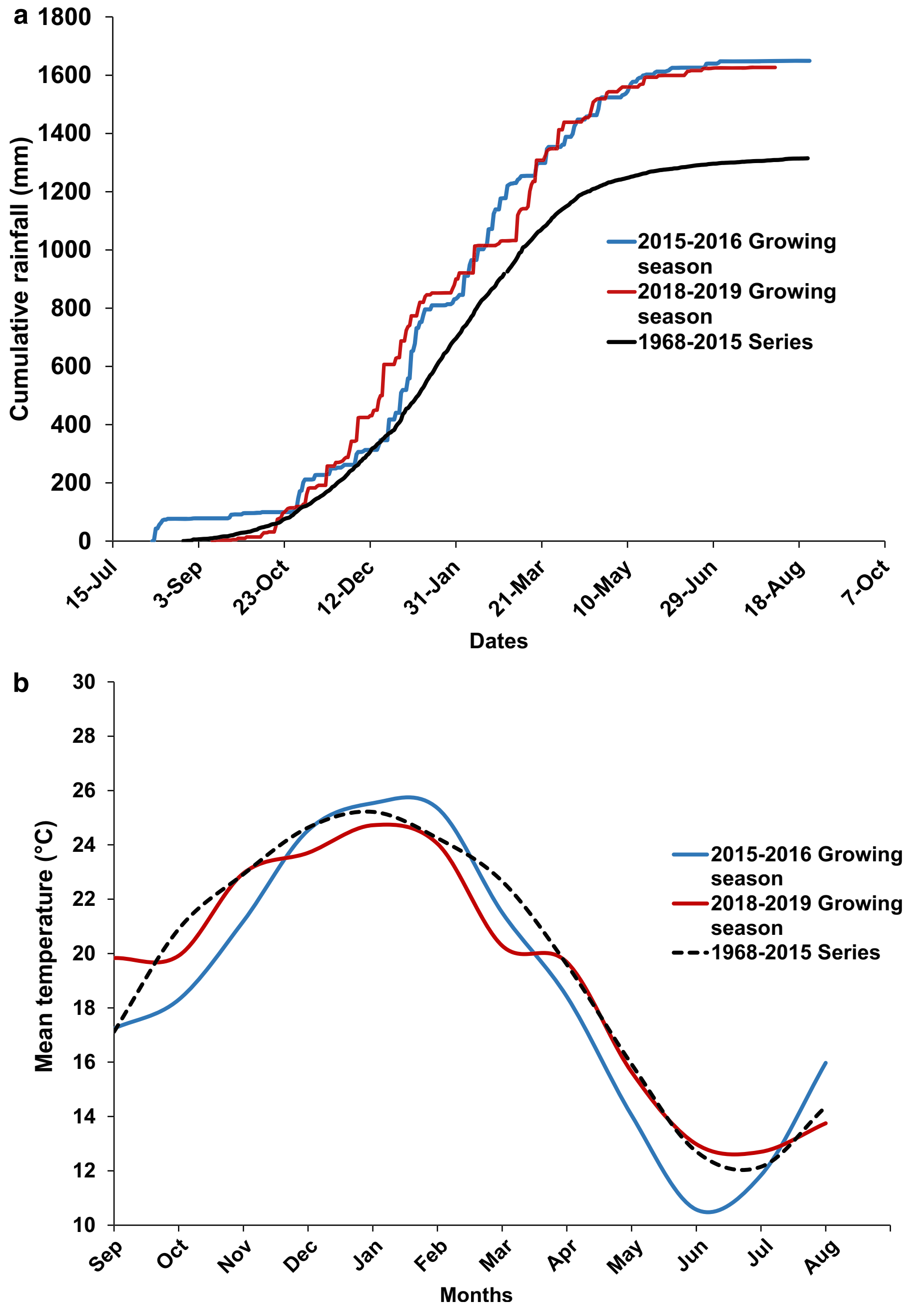

Fig. 1 Cumulative rainfall (**a**) and mean temperature (**b**) during the 2015–2016 and 2018–2019 sugarcane growing seasons in Tucuman, Argentina. Black lines represent the mean values for the 1968–2015 series

Purity = Pol in juice/°Brix × 100 (Hugot 1960). Sugar yield was calculated as the product of cane yield and sugar content.

The remaining cane/stalks and harvest residues of each sample (the sub-sample that was oven-dried) were used to determine vegetative nitrogen content. Dried subsamples were milled and N contents of canes and residues were determined using the Kjeldahl method (Bremner 1965b). Total N in biomass (kg ha^{-1}) was determined by adding the products of total residues weight × N content in residues and cane yield × N content in canes.

Soil measurements

During each sampling, soil temperature and gravimetric soil moisture were measured using manual digital thermometers and soil core-samplers in both experiments. Gravimetric soil moisture was converted to water-filled pore space (WFPS) as in Araujo et al. (2021). Inorganic N content in the soil, as nitrates (NO_3^-–N) and ammonium (NH_4^+–N), was also determined at all gas-sampling dates at 0.1 m soil depth (mgN kg^{-1} of dry soil) in the second experiment. For this, composite soil samples were collected from within each chamber and soil solutions (a mixed solution of KCl (1 N) and soil (5:1)) were prepared. Nitrates and ammonium were determined by steam distillation (Keeney and Nelson 1982).

