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Impact of urease inhibitor on greenhouse gas emissions and rice yield in a rainfed transplanting rice system in Costa Rica

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Rice crop production intensification has become one of the most important sources of greenhouse gases. In rainfed rice production, urea is the most common nitrogen (N) fertilizer used in Costa Rica. Urea has low efficiency in crops, which is associated with high risk of N gaseous losses. The use of urea coated with the urease inhibitor NBPT has been identified as a mitigation strategy for ammonia losses. However, this can increase N input to the system, potentially leading to higher N_2O and CH_4 emissions in rice fields. In 2022, a rainfed rice transplanting trial was conducted on a tropical Inceptisol in Costa Rican Central Pacific region to analyze yield and quantify N_2O and CH_4 emissions. The plots of 6m x 6m, with an experimental design of five complete randomized blocks, were treated with three N-fertilization treatments: urea (U; 144 kg N ha^{-1}), urea plus NBPT (UI; 144 kg N ha^{-1}) and control plots (without N). Total N was splitted in four applications. The yield did not exhibit a significant difference ($p>0.05$) between U and UI treatments (U: 5.72 ± 0.97 t ha^{-1}, and UI: 5.86 ± 1.12 t ha^{-1}). There were no significant differences in yield-scaled N_2O emissions (U: 4.4 ± 1.9 ug N_2O-N $kg^{-1}{}_{rice}$, UI: 4.2 ± 1.9 ug N_2O-N $kg^{-1}{}_{rice}$) or yield-scaled CH_4 emissions (U: 0.32 ± 0.20 mg CH_4 $kg^{-1}{}_{rice}$, UI: 0.33 16 ± 0.18 mg CH_4 $kg^{-1}{}_{rice}$). Environmental factors and soil conditions such as temperature, pH, clay content, and specific cation exchange capacity could reduce the efficacy of NBPT. Under the experimental conditions, NBPT did not promote economic benefits, nor did it have an impact on greenhouse gas emissions.

KEYWORDS

greenhouse gases, NBTP, rice yield, tropical soils, urea

1 Introduction

The projected 34% increase in global population by 2050 presents a significant challenge for food security, driving the intensification of rice production (Tesfaye et al., 2021). In Costa Rica, rice cultivation covered 24 258 ha across five geographical regions during the 2022 to 2023 period, with rainfed production accounting for 57% of the total planting area. In Parrita, located in the Central Pacific region, where this study was conducted, the average annual yield from 2022 to 2023 was 3.4 t ha^{-1}, with a recommended nitrogen (N) application rate of 100-140 kg ha^{-1} for rainfed conditions (Badilla et al., 2020; CONARROZ, 2023).

In the agricultural sector, rice cultivation is estimated to contribute 30% of global methane (CH_4) emissions and 11% of nitrous oxide (N_2O) emissions (Gupta et al., 2021; Mboyerwa et al., 2022). Emissions of CH_4 and N_2O in rice fields exhibit great variability due to factors such as the cropping system, soil organic matter content, N-fertilization management, crop phenological stage, topography, geomorphology, and the existing greenhouse gases (GHGs) levels in both soil and atmosphere (Liu and Greaver, 2009; Herrera et al., 2013; Turbiello et al., 2015). Nitrogen fertilization is of particular concern, as N plays a key role in both the production and consumption of GHGs by microorganisms, which can alter the fluxes of the three main biogenic gases (CH_4, CO_2 and N_2O) (Liu and Greaver, 2009; Beek et al., 2010).

In rice crops, N demand is mainly supplied with urea due to its lower cost compared to other fertilizers. However, the N-use efficiency (NUE) of urea is low (30-35%), as a significant portion of the applied N lost by leaching, denitrification, nitrification and primarily through volatilization of N as ammonia (NH_3) (Ferdous et al., 2023). This highlights the need of alternative management strategies such as the use of slow-release fertilizers, like urea coated with the N-(n-butyl) thiophosphoric triamide (NBPT) (Beek et al., 2010; Jiafeng and Qiuliang, 2024).

NBPT temporarily inhibits soil ureases, delaying the hydrolysis of urea into ammonium (NH_4^+) and carbon dioxide (CO_2). This process reduces NH_4^+ losses due to NH_3 volatilization and improves the synchronization between N availability and crop demand (Abalos et al., 2014; Liu et al., 2018; Li et al., 2017; Cantarella et al., 2018). The resulting increase in NH_4^+ affects GHGs emissions in complex ways, particularly in flooded rice systems using ammonium-based nitrogen fertilizers. Elevated NH_4^+ levels can reduce CH_4 oxidation by competing for the methane monooxygenase enzyme used by methanotrophs, resulting in higher CH_4^+ emissions. Additionally, high NH_4^+ concentrations enhance nitrification, leading to the production of nitrite (NO_2^-) and nitrate (NO_3^-), which can increase N_2O emissions through denitrification under anaerobic conditions (Liu and Greaver, 2009; Hussain et al., 2015).

In Asia, where 90% of global rice production occurs, continuous flooding often results in high CH_4 emissions due to anaerobic conditions. To mitigate these emissions, the alternate wetting and drying (AWD) system has been adopted; however, this method can increase N_2O emissions and lower yields compared to continuously flooded fields (Dahlgreen and Parr, 2024; Yadav et al., 2024). In contrast, Costa Rica experiences episodic emissions caused by fluctuating aerobic and anaerobic conditions of rainfed farming and its diverse climate and soil types. Heavy rainfall followed by clear skies can intensify N_2O peaks by enhancing nitrification and denitrification in soils rich in NH_4^+ from fertilizers (Pérez-Castillo et al., 2021). Additionally, saturated soils with low O_2 can reduce NO_3^- to N_2, resulting in lower N_2O emissions or even negative N_2O emissions. Lower CH_4 emissions are expected in Costa Rican rainfed farming system compared to flooded or AWD systems (Minamikawa et al., 2015; IPCC, 2019a; Veçozzi et al., 2024).

