



Fertilizer Application Method Provides an Environmental-Friendly Nitrogen Management Option for Sugarcane

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Received: 28 August 2023 / Accepted: 26 March 2024
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Abstract

Purpose Nitrogen fertilizer management is an important agricultural tool that must be optimized to promote sustainable practices since the nitrogen-fertilizer recovery by plants (NRP) is low, leading to nitrogen losses to the environment. In sugarcane, N-fertilization has been investigated over the years but little attention has been given to N-fertilizer application methods. Sugarcane crop production and environmental impact regarding N-fertilizer application methods (i.e., applied onto the sugarcane straw layer and incorporated into the soil) were investigated in the present study aiming to achieve an environmental-friendly cropping system.

Methods Sugarcane yield and NRP, N₂O emissions, relevant components of the soil microbiological community and N-fertilizer retention in soil layers were quantified. The experiment was carried out in field conditions where N-fertilizer application methods using ¹⁵N-labelled ammonium nitrate (¹⁵NH₄¹⁵NO₃) were compared to a control treatment with no N-fertilization.

Results Incorporation of N-fertilizer into the soil increased the sugarcane yield by 17% (two-year average) compared to N-fertilizer applied onto the sugarcane straw layer, which was similar to control treatment. There was an increase in NRP-fertilizer of 79% due to the application of N-fertilizer incorporated into the soil. Furthermore, soil incorporation of N-fertilizer decreased N₂O emission by 22% with the fertilizer N emission factor reduced four-fold. The N₂O emissions were mostly associated with ammonium-oxidizing bacteria (AOB).

Conclusions Our results show that application of N-fertilizer incorporated into the soil is an environmental-friendly N-fertilization management which will improve agricultural sustainability and reduce environmental impacts.

Keywords Nitrogen-fertilizer recovery · Labelled nitrogen · AOA · AOB · N₂O emission · Sugarcane yield

1 Introduction

The global pursuit of renewable energy to reduce the reliance on fossil fuels and mitigate greenhouse gas emissions (GHG), mainly nitrous oxide (N₂O), has brought ethanol to prominence (Carvalho et al. 2019; Jaiswal et al. 2017; Long et al. 2015). While the USA is the world's largest ethanol producer, the N₂O emission factor for ethanol derived from maize is 4.6 higher than that from sugarcane (Otto et al. 2022).

Brazil is the world's largest sugarcane producer, contributing 40% to global production. Over the past decade, concerns have arisen regarding Brazilian sugarcane production due to the country's stagnant total volume (~630 million

tons) and low yield (≤ 75 Mg ha⁻¹ of stalk) (FAO 2018). Under favorable edaphoclimatic conditions and proper agronomic management, the potential sugarcane yield in Brazil reaches 310 Mg ha⁻¹ of stalk (Dias and Sentelhas 2018; Waclawovsky et al. 2010). Factors limiting productivity encompass soil quality degradation (Bordonal et al. 2018a, b), extensive agricultural machinery traffic (Castioni et al. 2019), weather adversities throughout crop development (de Castro et al. 2022; Lisboa et al. 2018; Ruiz Corrêa et al. 2019), and the adoption of cost-effective agricultural practices rather than those beneficial to the crop, especially concerning N-fertilizer management (Otto et al. 2016; Quassi de Castro et al. 2018).

Effective N-fertilizer management plays an important role in ensuring productivity and sustainability of sugarcane (Cantarella and Rossetto 2014; de Castro et al. 2017;

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Thorburn et al. 2011). Understanding the dynamics of N-fertilizer in the soil–plant–atmosphere system is crucial in addressing the low nitrogen-fertilizer recovery (NRP) by sugarcane plants when N-fertilizer is applied to the soil (Boschiero et al. 2019; Franco et al. 2011; Otto et al. 2016; Thorburn et al. 2011). Inadequate N-fertilizer use contributes to soil and water contamination (Bowles et al. 2018a; Ghiberto et al. 2015; Khan et al. 2016; Skocaj et al. 2013), along with the emission of gases, for example, N₂O and ammonia (NH₃) (Degaspari et al. 2020; Gonzaga et al. 2018; Pan et al. 2016; Soares et al. 2015). To bypass NH₃ emission from urea application, ammonium nitrate can be employed as N source in sugarcane fields (Boschiero et al. 2018). Nevertheless, there is a knowledge gap regarding nitrogen losses (e.g., leaching, runoff and N₂O emission) and NRP when ammonium nitrate is applied in sugarcane fields using different N-fertilizer application methods.

The two most widespread methods for applying N-fertilizer in sugarcane fields are either onto the sugarcane straw layer or incorporated into the soil. To meet plant nutritional requirements under high yield conditions (> 100 Mg ha⁻¹), new recommendations advocate for higher N-fertilizer rates (approx. 150 kg N ha⁻¹ — Castro et al. 2019; Otto et al. 2019; Sanches et al. 2019). It is noteworthy that sugarcane is mechanically harvested without prior burning in Brazilian fields, leaving a layer of sugarcane straw residue on the soil surface (Carvalho et al. 2019). This aroused challenges in fertilization management since the straw layer hamper the incorporation of N-fertilizer into the soil. Then, application of N-fertilizer onto the sugarcane straw layer is the wide usage method, nevertheless it may lead to N-fertilizer immobilization during straw decomposition (Otto et al. 2016; Quassi de Castro et al. 2021) and significant emission of N₂O (Borges et al. 2019; Carmo et al. 2013; da Silva Paredes et al. 2014; Signor and Cerri 2013; Soares et al. 2015).

The increased N₂O emissions associated with sugarcane N-fertilization contradict recent Brazilian sectoral policies (Renovabio) and the Conference of the Parties (COP 23), both aiming to promote initiatives for the mitigation of GHG emissions while generating revenue for the sugar-energy sector by the commercialization of decarbonization credits (Gonçalves et al. 2021; Grangeia et al. 2022). Therefore, adjustments in crop agronomic management, particularly in N-fertilization, will enhance environmental and economic sustainability through reducing N-fertilizer losses from the soil–plant system by increased NRP (Bowles et al. 2018b; Otto et al. 2019; Thorburn et al. 2017). An important part of improving NRP involves identifying the N loss pathway (e.g., via gaseous emissions) and correlating those GHG emissions with the soil microbial communities that generate these emissions. There is over 60 types of bacteria and archaea associated to N₂O emissions (Pitombo et al. 2016),

with ammonia-oxidizing bacteria (AOB) and archaea (AOA) standing out (Lourenço et al. 2018; Soares et al. 2016).