Efficiency indices calculation

We considered three efficiencies: the nitrogen use efficiency (NUE), the nitrogen uptake efficiency (NUpE) and the nitrogen utilization efficiency (NUtE). They were determined as follows (Acreche 2017; Congreves et al. 2021):

$$NUE\left(\frac{kg\ cane}{kg\ N}\right) = \frac{Cane\ yield\ (kg\ ha^{-1})}{Inorganic\ N\ available\ in\ the\ soil\ during\ the\ crop\ cycle\ (kgN\ ha^{-1})}$$

$$NUpE\left(\frac{kg\ N\ in\ biomass}{kg\ N\ in\ soil}\right) = \frac{Total\ absorbed\ N\ in\ the\ biomass\ (kgN\ ha^{-1})}{Inorganic\ N\ available\ in\ the\ soil\ during\ the\ crop\ cycle\ (kgN\ ha^{-1})}$$

$$NUtE\left(\frac{kg\ cane}{kg\ N\ in\ biomass}\right) = \frac{Cane\ yield\ (kg\ ha^{-1})}{Total\ inorganic\ N\ absorbed\ in\ the\ biomass\ (kgN\ ha^{-1})}$$

Inorganic N (NO_3^-–N + NH_4^+-N) available in the soil during the crop cycle was calculated as follows:

$$\begin{aligned} &Inorganic\ N\ available\ in\ the\ soil (during\ the\ crop\ cycle)\\ &\quad = kgN\ ha^{-1} from\ previous\ harvest\ residues\\ &\quad + kgNha^{-1} from\ fertilizers\\ &\quad + kgN\ ha^{-1} of\ inorganic\ N\ from\ soil \end{aligned}$$

The N from previous harvest residues was determined as the product between the total N content in residues and the average residue decomposition rate reported for Tucuman (Digonzelli et al. 2011). Inorganic N from soil (NO_3^-–N + NH_4^+–N) during the crop cycle was estimated for each site using sugarcane plots without N fertilization as follows:

$$\begin{aligned} &Inorganic\ N\ from\ soil\ \left(kgN\ ha^{-1}\right)\\ &\quad = kgN\ ha^{-1} in\ biomass\ at\ harvest\\ &\quad - kgN\ ha^{-1} from\ previous\ harvest\ residiues \end{aligned}$$

ese values were similar to those measured in the same experimental area (Acreche 2017). Yield-scaled N_2O emission or N_2O emissions intensity (Mosier et al. 2006) was calculated as follows:

$$Yield - scaled\ N_2O\ emissions\ \left(\frac{gN_2O - N}{t\ cane}\right) = \frac{Cumulative\ N_2O\ emissions\ (gN_2O - Nha^{-1})}{Cane\ yield\ (tha^{-1})}$$

Statistical analyses

In order to assess treatment effects on N_2O fluxes along measurement periods, ANOVAs were performed for each site by adjusting and selecting mixed models under akaike information criterion (AIC) (Chalco Vera et al. 2017). Two-way ANOVAs were performed to assess treatments and sites effects on sugar- and cane-yield, total biomass, total N in biomass, cumulative N_2O emissions, yield-scaled N_2O emissions, NUE, NUtE and NUpE. In addition, correlation analyses were performed using Pearson's correlation coefficient. These analyses were performed in InfoStat software (Di Rienzo et al. 2018).

To determine the best mathematical functions for defining relationships between yield components and/

or N use efficiencies and cumulative N_2O emissions, optimization models that fitted the experimental data were estimated using linear and no-linear regression models. Then we followed the AIC criterion and considered the contribution of each equation coefficient (quadratic and linear) to be confident in the data interpretation.

To analyse associations among variables, between variables and treatments and find an optimal graphical representation of the variability of the data, a principal component analysis (PCA) was performed. The associations among variables were determined only for experiment 1 because it has the complete set of measured variables (which include cumulative N_2O emissions and yield-scaled N_2O emissions). Results of PCA were plotted by using the FactoMineR package of R statistic software (R version 3.6.0) and R studio interface software (RStudio Team 2016). The association between variables and treatments was made by summarizing the contribution (direction and magnitude) of each variable to each principal component through the broken-stick method (King and Jackson 1999). It considers, as significant, contribution values that are equal or greater than 2/3 of the highest contribution value within the analysed principal component. Treatments were grouped by the proximity among them according to their relationship with the direction and magnitude of each variable.

Results

N_2O emissions

In the first experiment (sites 1 and 2 of the 2015–2016 growing season), nitrous oxide emissions significantly differed among treatments and sampling dates, with a significant interaction between them ($p < 0.001$) (Online Resource 1 and 2). As expected, N_2O emissions throughout the growing cycle were higher with higher N fertilization rates (Fig. 2a, b). At high N rates (110 kgN ha^{-1}), fertilization with ammonium nitrate produced higher N_2O emissions than with urea (Fig. 2a, b). These same patterns were observed for cumulative N_2O emissions at both sites where significant differences were detected for treatments and sites ($p < 0.0001$) with no significant interaction between them ($p > 0.05$) (Table 4). On the other hand, N_2O emission patterns throughout the growing season were similar among fertilization treatments, since fluxes were mostly positive (emissions) throughout all the growing season and the highest N_2O emissions occurred immediately after N fertilization (during December) (Fig. 2a, b) (Online Resource 1 and 2). These seasonal trends were more associated with the role of timing after fertilization than soil temperature dynamics and/ or WFPS changes (Fig. 2c). The observed minimum and maximum values of N_2O emission rates ($\pm$ standard error) during the crop cycle were 4.7 ± 1.8 to 88.1 ± 32.4 for treatments without fertilizer; 2.9 ± 0.5 to 106.3 ± 23.9 for treatments with low urea; 0.6 ± 2.8 to 191.9 ± 16.0 for treatments high urea; 1.7 ± 4.9 to 223.7 ± 131.9 μg N_2O–N m^{-2} h^{-1} for treatments with ammonium nitrate.