In the tropics and subtropics, research on the impact of NBPT on GHG emissions in rice crops, particularly in rainfed systems, is limited, highlighting the need for site-specific studies in Costa Rica. Most research has focused on CH_4 emissions in flooded systems and soil water management (Herrera et al., 2013; Tang et al., 2016; Veçozzi et al., 2024; Yadav et al., 2024), with limited research on NBPT's effect on NH_3 losses in Costa Rican rice (Pérez-Castillo et al., 2024). Simultaneous measurement of CH_4 and N_2O emissions is crucial for assessing the global warming potential of these systems (Malla et al., 2005). This study aims to evaluate the impact of NBPT on CH_4 and N_2O emissions and rice yield in a rainfed system in Parrita, Costa Rica, to develop GHG mitigation strategies.

2 Materials and methods

2.1 Site description

The research was conducted at La Bandera Experimental Farm of the National Rice Corporation in Parrita, Costa Rica (coordinates 9°30'55.02" N, 84°22'2.56" W) from May 1st to September 9th, 2022. The soil is classified as an Inceptisol with an ustic moisture regime, low organic matter content, and medium to high fertility, as previously described by Pérez-Castillo et al. (2024). Additional physical and chemical soil characteristics are provided in Table 1.

In the period 2013-2021, the average monthly temperature ranged between 26 to 29°C. The average accumulated precipitation per year ranged from 2000 to 3000 mm, with the highest rainfall occurring in October and a marked reduction during the dry season, mainly in February. The average daily temperature and daily precipitation during the growing cycle are shown in Figure 1D. The recorded average temperature and the total accumulated precipitation were 25.7 ± 2.0°C and 509.2 mm, respectively. The rainfall distribution was influenced by the La Niña phase of the El Niño-Southern Oscillation (ENSO), resulting in an increase of approximately 75% in the monthly rainfall compared to the historical average for the Parrita region since 2015.

Volumetric soil moisture was determined using a moisture sensor (model MP406) connected to an MPM-160 meter for immediate readings. Two measurements were taken for each point during gas flow sampling from August 9th to September 8th, 2022. The Water Filled Pore Space (WFPS) in the soil was calculated using the volumetric soil moisture content and soil porosity. The average WFPS was around 77% for the three fertilization treatments (77.3% CK, 77.5% U, and 77.7% UI). Data prior to the mentioned period are unavailable due to a malfunction of the 10 cm soil moisture sensors deployed at the experimental field at the beginning of the crop cycle (Giraldo-Sanclemente, 2024).

TABLE 1 Physical and chemical properties of the surface layer of soil (0-20 cm deep) in La Bandera farm, Parrita, 2022.

Characteristic	Value
Bulk density (g cm^{-3})	1.14 ± 0.08
C content (g kg^{-1})	18
N content (g kg^{-1})	2,2
C/N ratio	8,2
pH	6.15 ± 0.00
Exchangeable acidity (mg kg^{-1})	8.7
Effective cation exchange capacity (g kg^{-1soil})	4.25
Ca (mg kg^{-1})	18.2 ± 1.7
Mg (mg kg^{-1})	8.62 ± 0.82
K (mg kg^{-1})	0.33 ± 0.01
P (mg kg^{-1})	13.50 ± 0.71
B (mg L^{-1})	0.47 ± 0.35
Zn (mg kg^{-1})	0.95 ± 0.21
Cu (mg kg^{-1})	7.00 ± 0.00
Fe (mg kg^{-1})	47.0 ± 1.4
Mn (mg kg^{-1})	20.0 ± 2.8
Acid saturation (%)	0.04 ± 0.00
Electrical conductivity (mS cm^{-1})	0.15 ± 0.07

Values are derived from a composite sample taken from 30 sampling points per hectare (Giraldo-Sanclemente, 2024).

2.2 Field trial

Nursery trays were prepared with 180 g of CONARROZ-3 variety seeds and a substrate composed of a mixture of strained soil and rice husk ash in a 2:1 ratio, respectively. The trays were placed in the open air, protected by a saran shade cloth at a height of 1 m. The seedlings were watered daily and transplanted 17 days after germination. A multimineral foliar biostimulant was applied at a concentration of 10 ml L^{-1}, 11 days after germination.

Two days before transplanting, the experimental area was flooded to create a water layer of 2-3 cm. Three rotavator passes were made to disaggregate the surface soil clods and the soil particles were left to settle for one day. Transplanting was carried out using a manual transplanting machine calibrated to place the plants 15 cm apart, with rows separated 30 cm, for a planting density of 90 kg seed ha^{-1}. The crop cycle was conducted in the rainy season, from May 17 to August 31, 2022 (Giraldo-Sanclemente, 2024).

2.3 Experimental design and applied treatments

Fifteen 6 m x 6 m plots, planted with the CONARROZ-3 rice variety, with 2.5 meters of separation between them, were established following a completely randomized block design with five blocks, three N-fertilization treatments and five repetitions per treatment.