Another pathway of N-fertilizer loss from the soil is via leaching, primarily of the nitrate anion. Nitrate leaching from N-fertilizer application in sugarcane fields in South-Central Brazil can reach up to 6% of the total N-fertilizer applied (Otto et al. 2016). In general, the magnitude of N-leaching losses from sugarcane ratoon fields is related not only to the frequency and intensity of rainfall but also to the season and N-fertilizer application methods (Khan et al. 2016; Otto et al. 2016).

Based on these statements, the present research aimed to test the hypothesis that the application of granular N-fertilizer incorporated into the soil reduces N losses and increases NRP by sugarcane plants compared to N-fertilizer applied onto the sugarcane straw layer. To examine this hypothesis, the research objectives were as follows: i) quantify the N-fertilizer recovery by sugarcane using different N-fertilizer application methods; ii) quantify N₂O emissions throughout the crop cycle; iii) assess N-fertilizer losses via leaching and N-fertilizer retention in soil layers; and iv) quantify the microbial communities associated with N₂O-N emissions.

2 Material and Methods

2.1 Field Description

The experiment was carried out in Sales Oliveira, São Paulo, Brazil (20°51'S 47°56'W), in a cultivated field that had grown sugarcane for eight years (experiment was established in the 7th ratoon). Mechanized harvesting without prior sugarcane straw burning was conducted in the three years preceding the experiment set up. The sugarcane variety was SP81-3280, harvested at the end of the crop season (from September to November). The mean annual precipitation and temperature of the field were 1553 mm and 23 °C, respectively. Before initiating the experiment, soil samples were collected for chemical and physical characterization (Online Resource 2), according to the methodology described by Raj et al. (2001). The soil was classified as Rhodic Eutrudox (Soil Survey Staff 2014).

2.2 Experimental Design and Treatments

The experiment was conducted in a randomized block design encompassing three treatments and three replicates over two consecutive years (1st and 2nd year, corresponding to the 7th and 8th sugarcane ratoon). The treatments consisted of i. control [without N-fertilization], and two N-fertilizer application methods, namely ii. Surface [N-fertilizer applied onto the sugarcane straw layer], and iii. Incorporated [N-fertilizer

applied in a band at 0.08 m soil depth, positioned 0.15 m away from the sugarcane row, methodology described by Castro et al. (2017)]. Each plot consisted of seven sugarcane rows with inter-row spacing of 1.5 m and a length of 2 m, totaling an area of 21 m². To evaluate the N-fertilizer losses by leaching and surface runoff, the plots were delimited by a galvanized steel gutter.

Nitrogen, phosphorus, and potassium (NPK) were applied 30 days after the harvest in both experimental years, except in the control treatment, in which N was not applied. The rates and sources adopted for NPK fertilization were 120 kg N ha⁻¹ (applied as ammonium nitrate — 33% N), 35 kg P ha⁻¹ (applied as triple superphosphate — 41% P₂O₅) and 120 kg K ha⁻¹ (applied as potassium chloride — 60% K₂O), respectively. In the 2nd year, microplots containing double-labeled ¹⁵N ammonium nitrate (¹⁵NH₄¹⁵NO₃, with 5 atom% ¹⁵N) were installed at the center of each plot.

2.3 Sugarcane Stalk Population and Yield

Sugarcane stalk population (stalks ha⁻¹) and yield were evaluated in both years. The stalks in each plot were counted and the aboveground biomass of sugarcane, comprising three plant tissues (dry leaves, tops and stalks), was quantified. The fresh mass of each plant tissue was determined to calculate the stalk yield per hectare.

2.4 Nitrogen-Fertilizer Losses via Leaching and Runoff

N-fertilizer losses through leaching and runoff were evaluated in the 2nd year following the methodology described by Scarpore et al. (2023). N-leaching was assessed using lysimeters installed at a depth of 0.90 m in the microplot. N-fertilizer at this depth was considered to have leached beyond the effective root zone since 80% of the sugarcane plant root system is located in the first 0.40 m of soil depth, with long roots extending to 0.60 m (Otto et al. 2009; Rossi Neto et al. 2018). To maintain lysimeters operational, weekly vacuuming was performed. Surface water runoff from the entire plot was collected in water tanks, where water sub-samples were obtained. Collected solutions were stored in a freezer to prevent N transformation until analysis.

Leachate and runoff water were analyzed using a mass spectrometer interfaced with an automatic nitrogen and carbon analyzer (IRMS-ANCA-GSL, Sercon Inc., Crewe, GBR) for determining total-N content and isotopic abundance, according to Ghiberto et al. (2009). As no difference in isotopic abundance between treatments was observed, ammonium and nitrate content in surface water runoff and leachate water were determined by flow injection analysis

by conductimetry (Reis et al. 1997) and colorimetry (Giné et al. 1980), respectively.

2.5 N₂O Emission

N₂O emission was evaluated in the 2nd year. Gas sampling started one day before the N-fertilizer application and continued daily for 18 days. Afterward, sampling was conducted three times a week for three weeks, twice a week for the following three weeks, and once a week until the onset of sugarcane harvesting (46 sampling events). Flux of N₂O were measured using static chambers (PVC cylinders with 0.20 m in height and 0.30 m in diameter) placed in row and inter-row. The chambers were sealed with caps and air samples were collected using BD nylon syringes (Becton Dickinson Industry Co. Ltd) after 0, 15, and 30 min of incubation, and transferred to evacuated glass vials. Simultaneously, soil moisture and temperature were measured with a MPS 2 Decagon® device installed at a 0.10 m depth.

Gas samples were analyzed using a Shimadzu® (GC-2014) gas chromatograph equipped with a packed column and an electron capture detector for N₂O analyses. The N₂O flux was calculated based on the linear change in concentration within the chamber during the incubation time (30 min). Atmospheric pressure, chamber height, and air temperature were measured simultaneously with gas sampling for determining air chamber volume and calculating N₂O emissions. Daily N₂O flux was calculated according to Eq. 1 (Parkin and Venterea 2010).

$$F = [\Delta C \div \Delta t] \times (V \div A) \times (m \div Vm) \quad (1)$$

where: **F** is the N₂O flux (μg N₂O-N m⁻² h⁻¹), $[\Delta C/\Delta t]$ is the gas concentration exchange rate inside the chamber during the incubation time (μg mol⁻¹ h⁻¹), **V** is the chamber volume (L), **Vm** is the molar volume of the gas at the sampling temperature (L mol⁻¹), **m** is the molecular mass of N₂O (N=14), and **A** is the soil area covered by the chamber (m²).