In the second experiment (site 3 of the 2018–2019 growing season), N_2O emissions significantly differed among treatments during the first 15 days after fertilization ($p < 0.0001$) (Online Resource 3). Nitrous oxide emissions peaked within the first 48 h after ammonium nitrate fertilization, whereas with urea fertilization (high and low urea treatments) N_2O emissions peaked later, on the fifth day after N fertilization (Fig. 3a). From the 5th day onwards, N_2O emissions decreased in all treatments (Fig. 3a). Mean N_2O emissions rates for all gas samplings performed within 15 days after N fertilization also increased with the N dose and ammonium nitrate fertilizer type. Average N_2O emissions were 11.2 ± 1.7, 30.2 ± 4.7, 38.2 ± 3.6 and 75.2 ± 9.1 μg N_2O–N m^{-2} h^{-1} for without fertilizer, low urea, high urea and with ammonium nitrate, respectively. Precisely, the highest N_2O emissions shown under the ammonium nitrate treatment coincided with an early and relatively low increase in the inorganic N content of the soil with respect to the high urea treatment (Fig. 3a, b). In fact, N_2O emissions were associated with changes in the soil inorganic N content only in fertilization treatments with high N rates (Table 2) and were not related to soil temperature and/or WFPS variations ($p > 0.5$) (Fig. 3c). Specifically, N_2O emissions in the high ammonium nitrate treatment were associated with both NO_3^-–N and NH_4^+–N forms, while under the high urea treatment they were associated only with the NH_4^+–N form (Table 2).

Yield components

Cane yield, sugar yield and total biomass production were significantly different among sites and