The treatments were: Control (CK), where N was not applied; Urea (U), where commercial urea was applied for each N-fertilization stage, Urea with urease inhibitor (UI), where N was applied as urea impregnated with the urease inhibitor NBPT (Nitro-Xtend). The total N rate for U and UI treatments (144 kg ha^{-1}) was divided as follows: transplant (23.5 kg ha^{-1}), beginning of tillering (62.1 kg ha^{-1}), active tillering (29.3 kg ha^{-1}), and pre-flowering (29.3 kg ha^{-1}) (Giraldo-Sanclemente, 2024).

2.4 Agronomic management

Every plot received 60 kg P ha^{-1}, applied at sowing as granular calcium hydrogenphosphate, and 80 kg K ha^{-1}, applied as potassium chloride, which was split into three applications: sowing (30%), active tillering (35%) and differentiation of the floral primordium (35%).Prior to sowing, chemical depletion of weeds was carried out using herbicides with different modes of action to prevent resistance in *Echinochloa colona*, the primary weed identified in the experimental area. At the time of transplanting, weed management included the use of a post-emergence systemic herbicide (a.i. Halosulfuron Methyl) to control *Cyperaceae* sp., along with a multimineral foliar biostimulant. This chemical control was complemented with a pre-emergence and post-emergence selective systemic herbicide (a.i. Clomazone), targeting broadleaf weeds and grasses. The weed sectors not chemically controlled were managed by manual weeding. Control of insect pests and diseases was conducted through regular monitoring. The presence of *Pyricularia oryzae* prompted the application of a fungicide (a.i. Isoprothiolane) (Giraldo-Sanclemente, 2024).

All these practices, including fertilization, weed control, and pest management, were applied uniformly across both experimental and CK plots.

2.5 Yield and N use efficiency estimation

Rice yield was estimated from five 8 m^2 subplots for each treatment. The fresh weight of the grain was measured. Two composite samples of 4 kg per treatment were dried at room temperature for 72 h. Then, grain moisture was determined using the Dickey-John GAC 2500 UGMA International Grain Moisture Analyzer. Finally, the grain yield was normalized to 13% moisture (the standard for commercial sale in Costa Rica).

Nitrogen Use Efficiency (NUE) was calculated using the N difference method, which consists of the difference in N content between the grain from U or UI treatment and the CK treatment, divided by the total N applied per area (IAEA, 2001). Total N content of the grain was measured by dry combustion using an N autoanalyzer and the Dumas method (Horneck and Miller, 1998).

2.6 Emissions of CO_2, CH_4 and N_2O

From May 17th to September 8th, 2022, CO_2, CH_4 and N_2O emissions were monitored using the non-steady-state chambers

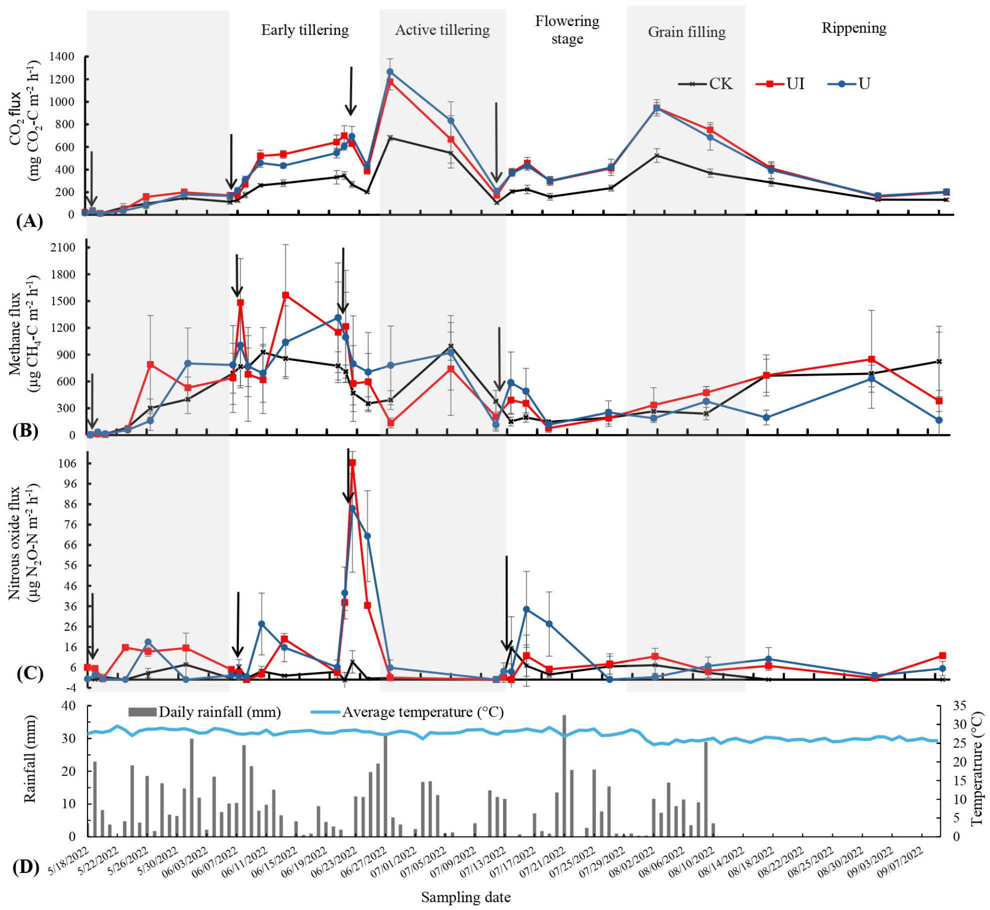

FIGURE 1
(A) CO_2, **(B)** CH_4, and **(C)** N_2O daily fluxes under three fertilization treatments control (CK), urea (U) and urea with urease inhibitor (UI), during a crop cycle of CONARROZ-3 rice variety, conducted from May 18, 2022, to September 8, 2022. The rice was planted by transplant in La Bandera, Parrita, Costa Rica. **(D)** Daily rainfall and average temperature were recorded by an automatic station operated on the farm. Vertical bars represent the standard error (n=5). Arrows indicate N-fertilization events.