The N₂O flux were converted and expressed in g N₂O ha⁻¹ day⁻¹ using linear interpolation between the dates adjacent to the samples (Whittaker and Robinson 1967). The cumulative N₂O emissions from the fertilized band were considered to represent 20% of the total area, with the area between rows accounting for 80%, as used in other studies (Borges et al. 2019; Carmo et al. 2013; Gonzaga et al. 2019). The N₂O emission factors were calculated based on the N-fertilizer rate applied within the chamber according to Eq. 2.

$$EF_{N_2O} = (N_2O_{treatment} - N_2O_{control}) \div N_{fertilizer} \quad (2)$$

where: EF_{N₂O} is the emission factor (N₂O as percentage of nitrogen fertilizer applied); N₂O_{treatment} and N₂O_{control} are the cumulative emissions of N₂O from a specific treatment and a

specific control; and $N_{\text{fertilizer}}$ is the N-fertilizer rate applied to soil.

2.6 Nitrogen-Fertilizer Recovery by Aboveground Plant Tissues

The N-fertilizer recovery by the aboveground of sugarcane plant was determined by summing up the N-fertilizer recovery by each plant tissue (tops, stalks, and dry leaves) in the 2nd year. The plant tissues were sampled in both the central row and the two adjacent rows (Trivelin et al. 1994). After determining the fresh mass of each plant tissue, they were ground and sub-samples were taken for moisture and isotopic abundance determination. The sub-samples were oven-dried at 65 °C until reaching a constant mass followed by grinding in a Wiley mill with a 0.5 mm mesh sieve. The moisture content of the plant tissues was calculated, and their yield was corrected to dry matter, serving as the basis for calculating the NRP. The ground and dried samples were weighted (Mettler Toledo XPR6U, Columbus, EUA) in tin capsules and analyzed in a mass spectrometer coupled to an automatic nitrogen analyzer (IRMS-ANCA-GSL, Sercon Inc., Crewe, GBR). The results obtained were the isotopic abundance of the samples (atom% ^{15}N) and the total-N content (Total-N, g kg^{-1}). The results were used to calculate the nitrogen in plant derived from fertilizer (NPdff), in percentage, the amount of nitrogen in plant derived from fertilizer (ANPDFF, kg ha^{-1} , and the ^{15}NRP in each plant tissue, following the equations provided (Trivelin et al. 1994).

$$\text{NPdff}(\%) = (a \div b) \times 100 \quad (3)$$

$$\text{ANPDFF}_{\text{CR}}(\text{kg ha}^{-1}) = [\text{NPdff}_{\text{CR}}(\%) \div 100] \times \text{Total} - \text{N} \quad (4)$$

$$\text{ANPDFF}_{\text{AR}}(\text{kg ha}^{-1}) = [\text{NPdff}_{\text{AR}}(\%) \div 100] \times \text{Total} - \text{N} \quad (5)$$

$$\text{ANPDFF}_{\text{total}}(\text{kg ha}^{-1}) = \text{ANPDFF}_{\text{CR}} + \text{ANPDFF}_{\text{AR}} \quad (6)$$

$$^{15}\text{NRP}(\%) = (\text{ANPDFF}_{\text{total}} \div \text{NR}) \times 100 \quad (7)$$

where NPdff is the N in each aboveground plant tissue derived from fertilizer (%); “a” and “b” are the isotopic abundance (atom % ^{15}N excess) in the plant and in the fertilizer, respectively; ANPDFF_{CR} and ANPDFF_{AR} is the amount of N in the plant derived from the fertilizer at the center row and at the adjacent rows, respectively; Total-N is the plant N content (kg N ha^{-1}); ^{15}NRP is the N-fertilizer recovery by each aboveground plant tissue; and NR is the N-fertilizer rate applied (kg N ha^{-1}).

2.7 Nitrogen-Fertilizer Recovery in the Soil and by the Belowground Plant Tissues

In the 2nd year of the experiment, the soil was sampled up to a depth of 0.9 m within the chamber, to assess the ^{15}N -fertilizer recovery in the soil. Before soil sampling, the litter inside the chamber was collected, encompassing material from the superficial soil layer and residual straw (referred to as straw-soil). Soil samples were obtained using a Dutch auger at depths of 0.0–0.1, 0.1–0.2, 0.2–0.4, 0.4–0.6, and 0.6–0.9 m. Soil samplings were oven-dried at 50 °C for 72 h with air circulation and renovation. A sub-sample was ground in a ball mill, and analyzed for total-N (g kg^{-1}) and isotopic abundance (atom% ^{15}N) using a mass spectrometer (IRMS-ANCA-GSL, Sercon Inc., Crewe, GBR). The soil mass of each soil layer was determined by the ring volumetric method (Blake and Hartge, 1986). The average soil density at depths of 0.0–0.1, 0.1–0.2, 0.2–0.4, 0.4–0.6, and 0.6–0.9 m was 1.25, 1.18, 1.16, 1.16, and 1.15 g cm^{-3} , respectively, in N-fertilizer applied incorporated into the soil treatment, and 1.28, 1.17, 1.15, 1.22, and 1.13 g cm^{-3} , respectively, in N-fertilizer applied onto the sugarcane straw layer treatment. The N-fertilizer recovery in each soil depth (^{15}NRS) was calculated according to Eqs. 3, 4, and 7.

After soil sampling, the sugarcane root system was sampled through trenches (1.5 × 2.0 × 1.0 m, width × length × depth). The samples were washed for soil removal and compartmentalized into two plant tissues (roots, and rhizome). The root system tissues were dried, ground, and analyzed in a mass spectrometer (IRMS-ANCA-GSL, Sercon Inc., Crewe, GBR) obtaining the total-N (g kg^{-1}) and isotopic abundance (atom% ^{15}N), following procedures described for the aboveground plant samples. The ^{15}N -fertilizer recovery by the belowground plant tissues was calculated according to Eqs. 3, 4, and 7.

2.8 Microbiological Analyses: Genomic DNA Extraction and Quantification of *amoA* Genes

Soil samples for genomic DNA extraction was collected from the surface layer (0–0.1 m) where N-fertilizer was applied in the 2nd year of the experiment; samples were stored at -80 °C until the moment of use. The DNA extraction was performed using the PowerSoil PowerLyzer DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) following the instructions of the manufacturer. All genes used were quantified by applying the quantitative PCR technique (qPCR) with the use of the ViiA7 Real-Time PCR System (Applied Biosystems, Foster City, USA).