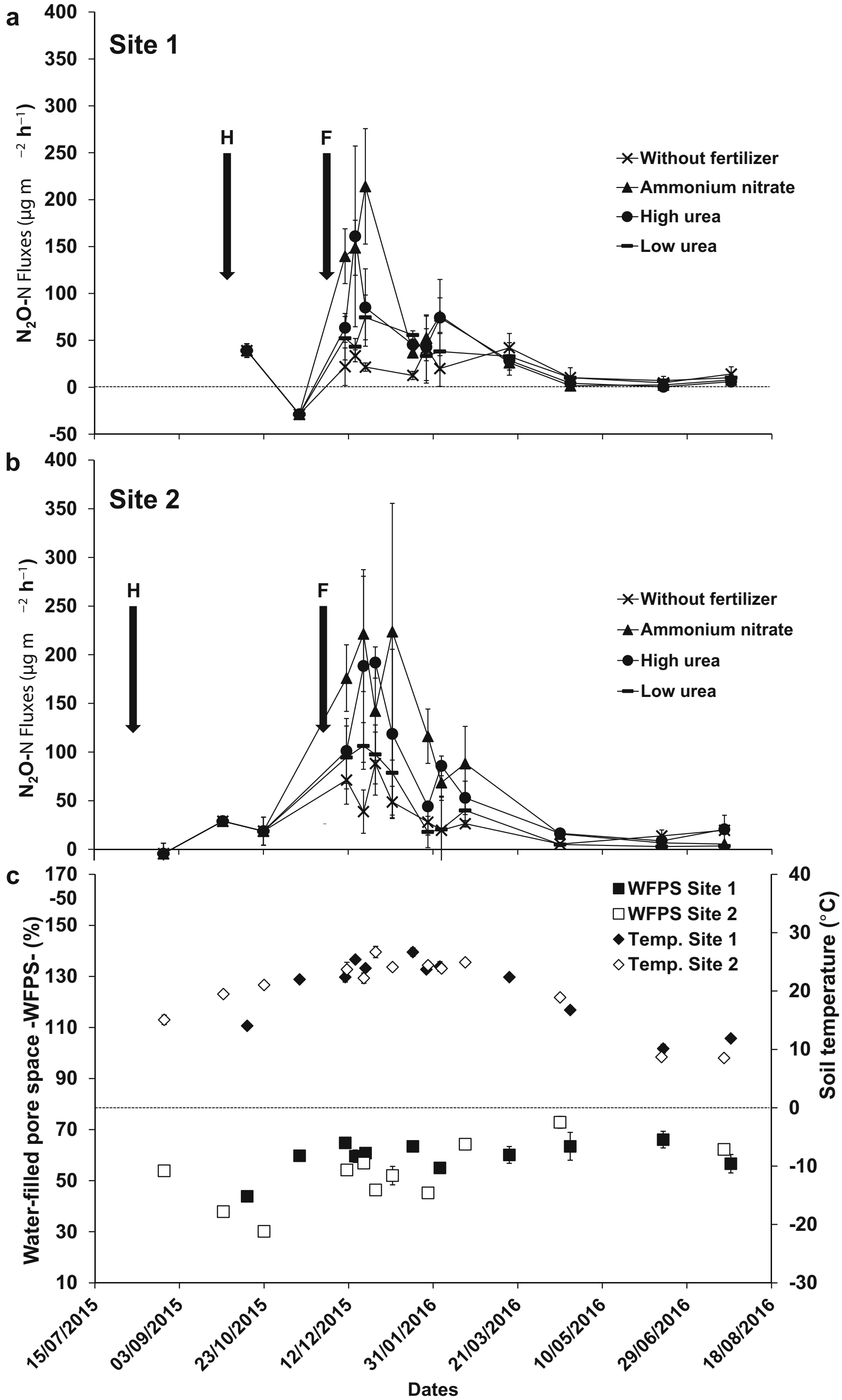

Fig. 2 N_2O emissions for site 1 (**a**) and 2 (**b**) and dynamics of water-filled pore space (WFPS) and soil temperature (**c**) for the 2015–2016 growing season of sugarcane in Tucuman, Argentina. Arrows indicate harvest (H) and fertilization (F) dates. Bars represent the standard error

treatments ($p < 0.01$), with no significant interactions between these factors (Table 3). Cane yield, sugar yield and total biomass increased with N fertilization rates. When comparing these variables for different N fertilizer types at the same rate (110 kgN ha^{-1}), the N supply as ammonium nitrate generated a significant lower cane yield, sugar yield and total biomass than the high urea treatment (Table 4). In fact, the low urea

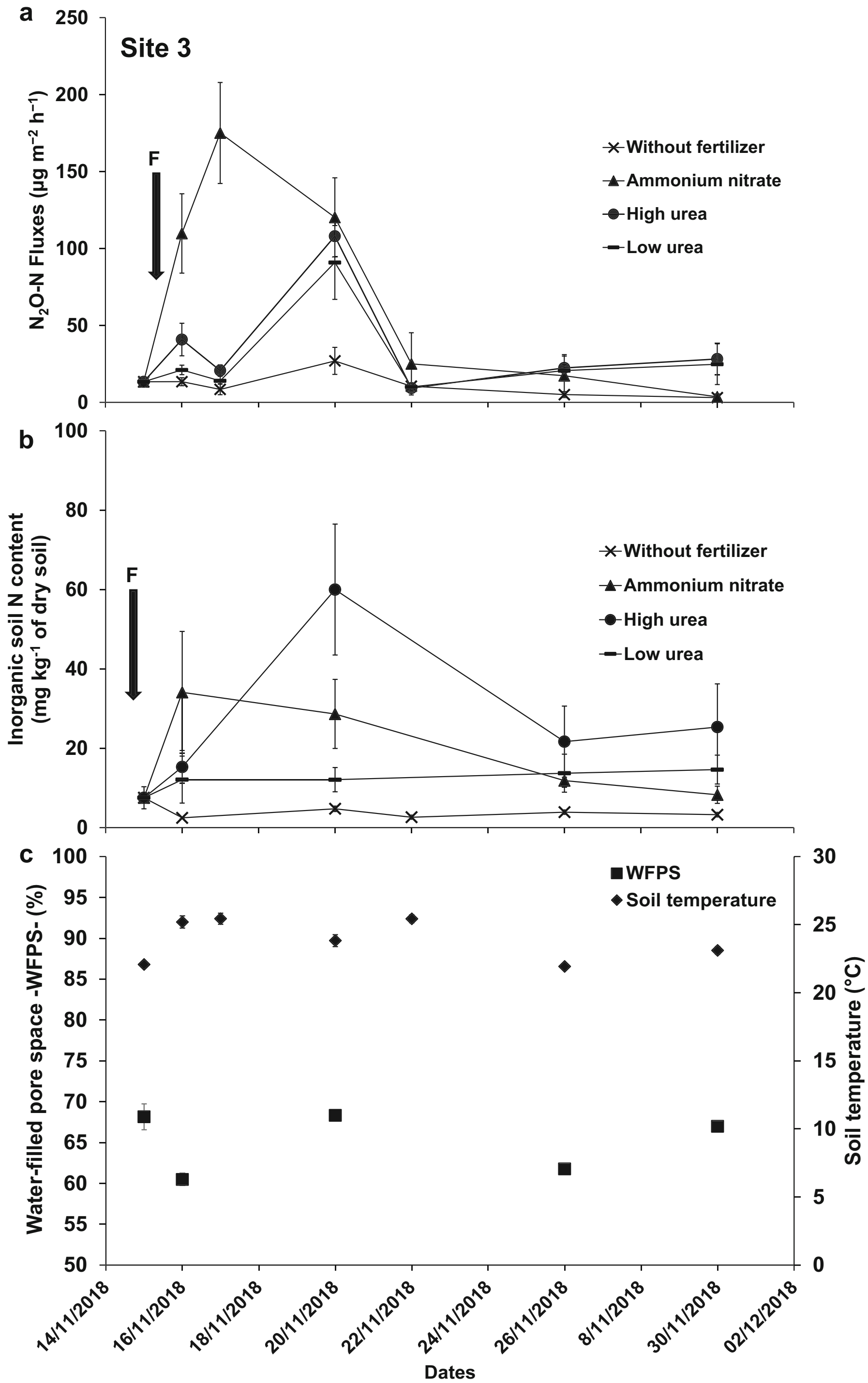

Fig. 3 N_2O emissions (**a**), inorganic soil N content (**b**) and dynamics of water-filled pore space (WFPS) and soil temperature (**c**) for the 15-days period since N fertilization for site 3 of the 2018–2019 growing season of sugarcane in Tucuman, Argentina. Arrows indicate harvest (H) and fertilization (F) dates. Bars represent the standard error