methodology. Semi-static chambers were set on frames inserted 15 cm below the soil surface at the time of crop transplant. Two bases were placed per experimental unit to alternate the chamber position during the different sampling days. The chambers, with an area of 0.16 m^2, and adjustable height according to the phenological stage of the crop, were covered with an insulating material. A small fan was used to homogenize the gas inside the chamber throughout the measurement period. A bag was placed in each chamber to balance the internal-external pressure.

The experiment included a total of 27 sampling days. Samples were collected on days 0, 1, 2, 4, 7, and 14 after each N fertilization application. After the last fertilization cycle, samples were taken every seven days until one week after harvest, including the day before harvest. Samples were collected at 0, 20, and 40 min after closing the chamber and were injected into vacuum vials that were covered with Teflon. CO_2, CH_4, and N_2O concentrations were determined using a gas chromatograph equipped with a methanizer, a hydrogen ionizing flame detector (FID), and an electron capture detector (ECD) (Agilent 7890A, USA) coupled to an auto sampler (Agilent 7697A, USA). The equipment was calibrated for each analyte with a four-level calibration curve at the beginning, after every 24 h of continuous injection, and at the end of the injection sequence. The concentrations of the standard gases, with air as the balance gas, had an uncertainty of 5%. For equipment verification, a standard mix of CO_2, CH_4, and N_2O was analyzed after every group of 14 sample vials. Deviations from the standard certified concentration value were within the 20% limit. Ambient concentration variations were monitored with two air samples per sampling day. The integrity of the samples was ensured using four vials filled with two standard mixtures (each prepared in duplicate) and a blank with of ultrapure nitrogen. The hourly concentration change for each GHG ($\Delta Cn_i/\Delta t$) was determined using a linear regression. Fluxes of each GHG (f_i) were calculated using the Equation 1

$$f_i = \frac{\Delta Cn_i}{\Delta t} * \frac{P * MM_i}{8.314 * (273.15 + T)} * h \tag{1}$$

where atmospheric pressure (P in Pa) and temperature in the headspace (T in °C) were recorded for each round measurement with a Kestrel 4000 weather meter (Loftopia LLC., MI, USA). The molar mass (MM_i) is 12 µg C μmol^{-1} for CO_2 and CH_4 or 28 µg N μmol^{-1} for N_2O, and the ideal gas constant is expressed as 8.314 J K^{-1}

mol^{-1} (Dawar et al., 2021). The height (h in m) was adjusted according to crop development and by adding the space between the frame level and the soil surface.

Data analysis for GHG was conducted under the following criteria: CO_2, CH_4 and N_2O fluxes were rejected if the coefficient of determination (r^2) of the linear regression for the change in CO_2 concentration was < 0.95, as this indicates a leak in the system. The threshold for accepting CH_4 and N_2O fluxes was set at a $r^2 = 0.83$ and $r^2 = 0.85$, respectively. If N_2O concentration during the chamber closing period remained within the mean air concentration range ± 2μ, the fluxes were set at zero when the r^2 was below the threshold. Additionally, if $r^2 < 0.85$ and the fluxes were below the quantification limit (N_2O: 0.00049 μg m^{-2} h^{-1}), they were replaced with the detection limit (N_2O: 0.0032 μg m^{-2} h^{-1}). To ensure data quality, 18% of N_2O and 20% of CH_4 fluxes were discarded.

2.7 Calculation of the emission factor and yield-normalized emissions

Cumulative emissions of CH_4 and N_2O were calculated using the trapezoidal method (Pérez et al., 2021) and the median flux for each specific treatment and sampling day, when a flux value was rejected based on the criteria specified in the previous section.

The CH_4 emission factor (EF_{CH4} in kg CH_4 ha^{-1} d^{-1}) for the rainfed rice crop sown by transplanting was calculated by dividing the cumulative emissions of CH_4 by the chamber area and the monitoring period in days. This emission factor was then compared with the value obtained using Equation 2 (IPCC, 2019a; Vo et al., 2020):

$$EF_{CH4} = EF_C \ * \ SF_W \ * \ SF_P \ * \ SF_{OR} \tag{2}$$

where the EF_C (1.19 kg CH_4 ha^{-1} d^{-1}) denotes the crop emission baseline according to crop conditions, the SF_W is a scaling factor that accounts for the water regime preceding crop establishment, the SF_P represents the moisture regime during the crop cycle, and the SF_{OR} accounts for the application of organic amendments. For the SF_W parameter, a scaling factor of 0.16 was applied (IPCC, 2019a) for rice crops subject to flooding from rainfall but also prone to drought. For SF_P *parameter*, a scaling factor of 0.89 was used (IPCC, 2019a) for rice crops with at least 180 dry days prior to crop establishment, and the SF_{OR} parameter was excluded since no organic amendments were applied.