Reactions were performed with 1 μL BSA, 5.5 μM of each primer with concentration of 10 nM, 10 μL of qPCR SYBR master mix 2x (Applied Biosystems, Foster City, CA, USA) and 10 to 20 ng of DNA. The primers used (Online

Resource 3) were 16S rRNA of *bacteria* and *archaea* (Bakke et al. 2011; Yu et al. 2005), *nitrogenase* (*nifH*; Wallenstein and Vilgalys 2005), *ammonium-oxidizing* (*amoA*) for *bacteria* (AOB; Rotthauwe et al. 1997) and *archaea* (AOA; Leininger et al. 2006). The standard curve was undertaken with pre-amplification of *primer* sets, using known genes in serial dilution of 10^2 to 10^7 μL^{-1} genes. The amplifications were performed as described in Table S2; the specificity of the *primer* set was checked by observing a single *melting* peak (60 °C to 95 °C), thus validating the purity of the generated products and also for presenting a single band in the 1% agarose gel, corroborating the size of *amplicons*. Only r^2 above 0.99 of the standard curve were accepted. Amplification efficiencies of quantitative PCR were of $97\% \pm 9$.

2.9 Weather Conditions during the Experimental Period

Climatological data was monitored by a meteorological station (Vantage Pro II, Decagon Devices, CA, USA). The water balance (Online Resource 1) was calculated according to the Penman–Monteith method (Howell and Evett 2004).

The precipitation was evenly distributed throughout the maximum plant growth season, from November to December, resulting in a slight water deficit in the soil (≤ 40 mm) during the autumn and winter (March to September). It is noteworthy that after N-fertilization a substantial volume of precipitation occurred (1090 mm accumulated precipitation), favoring the incorporation of N-fertilizer into the soil solution.

2.10 Statistical Analysis

The data set was subjected to analysis of variance (ANOVA) of the entirely randomized block design using the ‘ExpDes’ package (Ferreira et al. 2014), after performing the Shapiro–Wilk normality test. Means among treatments were compared by the Tukey test at $p < 0.05$ significance. The agronomic traits, such as stalk yield and population, and recovery of ^{15}N -fertilizer in both aboveground and belowground plant tissues, as well as in the soil depths, and the environmental variables (GHG emissions, flux, and emission

factor) were subjected to a non-metric multidimensional scaling (N-MDS) using Bray–Curtis dissimilarity matrix. All analysis were performed using Bray–Curtis dissimilarity matrix (R Core Team 2021).

3 Results

3.1 The Application of N-fertilizer Incorporated into the Soil Increased the Population and Yield of Sugarcane

The incorporation of N-fertilizer into the soil increased the number of stalks per hectare compared to the control treatment (Table 1). In the 1st year, the stalk population in incorporated treatment was greater than the surface application. In the 2nd year, the stalk population did not differ among the N-fertilizer application methods. The N-fertilizer application method influenced sugarcane stalk yield. Stalk yield in the incorporated treatment was 15 and 9 Mg stalk ha^{-1} greater than the control in the 1st and 2nd year, respectively (Table 1). In the 2nd year, sugarcane stalk yield in the incorporated treatment was also greater than surface treatment.

3.2 N-Fertilizer Recovery by the Plant (^{15}NRP) Increased by Applying N-Fertilizer Incorporated into the Soil

The ANPDDF of aboveground plants in incorporated and surface treatment were 59.4 and 33.3 kg N ha^{-1} , respectively (Table 2). The difference among N-fertilizer application methods is due to the ANPDDF in stalk and dry leaves. The average ANPDDF in the belowground did not differ among treatments, with an average of 4.8 kg N ha^{-1} .

The ^{15}NRP in the aboveground plant was 49.6% and 27.7% for incorporated and surface treatments, respectively (Table 2). N-fertilizer incorporated into the soil enhanced the N-fertilizer recovery in the stalk and the dry leaves (Table 2). The average of ^{15}NRP in the belowground plant was 4%. The N-fertilizer recovery by the belowground tissues and in the top did not differ among the N-fertilizer application methods (Table 2). Sugarcane stalk was the main

Table 1 Sugarcane stalk population (number of stalks ha^{-1}) and fresh weight stalk yield (Mg ha^{-1}) in the 1st and 2nd year of the experiment in the control treatment and in each N-fertilizer application method

Treatment	Stalk population (stalks ha^{-1})		Stalk yield (Mg ha^{-1})	
	1st year	2nd year	1st year	2nd year
Control	66,700 \pm 4,193 b	66,700 \pm 4,368 b	55 \pm 0.40 b	71 \pm 0.58 b
Surface	66,700 \pm 2,061 b	80,000 \pm 1,874 a	62 \pm 1.10 ab	66 \pm 2.50 b
Incorporated	80,000 \pm 4,225 a	80,000 \pm 2,445 a	70 \pm 0.55 a	80 \pm 1.70 a

The data represent the average of three independent biological replicates \pm standard error. Lowercase letters indicate differences among the treatments within each experiment year, according to Tukey post-hoc test ($p < 0.05$).

Table 2 Amount of nitrogen in plant derived from fertilizer (ANP-DFF) and ^{15}N -fertilizer recovery by the plant (^{15}NRP) in each above-ground tissue (i.e., stalk, top, and dry leaves), and belowground tis-

sue (i.e., roots and rhizome) of sugarcane plant in the 2nd year of the experiment in each N-fertilizer application method

Application method	Stalk	Top	Dry leaves	Roots	Rhizome	Total
ANPDFF (kg N ha⁻¹)						
Incorporated	41.9 ± 2.09 a	9.8 ± 0.39 a	7.7 ± 0.44 a	0.32 ± 0.07 a	5.4 ± 1.17 a	65.1 ± 1.20 a
Surface	19.8 ± 2.39 b	7.9 ± 0.66 a	5.6 ± 0.13 b	0.22 ± 0.02 a	3.6 ± 1.16 a	37.1 ± 2.47 b
^{15}NRP (%)						
Incorporated	35.0 ± 1.74 a	8.2 ± 0.32 a	6.4 ± 0.37 a	0.27 ± 0.06 a	4.5 ± 0.98 a	54.4 ± 1.00 a
Surface	16.5 ± 1.99 b	6.6 ± 0.55 a	4.6 ± 0.11 b	0.18 ± 0.02 a	3.0 ± 0.97 a	30.9 ± 2.05 b

The data represent the average of three independent biological replicates ± standard error. Lowercase letters indicate differences among the N-fertilizer application methods in each plant tissue sampled according to Tukey post-hoc test ($p < 0.05$)