treatment was not significantly different from the ammonium nitrate treatment for cane yield, sugar yield and total biomass (Table 4).

Effectiveness of N use

Nitrogen use efficiency, NUpE, NUtE and total N in biomass were significantly different among N fertilization treatments, while NUpE, NUtE and total N in biomass varied also among sites ($p < 0.01$) (Table 3). In general, higher N rates decreased NUpE, NUtE and NUE and increased yield-scaled N_2O emissions (Table 4).

Adding N as ammonium nitrate also decreased the NUtE and NUE and increased the yield-scaled N_2O emissions (Table 4). The ammonium nitrate fertilizer emitted significantly higher amounts of nitrous oxide per ton of dry cane yield (156.6 ± 16.6 g N_2O–N per tonne of dry cane yield) than the other treatments that had similar yield-scaled N_2O emissions among them (average of 89.8 ± 4.34 g N_2O–N per tonne of dry cane yield) (Table 4).

Total N in biomass was positively associated with yield components and N_2O (rates or cumulative) emissions (Fig. 4a, b).

Associations among variables and between variables and treatments

The PCA showed general associations among variables, treatments and sites (Fig. 4). Based on this, the relationships between cumulative N_2O emissions or cane yield with NUE were deepened (Fig. 5). Nitrous oxide emissions (rates or cumulative) were negatively associated with N use efficiencies (NUE, NUtE and NUpE; $p < 0.05$) (Figs. 4a, 5a) and positively associated with yield-scaled N_2O emissions and yield components ($p < 0.01$) (Fig. 4a). It is important to note that the curves fitting in Fig. 5a, b were confident with linear functions since the polynomial ones (with the best AIC) had their quadratic coefficient two to three orders of magnitude smaller than the linear one. Data showed high N_2O emissions associated with low NUE at high N fertilization treatments, and low N_2O emissions associated with high NUE at low or no N fertilization treatments.

There was not a strong relationship between cane yield and NUE (Fig. 5b). For site 1, a slight negative association indicated that cane yield decreased with low or no fertilizer additions (Fig. 5b). For site 2, with the exception of the high urea treatment, all data lie in a very narrow band between 25 and 30 tons (DM) of cane per hectare.

The association among variables and treatments revealed that the site (i.e. soil properties) modify the magnitudes of the treatment effect (Fig. 4b). For example, the low urea treatment of site 2 showed higher NUE and NUtE than the high urea treatment of site 1 but it had similar N_2O emissions; the without fertilizer treatment of site 2 showed higher NUE and

Table 2 Pearson's correlation between N_2O emissions and N available in soil (0.1 m depth) for each N fertilization treatment (n = 22) during the second experiment

Treatment	Variable 1	Variable 2	Pearson coefficient
Without N-fertilizer	ug N_2O–N m^{-2} h^{-1}	NH_4–N (mg kg^{-1})	0.35
		NO_3^-–N (mg kg^{-1})	0.09
		Total soil N	0.21
Low urea (55 kgN ha^{-1})	ug N_2O–N m^{-2} h^{-1}	NH_4–N (mg kg^{-1})	0.06
		NO_3^-–N (mg kg^{-1})	− 0.23
		Total soil N	− 0.13
High urea (110 kgN ha^{-1})	ug N_2O–N m^{-2} h^{-1}	NH_4–N (mg kg^{-1})	0.66**
		NO_3^-–N (mg kg^{-1})	0.26
		Total soil N	0.65**
Ammonium nitrate (110 kgN ha^{-1})	ug N_2O–N m^{-2} h^{-1}	NH_4–N (mg kg^{-1})	0.67**
		NO_3^-–N (mg kg^{-1})	0.64*
		Total soil N	0.69**

*, **, ***Indicate significance at 0.05, 0.01 and 0.001 levels, respectively

Table 3 Mean squares of sugar yield, cane yield, total biomass, total N in biomass, nitrogen-use efficiency (NUE), nitrogen utilization efficiency (NUtE) and nitrogen-uptake efficiency (NUpE) for all sites and treatments according to two-way ANOVA and LSD Fisher's test (p-value < 0.05)

Source of variation	Sugar yield	Cane yield	Total biomass	Total N biomass	NUE	NUtE	NUpE
Site (S)	60.52***	315.39***	541.55***	10,207.54***	1431	21,354.04***	0.11**
Treatment (T)	16.48**	88.96**	149.06**	4773.40***	8068***	2361.41*	0.08**
S × T	0.98	8.32	10.42	487.33*	115.89	474.19	0.01
MSE	1.80	11.41	15.34	152.61	449.57	602.92	0.01