The emission factor of N_2O (EF_N, g N_2O-N kg of N applied^{-1} ha^{-1}) was calculated using Equation 3 (IPCC, 2019b):

$$EF_N = \frac{N_2O - N_{Ti} - N_2O - N_{CKi}}{N_i} \tag{3}$$

where $N_2O\text{-}N_{Ti}$ and $N_2O\text{-}N_{CKi}$ represent the cumulative emissions of N_2O-N from the evaluated N-treatment and the control treatment, respectively, and N_i is the total amount of N applied in the evaluated treatment.

Yield-scaled CH_4 and N_2O emissions were calculated by dividing the cumulative flux of each gas by the clean rice grain produced per hectare (in kg) adjusted to a 13% moisture content (Geng et al., 2021). Emissions expressed as CO_2e were calculated by multiplying the cumulative flux per hectare by a factor of 27 for CH_4 and a factor of 273 for N_2O based on their warming potential over a time horizon of 100 years (Forster et al., 2021).

2.8 Statistical analysis

Normality and homogeneity of variances were assessed with the Shapiro-Wilk and Levene's test, respectively. Outlier data were identified using Cook's distance method, and Dixon's Q test was applied to discard atypical values. One-way ANOVA, with block as an experimental error factor, was used to analyse the data. Mean comparisons between treatments results were evaluated using Tukey's honest significant difference. The cumulative fluxes of CH_4, N_2O and CO_2 were tested for Pearson correlation between greenhouse gases and temperature, relative humidity and precipitation. Treatment differences were considered significant with p-values below 0.05. Statistical analysis was conducted using RStudio software, version 4.2.3.

3 Results

3.1 Rice yield and nitrogen use efficiency

The grain yield did not show a significant difference between the UI and U treatments, with values of 5.72 ± 0.97 and 5.86 ± 1.12 t ha^{-1} for UI and U, respectively, both approximately 35% higher than the control, as expected. Similarly, grain NUE did not exhibit a significant difference between the UI (19.32 ± 3.99) and U (19.31 ± 6.77) treatments.

3.2 Daily fluxes of CO_2, CH_4 and N_2O

CO_2 emissions from the U and UI treatments were higher than those from CK throughout the crop cycle (Figure 1A). Emissions varied according to crop vegetative development. At the early tillering (June 6-20), CO_2 emissions increased, peaking during the active tillering stage (June 23-July 5). Subsequently, CO_2 fluxes decreased during floral primordium differentiation (July 9–17) but rose again during grain filling, reaching a second peak in August. Afterward, emissions declined steadily until the harvest on August 30.

There was a high variability in daily CH_4 emissions. The CH_4 fluxes from CK, U and UI did not show significant differences across the four fertilization events (Figure 1B; Supplementary Table 1). In the U and UI treatments, N_2O emissions increased during the first five days after each N-fertilization, peaking on June 22 (two days after the third N-fertilization) when fluxes increased around 90% compared to the CK (Supplementary Table 2). After these initial five-day periods, fluxes declined and fluctuated similarly to the CK treatment (Figure 1C).

3.3 Cumulative emissions

The fertilized treatments increased cumulative CO_2 emissions by 41% compared to the CK treatment (Figure 2A). The cumulative emissions of N_2O, although not significantly different between the U and UI treatments, were 76% higher than those of the CK treatment (Figure 2C). On the other hand, no significant differences were found in the cumulative CH_4 emissions across the U, UI, and CK treatments (Figure 2B).

Based on cumulative emissions, the randomized complete block design shown in Figures 2D–F was identified as the most appropriate spatial pattern for tracking GHG fluxes. In the experimental area, blocks 1 and 4 showed the highest cumulative CH_4, while blocks 1 and 3 exhibited the highest N_2O emissions.

Cumulative CO_2 emissions showed a low to moderate positive Pearson correlation with cumulative CH_4 emissions for CK, U and UI treatments (0.47, 0.37 and 0.31, respectively at $p < 0.001$) and a low correlation with cumulative N_2O for U and UI treatments (0.28 and 0.24, respectively at $p < 0.05$). Conversely, no significant correlation was found between CH_4 and N_2O emissions or GHG with the temperature, relative humidity and total daily rainfall.

3.4 Yield-scaled CH_4 and N_2O emissions and their emission factors

The yield-scaled CH_4 emissions were not significantly different between treatments, but the yield-scaled N_2O emissions were significantly higher ($p < 0.05$) in fertilized plots compared to CK (Table 2). The emission factors for CH_4 and N_2O were not significantly different between treatments (Table 2). Finally, the cumulative emissions expressed as CO_2e were not significantly different for CH_4 between treatments, but N_2O were significantly higher ($p < 0.05$) in fertilized plots than in CK (Table 2).

4 Discussion

4.1 Rice yield and nitrogen use efficiency

The yields in the U and UI treatments were higher (approximately 2 t ha^{-1}) than the average production reported for the 2022-2023 period