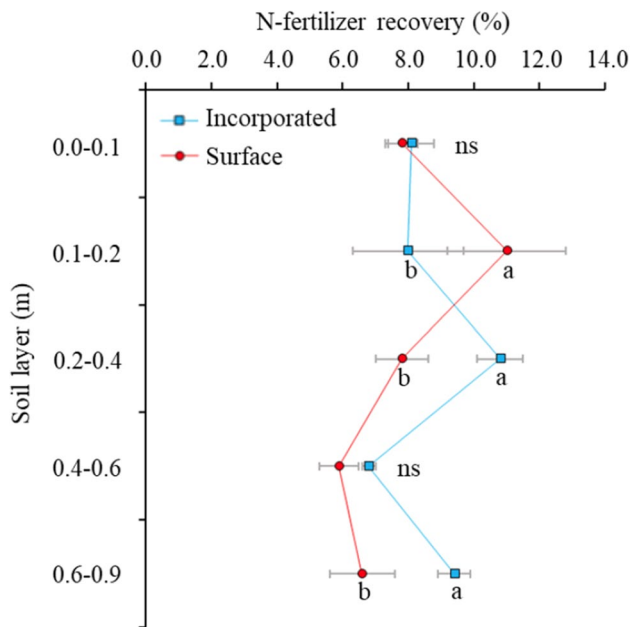


Fig. 1 ^{15}N -fertilizer recovery in each soil layer (^{15}NRS , %) within the N-fertilizer application method (i.e., Incorporated or Surface) in the 2nd year of the experiment. The data represent the average of three independent biological replicates ± standard error. Lowercase letters indicate differences among the N-fertilizer application methods within each soil layer according to Tukey post-hoc test ($p < 0.05$). “ns” means non-significant difference among N-fertilizer application methods

sink of N-fertilizer applied to the soil followed by top, dry leaves, rhizome and roots.

3.3 N-Fertilizer Application Method Altered the N-Fertilizer Recovery in Soil Layers (^{15}NRS)

The highest ^{15}N -fertilizer recovery in the soil (^{15}NRS) was observed in the 0.2–0.4 m and 0.1–0.2 m soil layers for incorporated and surface treatment, respectively (Fig. 1). In these soil layers treatments differed in the ^{15}NRS as well as at the deepest layer (0.6–0.9 m). The average of total NRS was

43.1 and 39.1% in the incorporated and surface treatments, respectively.

3.4 Influence of N-Fertilizer Application Method on the ^{15}N -Fertilizer in the Plant-Soil-Atmosphere System

The ^{15}N -fertilizer recovered in the plant-soil system is the sum of the total N-fertilizer recovery by the plant (NRP-total), in the soil (NRS-total), and in the litter (NRStaw-Soil). The total N-fertilizer recovery in the plant-soil system was 99.5 and 72.7% in the incorporated and surface treatments, respectively. The incorporated treatment increased by 76% the NRP-total compared to the surface treatment (Fig. 2). The NRS-total and the NRStaw-Soil did not differ among N-fertilizer application methods, an average of 41% and 2.4% respectively.

3.5 Ammonium and Nitrate Lost in the Water Leaching and Surface Water Runoff

The isotopic abundance in the water samples from water tanks (runoff) and lysimeters (nitrogen leaching) did not differ among treatments (i.e., solutions presented the natural abundance of ^{15}N , an average of 0.3663 atom% ^{15}N). Therefore, the ammonium and nitrate content were quantified in the water samples. N-fertilization increased the ammonium and nitrate content in the surface water runoff regardless of the application method (Table 3). Ammonium content was two-fold higher in surface treatment compared to incorporated treatment, meanwhile, they did not differ in terms of nitrate content. In the leached water solution, N-fertilization increased the nitrate content regardless of the N-fertilizer application method compared to the control (Table 3).

3.6 N_2O Emission Decreased when Applying N-Fertilizer Incorporated into the Soil

The N_2O emission occurred more intensively after N-fertilization for up to three months (Fig. 3A). N-fertilization

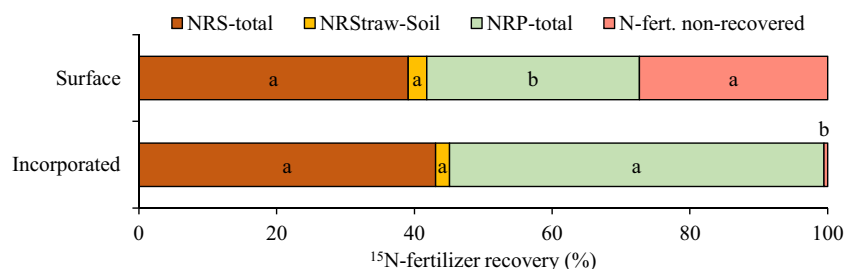


Fig. 2 ¹⁵N-fertilizer recovered in the soil–plant system in the 2nd year of the experiment within the N-fertilizer application method at sugarcane harvesting. NRS-total is the ¹⁵N-fertilizer recovery in the soil (sum of N-fertilizer recovered in each soil layer, from 0 to 0.9 m), NRStraw-Soil is the ¹⁵N-fertilizer recovered in the litter, and NRP-total is the ¹⁵N-fertilizer recovery by the plant (sum of N-fertilizer

recovered in the aboveground and belowground tissues). The data represent the average of three independent biological replicates. Lowercase letters indicate differences among N-fertilizer application methods within each evaluated system (soil, straw-soil, plant, and non-recovered nitrogen) according to Tukey post-hoc test ($p < 0.05$)

Table 3 Total ammonium and nitrate content in water samples collected in water tanks (nitrogen runoff) and lysimeter (nitrogen leaching) throughout the 2nd year of the experiment in each treatment and in the control

Cation/Anion	Surface	Incorporated	Control
Nitrogen runoff (g L ⁻¹)			
Ammonium (NH ₄ ⁺)	0.94 ± 0.47 a	0.48 ± 0.41 b	0.06 ± 0.01 c
Nitrate (NO ₃ ⁻)	188.5 ± 19.3 a	118.8 ± 18.4 a	Not detected
Nitrogen leaching (g L ⁻¹)			
Ammonium (NH ₄ ⁺)	0.40 ± 0.14 a	Not detected	0.35 ± 0.12 a
Nitrate (NO ₃ ⁻)	4.20 ± 1.45 a	2.50 ± 4.08 a	1.90 ± 0.77 b

The data represent the average of three independent biological replicates ± standard error. Lowercase letters indicate differences among the treatments within each parameter, according to Tukey post-hoc test ($p < 0.05$)

increased N₂O emission compared to control treatment, regardless of the application method. Additionally, applying N-fertilizer incorporated into the soil reduced by 50% the N₂O emissions compared to surface application. From the 3rd to 6th month after N-fertilization, a significant drop in N₂O emissions (below 100 μg N₂O m² day⁻¹) was observed (Fig. 3A). Following this period until the sugarcane harvest, the N₂O emissions from control and incorporated treatments were nearly nil, while surface applications continued to emit N₂O to the atmosphere (Fig. 3A).