MSE, mean squared error

*, **, ***Indicate significance at 0.05, 0.01 and 0.001 levels, respectively

Table 4 Statistical differences among means of sugar yield, cane yield, total biomass, total nitrogen in biomass, nitrogen use efficiency (NUE), nitrogen utilization efficiency (NUtE), nitrogen uptake efficiency (NUpE), cumulative and yield-scaled N_2O emissions for the experimented sites and treatments according to two way ANOVA and LSD Fisher's test (p-value < 0.05)

Site	Sugar yield (t ha^{-1})	Cane yield (t ha^{-1})	Total biomass (t ha^{-1})	Total N in biomass (kg ha^{-1})	NUE (kg cane kg available N^{-1})	NUtE (kg cane kg adsorbed N^{-1})	NUpE (kg absorbed N kg available N^{-1})	Cumulative N_2O emissions (kg N_2O-N ha^{-1} yr^{-1})	Yield-scaled N_2O emissions* (g N_2O-N t cane^{-1})
1	6.7a	18.5a	27.8a	118.8a	132.9a	160.9a	0.82a	1.8a	99.4a
2	11.0b	28.3b	40.8c	163.2b	140.7ab	174.9a	0.80a	3.2b	113.6a
3	10.1b	25.9b	37.3b	108.2c	154.5b	239.9b	0.65b	–	–
Treatment									
Without fertilizer	7.4a	20.2a	29.9a	99.6a	181.5a	208.1a	0.88a	1.6a	80.7a
Low urea	9.4b	24.5b	35.27b	124.9b	148.7b	196.6a	0.77b	1.9a	88.5a
High Urea	10.7b	27.8c	39.8c	147.4c	128.9bc	193.3ab	0.69bc	2.7b	100.3a
Ammonium nitrate	9.6b	24.4b	36.4bc	148.4c	111.8c	169.5b	0.68c	3.7c	156.6b

Different letter indicates significant difference at 0.05 level

*The cumulative and yield-scaled N_2O emissions were only calculated for sites 1 and 2 (2015–2016 sugarcane-growing season) because the cumulative N_2O emissions were not calculated for site 3 (2018–2019 sugarcane growing season)

NUtE than the high urea treatment of site 1 but it had similar N_2O emissions) (Fig. 4b).

Discussion

There are many studies in sugarcane reporting NUE and N_2O emissions, separately, as affected by different sources and rates of N (Robinson et al. 2007; Allen et al. 2010; Otto et al. 2016; Yang et al. 2019; Degaspari et al. 2020). However, this study revealed, in the same experiment, the relationship among N_2O emissions, NUE and yield for a subtropical sugarcane agroecosystem.

Increases in NUE decrease N_2O emissions but can penalize yield

Reducing the rate of N fertilization in sugarcane can decrease N_2O emissions and increase NUE, as was shown in our study; but what happened with cane yield? Our results indicated that NUE above *ca.*