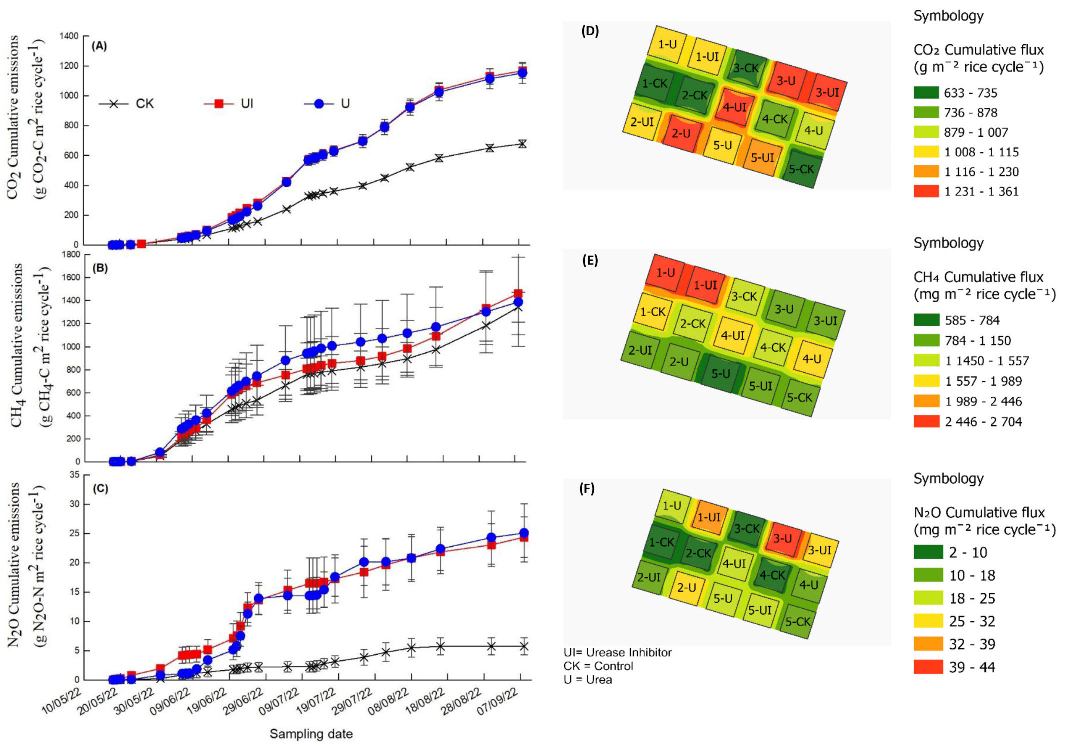

FIGURE 2
(A) CO_2, **(B)** CH_4, and **(C)** N_2O average cumulative emissions from the three fertilization treatments control (CK), urea (U) and urea with urease inhibitor (UI), during a crop cycle of rice variety CONARROZ-3 planted by transplant. Vertical bars represent the standard error (n=5). Series identified with different letters are different according to the Tukey test ($p < 0.05$). **(D)** CO_2, **(E)** CH_4, and **(F)** N_2O Spatial variability cumulative emissions on the field trial scale. Created by ArcGIS PRO 3. Study period from May 18th to September 8th, 2022, in La Bandera, Parrita, Costa Rica.

TABLE 2 Yield scaled emissions, emission factors and cumulative emissions expressed as CO_2e for CH_4 and N_2O from the treatments: control (CK), urea (U) and urea with urease inhibitor (UI), during a crop cycle of the rice variety CONARROZ-3 planted by transplant in La Bandera, Parrita Costa Rica, 2022.

Variable	Gas	Treatment		
		CK	UI	U
Yield scaled emissions	CH_4 (mg CH_4 kg^{-1} $_{ryce}$)	0.48 ± 0.10	0.33± 0.18	0.32 ± 0.20
	N_2O (µg N_2O-N kg $^{-1}$ $_{ryce}$)	1.5 ± 0.9**b***	4.2 ± 1.3**a**	4.4 ± 1.9**a**
Emission factor	CH_4 (kg CH_4 ha^{-1} d^{-1})	0.16 ± 0.03	0.17 ± 0.09	0.16 ± 0.10
	N_2O (g N_2O-N kg N $_{applied}$ $^{-1}$ ^{ha} $^{-1}$)		1.29 ± 0.53	1.34 ± 0.77
Global warning potential (CO_2e)	CH_4 (g CO_2e m^{-2})	36 ± 7.8	39 ± 22	37 ± 23
	N_2O (g CO_2e m^{-2})	1.6 ± 0.9**b**	6.7 ± 2.1**a**	6.8 ± 3.0**a**
	Total (g CO_2e m^{-2})	38 ± 7	46 ± 24	44 ± 23

*Series marked with different letters are significantly different according to the Tukey test ($p < 0.05$).

in the Parrita region (3.4 t ha^{-1}) (CONARROZ, 2023). This increase is likely attributable to the transplant system used, as no significant differences were observed between U and UI treatments. This result highlights the need to study other transplant conditions, such as plant densities to optimize yield scaled GHG emissions (Oo et al., 2018) and suggests that the adoption of NBPT may be limited under the experimental conditions.

The lack of effectiveness of NBPT may have been influenced by factors such as soil pH, texture, cation exchange capacity (CEC), and temperature (Abalos et al., 2014). The experimental site, characterized by clay loam texture, a pH of 6.15, and an average temperature of 28°C, may not have provided favorable conditions for NBPT. As it has been documented, high temperatures favor the NBPT degradation and instability (Cantarella et al., 2018; Martins et al., 2017; Silva et al., 2017) and fine-textured soil reduce the potential for N losses as NH_3 due to retention of the ammonium ion, which increases with higher CEC depending on the nature of the clay (Soares and Cantarella, 2022; Götze et al., 2023). Finally, in contrast to basic soil pH values (greater than 7), the slightly acidic soil at the experimental site favors the equilibrium shift toward the NH_4^+ form, reducing NBPT's effectiveness in preventing nitrogen losses as NH_3 (Linquist et al., 2013; Matczuk and Siczek, 2021; Ahmed and Akinremi, 2022; Soares and Cantarella, 2022). Additionally, studies have reported that the degradation rate of NBPT decreases by a factor of two to four when transitioning from slightly acidic to alkaline soils. Therefore, NBPT management may be more effective in alkaline soils than in acidic soils, as its greater stability enhances incorporation of urea into the soil under variable precipitation patterns (Engel et al., 2015; Lasisi and Akinremi, 2022).