The lowest accumulated N₂O emission (aN₂O) was obtained in the control treatment (Fig. 3B), which was 2-folder and 1.7-folder lower than the surface and incorporated treatment, respectively. On the other hand, the incorporated treatment reduced the aN₂O by 18% and the emission factor (EF%) by 75% (Fig. 3C) compared to surface treatment. No differences in N₂O emission intensity (iN₂O) were found among the control and incorporated treatments, meanwhile, the surface treatment doubled the iN₂O (Fig. 3D).

3.7 Microbiological Analysis

The control treatment presented higher abundance of bacteria (Fig. 4A) and archaea (Fig. 4B) compared to the N-fertilization treatments. Even considering the higher abundance in the control treatment, no differences were observed among treatments.

The abundance of biological nitrogen fixation (nifH) and nitrifying bacteria (AOB) exhibited variations among treatments (respectively, Fig. 4C and 4D). The lowest abundance of nifH was obtained in the incorporated treatment differing from the control and surface treatments. Surface and control treatments exhibited a similar abundance of nifH (Fig. 4C). The incorporated treatment also presented the lowest abundance of AOB and did not differ from the control treatment (Fig. 4D). An increase in the abundance of AOB was observed in surface treatment compared to incorporated treatment (Fig. 4D). The abundance of nitrifying archaea (AOA) was not influenced by N-fertilization regardless the application method (Fig. 4E).

3.8 Multivariate Analysis

The PCA (principal component analysis) accounted for 67% of the data variation (Fig. 5). The parameters related to plant (¹⁵NRP-total and yield) are directed towards the incorporated treatment, while they displayed a negative correlation with the parameters of N₂O emission (EF% and Daily N₂O emission). The N₂O emission parameters are directed towards the surface treatment and correlated positively to the abundance of AOB. It is noteworthy that the N-fertilizer recovered in the soil (¹⁵NRS-total) showed a negative correlation with the abundance of bacteria (Bacteria16S), archaea (Archaea16S), and AOA, being these directed toward the control treatment. Based on these statements, it indicates that applying N-fertilizer incorporated into the soil increases

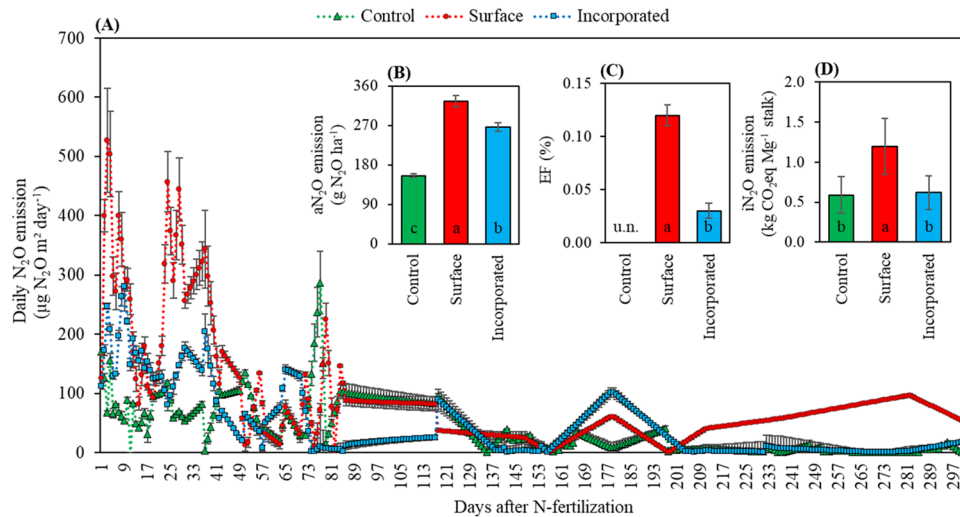
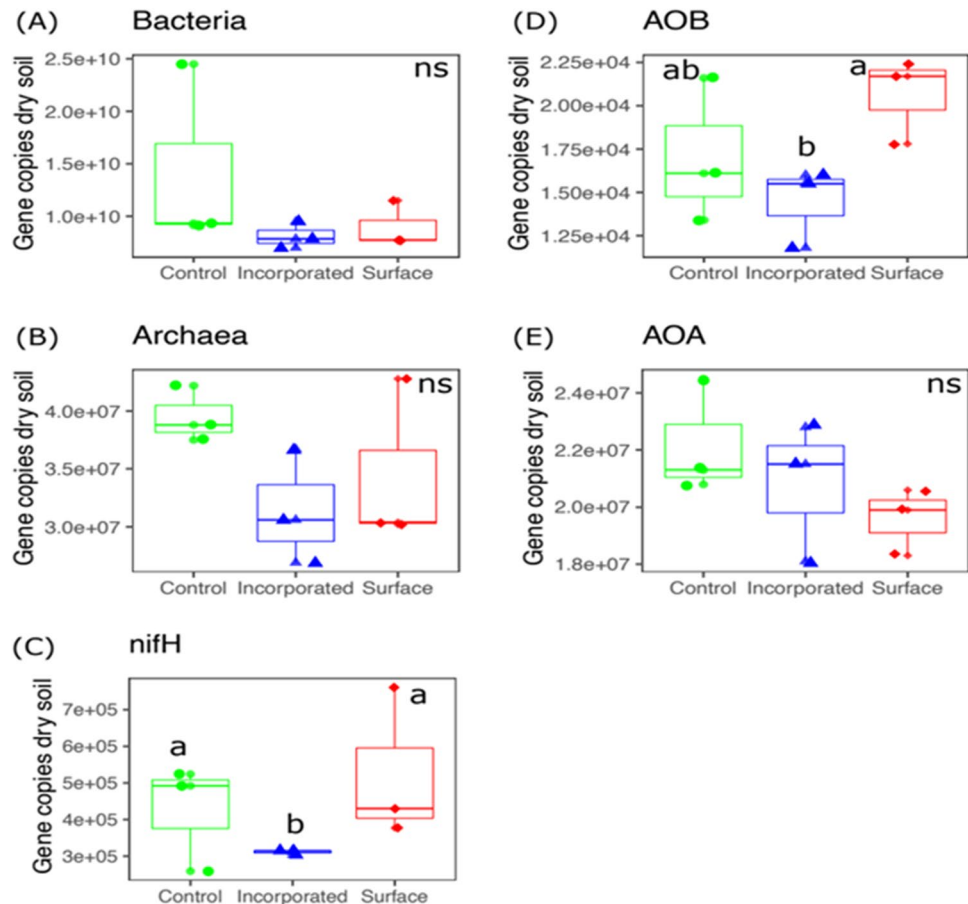