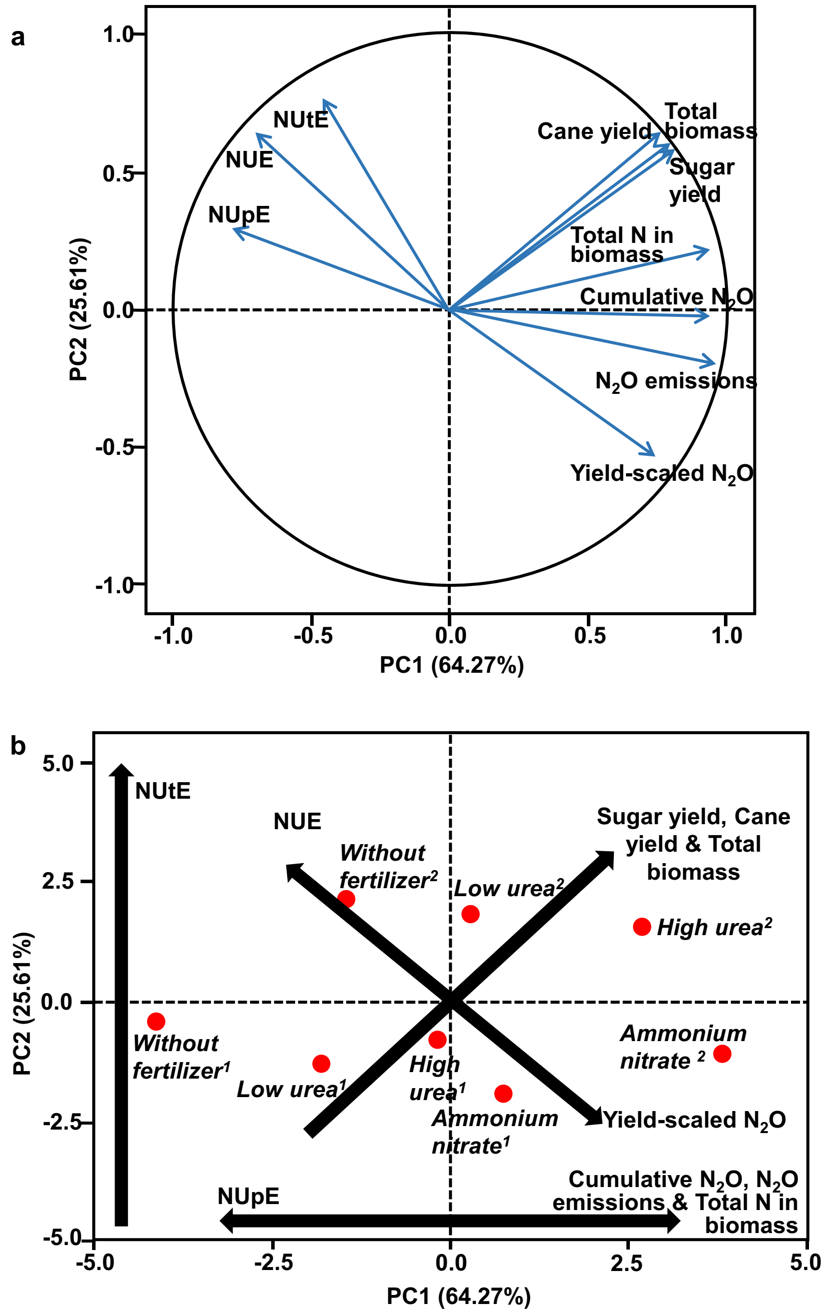

Fig. 4 Principal component (PC) analysis showing the variables factor map (**a**) and summarized factor map (**b**). In (**a**), blue arrows represent individual variables: nitrogen use efficiency (NUE), nitrogen utilization efficiency (NUtE), nitrogen uptake efficiency (NUpE), sugar yield, cane yield, total biomass, total nitrogen in biomass, mean nitrous oxide emissions, annual cumulative N_2O emissions and yield-scaled N_2O emissions. In (**b**), red points represent treatments, upper numbers indicate site, and black arrows indicate the way of increasing of the variables. (Color figure online)

160 kg of cane per kg of N available in soil (without fertilizer treatment; Fig. 5b) can penalize cane yield, whereas NUE below *ca.* 140 kg of cane per kg of N available in soil (high N fertilizer treatments; Fig. 5a) can penalize N_2O emission abatement. Hence, an enhanced NUE (by reducing rates or changing formulation of N fertilizer) does not necessarily mean higher yield but it does a reduction of N_2O emissions. Therefore, reaching NUE in the range of 140 to 160 kg of cane per kg of N available in soil, would lead to decrease N_2O emissions without a significant yield penalization. Barrow (1985, 2021) reported that the results of experiments comparing N sources with few levels of nutrient applications are site-limited. For this, more studies exploring the range of N fertilization from 55 to 110 kgN ha^{-1} should be conducted.