The limited effectiveness of NBPT in improving yield or NUE compared to urea alone has been previously reported in rice fields (Humphreys et al., 2018; Veçozzi et al., 2024). A meta-analysis by Hassan et al. (2024) suggested that urea-coated fertilizers are more effective in monsoonal climates. Positive results with NBPT have also been observed in rice systems on alkaline soils (Linquist et al., 2013; Matczuk and Siczek, 2021). Monsoonal climates, which are common in Asia, where 90% of the wold's rice is produced, strongly influence soil alkalinity and accelerate nitrogen and carbon cycles (Jia et al., 2021; Abbasi et al., 2020). While this study did not focus on climate or pH, local weather conditions and soil types likely explain the varying impacts of NBPT, as observed in tropical soils in Costa Rica (Pérez-Castillo et al., 2024) and subtropical soils in Brazil (Veçozzi et al., 2024) compared to Asian rice systems.

4.2 Daily fluxes of CO_2, CH_4 and N_2O

Aside from the anoxic conditions that favor the formation of CH_4 under waterlogged conditions, CH_4 and CO_2 emissions were primarily influenced by the phenological development of the crop (Figures 1A, B). The highest CO_2 emission rates, observed during the early and active tillering, coincided with increased CH_4 fluxes, as plant vegetative growth typically enhances root exudates, thereby boosting CH_4 emissions (Waldo et al., 2019). Furthermore, the development of plant aerenchyma during active tillering facilitated CH_4 release by preventing its oxidation to CO_2 by methanotrophic bacteria in the soil surface and accelerating the escape of 90% of the CH_4 produced during this growth stage (Mer and Roger, 2001; Rahalkar et al., 2021; Sahoo et al., 2021). In the early stages of the crop cycle, low CH_4 emissions observed were primarily due to the escape of CH_4 through bubbles and their vertical movement through the soil profile (Mer and Roger, 2001). In contrast, during the ripening stage, the diminution of the CH_4 emissions were mainly due to the reduced exudation of labile organic carbon by the roots of mature plants into the rhizosphere, which decreases the substrate available for methanogenesis (Figure 1B) (Wang et al., 2017).

The highest emission peaks were observed after the N-fertilizer applications (Figure 1C), consistent with other studies and associated with the nitrification and denitrification of applied nitrogen (Oo et al., 2018). Low daily precipitation in the two preceding days (3 mm and 2 mm, on June 20 and 21, respectively) along with no recorded precipitation on June 22, may explain the highest N_2O emissions observed on June 22 in the N-fertilization treatments. It is likely that the reduction of saturated WFPS in the soil was sufficient to favor the production of N_2O via nitrification due to an increase in the availability of C or nitrate (NO_3^-) during periods of soil drying (Coyne, 2008; Friedl et al., 2018). In contrast, WFPS values around 80% direct the denitrification process towards N_2 formation, since under conditions of permanent saturation, the processes of denitrification and dissimilatory reduction of ammonium compete for NO_3^-, favoring the final reduction of NO_3^- to N_2 (Coyne, 2008; Friedl et al., 2018). It has been found that as the soil dries, there is a greater accumulation of NO_3^- which is subsequently denitrified when the crop waterlogged again (Lagomarsino et al., 2013).

4.3 Cumulative emissions

In rainfed system, the spatial and temporal variability of GHGs emissions, a well-known limitation of the non-steady-state chamber technique (Maier et al., 2022), is exacerbated by high variability in soil moisture. To account for this additional variability in cumulative emissions, the sampling frequency and number of sampling days were increased to assess gas emissions throughout the entire rice cycle. The mosaic of experimental plots (Figures 2D–F), consisting of five blocks, allowed for an effective comparison between the U and UI treatments, highlighting how the microtopographic gradient could influence high emission points throughout the rice cycle (Maier et al., 2022).

As cumulative CO_2 emissions include aerial biomass, the difference between N-fertilized treatments and the control treatment is primarily due to the greater development of rice in the absence of nitrogen restriction, rather than the influence of N on soil respiration and its effects on microbial diversity, community structure, and co-occurrence networks (Sosulski et al., 2020; Wang et al., 2022).

As CH_4 emissions primarily occur through plant-facilitated transport via aerenchyma tissue, the lack of an NBPT effect on CH_4 emissions suggests that differences in aerenchyma tissue did not arise from the use of urea, either with or without NBPT (Humphreys et al., 2018).

N_2O emissions from the UI and U treatment did not differ throughout the complete crop cycle nevertheless, the urease inhibitor NBPT reduces N losses as NH_3, which would result in a greater amount of NH_4^+ available as a substrate for the N_2O formation process through nitrification (Pérez-Castillo et al., 2024). The reduction in N losses does not necessarily translate into a reduction in N_2O emissions. Scientific evidence suggests that nitrification inhibitors reduce N losses from fertilizers, whereas urease inhibitors only alter the N loss pathway, without significantly changing the total amount of N lost (Malla et al., 2005; Meng et al., 2020). Furthermore, the NBPT inhibitor, which delays the hydrolysis of urea by converting NBPT into its oxygen-analog form, may not have been effective under the rainfed waterlogging conditions observed in this experiment. In this case, the lack of expected results in the rainfed system under evaluation may reflect limitation associated with the specific experimental conditions of Costa Rica's rice systems. This hypothesis aligns with previous studies that have reported a reduction in overall N_2O emissions with NBPT under the AWD system, compared to urea alone. In AWD system, the soil is drained nearly twice during the rice crop cycle, promoting adequate soil aeration, which consequently reduces N_2O emissions and enhances the effectiveness of NBPT (Phong et al., 2017).