Fig. 3 Daily mean N₂O emission (A), accumulated of N₂O emission [aN₂O] (B), N₂O emission factor [EF] (C), and intensity of N₂O emission [iN₂O] (D) in the 2nd year of the experiment (from October 2015 to November 2016) after the application of ammonium nitrate incorporated into the soil (Incorporated), and over the sugarcane straw layer (Surface), and in the control treatment (without N-fertil-

zation). The data represent the average of three independent biological replicates \pm standard error. Lowercase letters indicate differences among the treatments according to Tukey post-hoc test ($p < 0.05$). "u.n." means unavailable once the N₂O emission factor is calculated in function of the control treatment

Fig. 4 Absolute quantification (copies per gram of dry soil) of microbial 16S rRNA genes from bacteria (A) and archaea (B) and genes associated with the biological nitrogen fixation [nifH, nitrogenase] (C), nitrifying bacteria [AOB, Ammonium-Oxidizing Bacteria] (D) and nitrifying archaea [AOA, Ammonium-Oxidizing Archaea] (E). The data represent the average of three independent biological replicates \pm standard error. Small letters indicate difference between treatments according to the post-hoc Tukey test ($p < 0.05$)



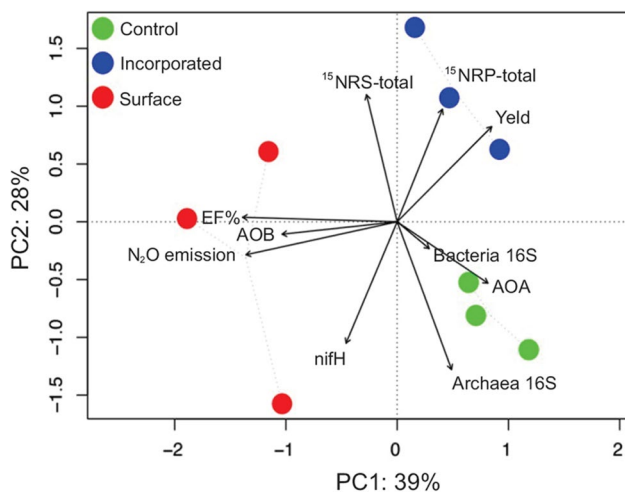


Fig. 5 Principal components analysis conducted on variables of interest. It includes the daily N_2O emission (N_2O emission), emission factor (EF%), total of ^{15}N -fertilizer recovered in the soil (^{15}NRS -total) and by the plant (^{15}NRP -total), sugarcane stalk yield (Yield), the abundance of nitrifying bacteria (AOB), nitrifying Archaea (AOA), and nitrogen-fixing bacteria (nifH), and the total abundance of bacteria (Bacteria 16S) and archaea (Archaea 16S). Black arrows indicate the correlation between biological and environmental parameters by different N-fertilizer application methods. The significance of these correlations was evaluated via the Bray–Curtis distance permutation test

the N-fertilizer recovery by the plant leading to a high yield and low N_2O emission.

4 Discussion

To achieve an environmental-friendly sugarcane production system, advancements have been made in N-fertilization management in the main producing countries, for example, Brazil (Otto et al. 2016), India (Ghosh et al. 2018), Australia (Bell 2014; Kroon et al. 2016), and Mexico (Bautista et al. 2019). However, the comparison of the application of granular N-fertilizer incorporated into the soil or onto the sugarcane straw layer exploring effects on soil, plant, and losses remains unexplored.

The understanding of the N-fertilizer management is crucial in Brazil since the absence of N-fertilization reduces the sugarcane yield (Sanches and Otto 2022). Furthermore, the tropical conditions, characterized by high temperatures, radiation, and precipitation during the spring and summer, potentially lead to high N-losses (Bowles et al. 2018a; Otto et al. 2016). These risks are associated with the length of the sugarcane growing season, 12 to 18 months in Brazil (de Castro et al. 2022), and the application of N-fertilization in several sugarcane stages (Franco et al. 2011).

To improve fertilization efficiency, a guide for best fertilizer management practices was developed by the

International Plant Nutrition Institute (IPNI). This highlights the importance of adjusting the fertilization method to achieve the goals of sustainable agriculture. Adjusting the N-fertilizer application method has been promoting great outcomes, such as a yield increase (de Castro et al. 2017), a reduction in N_2O emissions (Borges et al. 2019), and an enhancement in the N-fertilizer use efficiency (Boschiero et al. 2019; de Castro et al. 2022). However, further progress in refining N-fertilizer application methods is essential due to a lack of comprehensive studies providing information on both agronomic and environmental benefits. So, our work comes to fill this gap, highlighting that by applying N-fertilizer incorporated into the soil, great crop yield and lower nitrogen losses will be obtained.

Sugarcane commonly presents low NRP in soil-based N-fertilization (<40%) with potential high nitrogen losses (Bowles et al. 2018a; Franco et al. 2015; Thorburn et al. 2017; Trivelin et al. 2013). Recent studies highlighted the benefits of the application of N-fertilizer incorporated into the soil (Borges et al. 2019; de Castro et al. 2017, 2022), supporting findings outlined in our study (average yield increase of 17% compared to surface application). However, none of these studies quantified N-fertilizer recovery in the soil–plant system, leaving uncertainties regarding potential increases in this parameter due to the incorporation of N-fertilizer into the soil. Our investigation addressed this gap, conclusively demonstrating that the previously observed benefits are indeed linked to an increase in NRP (an enhancement of 23.5% in incorporated application compared to surface application).

The benefits observed stem from the placement of the N-fertilizer close to the sugarcane root system. This can influence the activity of microorganisms surrounding the fertilizer granule and potentially increase the availability of N-fertilizer for plants (Craig et al. 2021; Mariano et al. 2016). Soil microorganisms also benefit from the nitrogen from the fertilizer, crucial for balancing the C:N ratio to maintain their functionalities (Hodge et al. 2000; Kuzyakov and Xu 2013). For instance, they aid in the mineralization of sugarcane plant residues deposited onto the soil, characterized by a high C:N ratio (Fortes et al. 2013). Placing the N-fertilizer at a depth of 0.08 m bypasses the soil layer with the highest microbial activity, where N-fertilizer immobilization tends to be greater (Fracetto et al. 2017).