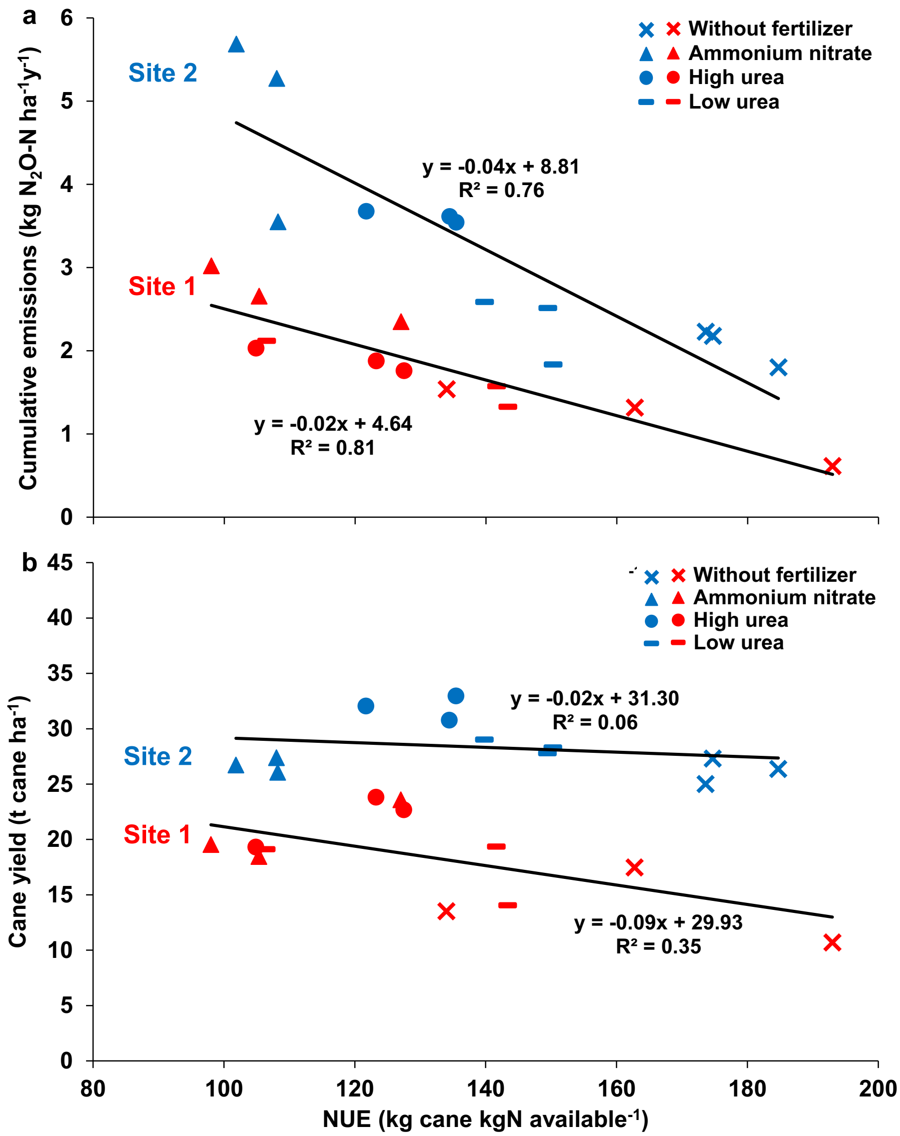

Fig. 5 Best regression models between NUE and **a** cumulative N_2O emissions, **b** cane yield, and **c** yield-scaled N_2O emissions. Significant parameters of function at 0.05 level

In contrast with NUE, total N absorbed in biomass was positively associated with yield and cumulative N_2O emissions. In fact, increases in N inputs produce a greater N availability as a substrate for both N_2O formation (Bouwman 1996; IPCC 1996; Kim et al. 2013) and biomass production. Moreover, our results showed that there was a surplus of inorganic N available in the soil immediately after N fertilization (see Fig. 3b) that was not exploited by the crop (Otto et al. 2016; Sainju et al. 2020) and produced high N_2O emissions and low NUE (as it was shown in our high N rates treatments). This could explain why high urea and ammonium nitrate treatments increased cumulative N_2O emissions and decreased NUE. Our results differ from those of Cardenas et al. (2019) that showed a negative correlation between N_2O emissions and grass N offtake for grassland systems since in our study they were positively correlated (Fig. 4a, b). Beyond the differences between species and environments, the differences could be associated with the extremely high N rates that that study used. In addition, they did not find correlations between NUE and N_2O emissions.

Synthetic nitrate results in higher and early N_2O emissions

Our results also showed that the high N_2O emissions and low NUtE and NUE generated by the use of ammonium nitrate instead of urea could be explained by the rapid N availability to the soil produced by ammonium nitrate fertilizers. Ammonium nitrate fertilizer supplies half of its N as NO_3^- and half as NH_4^+, which are readily available for plants or microorganisms. This fast release of NO_3^- into the soil may promote denitrification (Bremner and Shaw 1958; Knowles 1982; Soares et al. 2016; Lourenço et al. 2018). On the other hand, urea needs adequate urease activity to be hydrolysed to ammonia (NH_3) and then converted to nitrite (NO_2^-) and NO_3^- via the nitrification process (Prosser 1990), having a slower release of N into the soil. Due to operative reasons (rainfalls and/or height of sugarcane), N fertilization is traditionally performed early in the crop cycle when crop N uptake is not maximum (Robinson et al. 2011). Thus, the probable reason for the low N_2O emissions and high NUtE and NUE of urea could be the better synchronism between soil N offer and crop N demand. The advantage of reduced rate of N release from urea on reducing N_2O emissions in sugarcane was also reported by Kyulavski et al. (2019) comparing urea with organic fertilizers. This effect was also evident when urea was used with a nitrification inhibitor and/or as polymer-coated urea (Soares et al. 2015; Wang et al. 2016). These forms of urea also enhanced NUE in sweet corn (Liu et al. 2019). However, these technologies are still of limited access for small farmers of Tucuman (Argentina).

Soil nitrate and texture drive N_2O emissions

Chalco Vera et al. (2020) showed, in a long term study for the same experimental site as our experiments, that gravimetric soil moisture and soil inorganic N contents drive levels of N_2O emissions. In agreement, it was shown in our second experiment (the time-intensive experiment) that inorganic N contents (mainly as NO_3^-) in the soil seems to be the most important driver defining N_2O emissions (Fig. 3a, b). In addition, results from our first experiment highlight that a coarse soil texture (sites mainly differenced by sand content) could be another important driver for determining higher N_2O emissions (Site 2 > Site 1; Table 1, Figs. 4b and 5a). Under wet conditions, sand content probably enhanced the soil structural matrix and the oxygen supply (Weier et al. 1993; Schlüter et al. 2019) favouring at the same time both nitrification and denitrification processes (Firestone and Davidson 1989; Robertson 1989; Pihlatie et al. 2004). In Brazil, Borges et al. (2019) showed similar cane yield and lower cumulative N_2O emissions than our values for ammonium nitrate at similar N rates (120 kgN ha^{-1}). This could be attributed to the high soil clay content (almost 50%) in Brazil that could decrease N mineralization in the soil (McLauchlan 2006) and/or N_2O emissions due to worse soil moisture conditions for denitrification or nitrification processes (Araujo et al. 2021).

Conclusion

For the environmental conditions of the main sugarcane area of Argentina (Tucuman), it is possible to reduce N_2O emissions reaching NUE of approximately 140 to 160 kg of cane per kg of N available in the soil. Above this range of NUE, cane yield can be penalized. Selecting the suitable N fertilizer rate, type (i.e., fertilizers that avoid the readily release of nitrate) and/or promoting a better synchronization of N supply and demand could lead to mitigate N_2O emissions while maintaining sugar yields. However, more studies are needed reporting how to improve N synchronization and its effect on NUE and N_2O emissions.

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