Moreover, the soil characteristics at the La Bandera experimental site may have influenced the observed N_2O emissions. The high clay content (35-50%) of the soil may have contributed to the reduction in N_2O emissions as previous studies have associated clay content above 20% with lower N_2O emissions. This effect may be due to ammonium fixation in clay particles, microbial uptake, or shifts in the N_2O/N_2 product ratio (Götze et al., 2023). Also, the pH (6.15) at experimental site might have mitigated the effect of NBPT on N_2O emissions, as previous research has identified that NBPT stimulates N_2O emissions in alkaline soils, while it has no significant effect in acidic soils (Coyne, 2008; Fan et al., 2018; Meng et al., 2020).

A positive correlation between CH_4 and N_2O cumulative emissions could be expected, as the NH_4^+ input promotes an increase in the population of nitrifiers relative to methanotrophs, which reduces the CH_4 oxidation rate since methanotrophs oxidize CH_4 more efficiently than nitrifiers (Malla et al., 2005). However, no correlation was observed between CH_4 and N_2O emissions, nor between these emissions and environmental variables. This lack of correlation may be attributed to the influence of rice development and soil saturation conditions during the rainy season, which likely had a greater impact on GHG production. This finding is consistent with results reported in an AWD rice system (Wang et al., 2017).

4.4 Yield-scaled CH_4 and N_2O emissions and their emission factors

Yield-scaled CH_4 and N_2O emissions indicated that NBPT does not reduce CH_4 and N_2O release under split N applications and the prevailing experimental conditions (Table 2). These results align with studies showing that the use of urea with and without NBPT does not differ in yield-scaled GHGs emissions in subtropical rice system under continuous flood irrigation (Veçozzi et al., 2021, 2024). In contrast, a meta-analysis conducted by Yang et al. (2022), based on studies from Asia, primarily China, found that inhibitors (including NBPT) reduced yield-scaled CH_4 emissions by 10.3% under continuous flooding, 29.5% under intermittent flooding, and 10% under unflooded conditions. However, as discussed above, soil and climate conditions in Asia differ from those found in rainfed rice systems in Costa Rica and Brazil.

The CH_4 emission factor for the U and UI treatments (Table 2) is similar to the average predicted by the IPCC default values, adjusted for water regime during the crop season and prior to rice cultivation (0.17 kg CH_4 ha^{-1} d^{-1}) (IPCC, 2019a). The N_2O emission factors obtained in the U and UI treatments, although relatively low (Table 2), fall within the range of the IPCC default values for single and multiple drainage rice systems (5 g N_2O-N kg^{-1} N applied ha^{-1}, with a fluctuation from 0 to 16 g N_2O-N kg^{-1} N applied ha^{-1}) (IPCC, 2019b).

The experimental emission factors obtained apply to a rainfed rice system cycle where soil saturation conditions prevailed due to land preparation for the transplant system, and the influence of the ENSO phenomenon, which generated increased precipitation and a greater number of rainy days throughout the study months.

In accordance with previous studies, the global warming potential of cumulative CH_4 emissions in CO_2e contributes more than six times the global warming potential of cumulative N_2O emissions. Therefore, the most effective way to mitigate total greenhouse gas emissions from rice cultivation is to focus primarily on reducing CH_4 emissions from rice fields (Oo et al., 2018).

4.5 Refining data interpretation

Transplantation remains a promising technique to increase rainfed rice yields in Costa Rica; however, soil conditions, topography, and accessibility to resources by producers should be evaluated beforehand to successfully implement this system.

The use of NBPT as a strategy for N-fertilization practices must be evaluated, considering key factors at the experimental site, such as high temperatures, acidic soil pH, high clay content, and high soil cation exchange capacity. These factors may have affected the effectiveness of NBPT and could create conditions in which it does not improve yield or NUE compared to using urea alone.

Finally, the findings suggest that while NBPT may offer benefits under certain soil and environmental conditions, its effectiveness in reducing GHG emissions and enhancing NUE in rainfed rice systems, such as those in Costa Rica, appears to be limited. Further research is needed to alternative agronomic practices to optimize NBPT potential. New products on the market could enhance NBPT effectiveness under rainfed conditions. These include NBPT combined with duromide, other urease inhibitors such as N-(2-nitrophenyl) phosphoric triamide (2-NPT), or controlled release fertilizers. However, as previously discussed, it is essential to evaluate these alternatives in comparison with NBPT alone or under conditions where the impact of the inhibitor could yield greater benefits, such as alkaline Vertisol soils in Costa Rica where rice is cultivated (Vignola et al., 2018; Soares and Cantarella, 2022).

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

WG-S: Data curation, Formal Analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing, Visualization. AP-C: Data curation, Formal Analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing, Conceptualization, Funding acquisition, Project administration, Resources, Supervision. MM-M: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing. CC-S: Conceptualization, Writing – original draft, Writing – review & editing. LC-P: Conceptualization, Investigation, Resources, Writing – review & editing. MA-M: Investigation, Writing – review & editing. MZ: Conceptualization, Resources, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fagro.2025.1518802/full#supplementary-material

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