Currently, the primary concern on nitrogen loss relies on the escalation of greenhouse gas (GHG) emissions, prominently emphasizing N_2O emissions (Masson-Delmotte et al. 2018; Peters et al. 2013). In the sugarcane crop system, maintaining a straw layer on the soil surface alongside N-fertilization has the potential to elevate losses through GHG emissions (Carmo et al. 2013; Degaspari et al. 2020; Gonzaga et al. 2018; Sanches and Otto 2022). Conversely, the application of N-fertilizer incorporated into the soil has

minimized N₂O emissions compared to applying N-fertilizer onto the sugarcane straw layer (Borges et al. 2019). The majority of N₂O emissions during the sugarcane crop cycle occur within a short period following fertilization (Fracetto et al. 2017; Gonzaga et al. 2018), which was also observed in our study, that is, great emissions between the 7th and 25th days after N-fertilization. Moreover, applying the N-fertilizer into the soil reduced the N₂O emission factor throughout the crop cycle, contributing substantially to mitigate emissions linked to N-fertilization (Borges et al. 2019; Bowles et al. 2018b, a).

The N₂O emissions from agricultural soils rely on microorganism communities, mainly bacterial communities, which can increase the emission due to N-fertilization (Fracetto et al. 2017; Lourenço et al. 2018; Prosser and Martiny 2020; Soares et al. 2016; Vasconcelos et al. 2022). The incorporation of N-fertilizer into the soil can create localized regions with elevated nitrogen concentrations, leading to shifts in soil pH, influencing microbial activity and selecting microbial populations (Scarlett et al. 2021; Wang et al. 2019; Zhong et al. 2023). The interaction between N-fertilization, ammonia-oxidizing bacteria (AOB), and increased N₂O emissions has been observed (Fracetto et al. 2017; Vasconcelos et al. 2022). This interaction might be influenced by potential changes in soil pH and the NH₄⁺/NH₃⁻ ratio resulting from N-fertilizer application, which could favor the AOB community (Prosser and Martiny 2020). Moreover, soil characteristics such as pH, texture, organic matter content, and moisture levels are widely recognized to impact N₂O emissions (Oliveira et al. 2022; Otto et al. 2022). These factors play a significant role in regulating soil biological activity, which in turn affects the transformation of nitrogen in the soil, ultimately influencing the emission of nitrogen gases (Fracetto et al. 2017; Soares et al. 2016).

It is also noteworthy that the application of N-fertilizer incorporated into the soil emerges as a viable strategy to mitigate N loss by water runoff and water leaching. Rainfall intensity influences nutrient losses through water runoff (Khan et al. 2016; Skocaj et al. 2013). In our study, despite applying N-fertilization during the rainy season, isotopic abundance in surface water runoff did not exhibit significant differences between treatments. This could be attributed to the characteristics of the experimental field's soil, classified as Rhodic Eutrudox, featuring substantial A and B horizons and efficient water infiltration throughout the profile (Barbosa et al. 2018; Castioni et al. 2019; Prado et al. 2013). Additionally, the volume and distribution of rainfall during the experimental period (1,548 mm) played a crucial role; the proportion of water drained via surface runoff throughout the study accounted for less than 1% of the total rainfall.

N-leaching is also influenced by rainfall intensity as well as soil texture, and N-fertilization management (Fontes and Alleoni 2006; Ghiberto et al. 2009, 2015;

Scarpore et al. 2023). In soils with limited cation and anion retention, N-fertilization during high moisture periods can amplify N movement through the soil profile, increasing N-leaching (Bowles et al. 2018a). Ghiberto et al. (2015) showed a loss of 22 kg N ha⁻¹ by nitrate leaching. On the other hand, a recent study indicated that nitrogen leaching and runoff in sugarcane fields are lower where straw is kept onto the soil surface, respectively, 1.9 and 10 kg N ha⁻¹ (Scarpore et al. 2023). Similarly, experiments across Brazilian sites under N-fertilization reported low levels of N-fertilizer enriched in ¹⁵N atoms in leached water (Cantarella and Montezano 2010). Our study aligns with these findings, indicating an average N-leaching loss (¹⁵NRS at 0.6–0.9 m depth) of only 8%, regardless of the N-fertilizer application method.

5 Conclusion

The choice of the N-fertilizer application method holds significant relevance to achieving the goals of sustainable and environmental-friendly agriculture, promoting agronomic and environmental benefits. Agronomically, applying N-fertilizer incorporated into the soil (at 0.08 m depth) on both sides of the sugarcane row enhanced sugarcane stalk yield and the N-fertilizer recovery by the plant compared to the traditional surface method (application of N-fertilizer over the sugarcane straw layer). Environmentally, the application of N-fertilizer incorporated into the soil reduced N₂O emissions and minimized the N-fertilizer recovery in the soil that might otherwise be lost through N-leaching or runoff. So, the incorporation of N-fertilizer into the soil emerges as a practical and field-adaptable alternative. Opting for this method not only promotes an environmentally friendly N-fertilization system but also contributes to mitigating global warming by reducing greenhouse gas emissions.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s42729-024-01744-7>.

Acknowledgements The authors thank the São Paulo Research Foundation (FAPESP) for the financial support provided for this project (grant #2017/13619-0) and for granted PhD scholarship to SAQC (grant #2017/25489-3). We are also very thankful to Center of Isotopes of the São Paulo State University (CIE/UNESP) for the support granted to our research in isotopic measurement and analysis. Our special thanks to the Brazilian Biorenewables National Laboratory (LNBR/CNPEM) for the structural and operational support for this project.

Authors' Contribution Writing-original draft: SGQC, SAQC, JLNC and RAC. Data curation: SGQC, VEC, and CDB. Writing, revision and methodology: VEC, SAQC, JLNC, and CDB. Formal analysis: SGQC, OTK, and RAC. Project administration: SGQC and HCJF. Resources: SGQC, VEC and HCJF. All authors contributed to the article and approved the final manuscript version.

Funding Open access funding provided by Copenhagen University. This research was funded by São Paulo Research Foundation (FAPESP), grant #2017/13619–0 to SGQC. SAQC received Ph.D. scholarship from FAPESP, grant #2017/25489–3.

Data Availability The data presented in this study are available on request from the corresponding author.

Code Availability Not applicable.

Declarations

Ethics approval Not applicable.

Conflicts of Interest The authors declared that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

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