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Interaction of *Gluconacetobacter diazotrophicus* and nitrogen fertilization on nitrous oxide emissions in paddy rice

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ABSTRACT

This study aimed to investigate impacts of the combination of *Gluconacetobacter diazotrophicus* with reduced nitrogen inputs on N_2O emissions in paddy rice in the Mekong Delta region, Vietnam. The field experiment was arranged in a split-plot design, with method of inoculating rice seeds with *G. diazotrophicus* as the main-plot factor and N fertilizer rates as the subplot factor. Rice seeds were inoculated with *G. diazotrophicus* (G_d) and non-inoculated with *G. diazotrophicus* (G_o). The rates of N fertilizer included N_0, N_{50}, N_{75}, and N_{100} are received 0, 50, 75, and 100 kg N ha^{-1}, respectively. The results showed that the combination of *G. diazotrophicus* with N fertilizer at rates of 50–75 kg ha^{-1} show a trend toward reducing N_2O emissions by 13.5–19.2%, equivalent to a reduction of 191–259 kg CO_{2eq} ha^{-1}crop^{-1} in both cropping seasons. It is recommended that the further long-term studies in combination with N fertilizer application to comprehensive determine effect of *G. diazotrophicus* bacteria on the mitigation of N_2O emissions, GWP, and GHGI in the paddy rice system in the Vietnamese Mekong Delta region.

Keywords: global warming potential, greenhouse gas intensity, nitrogen-fixed bacteria, nitrous oxide, paddy rice.

INTRODUCTION

The Mekong Delta region is the largest rice-producing area in Vietnam, contributing over 90% of the country's annual total rice exports (Dung et al., 2021; Qui et al., 2024). In the intensive rice cultivation systems, farmers usually apply high rate of nitrogen (N) fertilizer to increase the grain yield (Long et al., 2024; Tho and Umetsu, 2022; Tran et al., 2024). However, the N use efficiency in this rice production system is low due to only 40–50% of the N input is taken up by rice plants, while the remaining is lost by leaching and greenhouse gas (GHG) emissions (Arai, 2022; Arai et al., 2021; Dung et al., 2023).

Recently, agricultural activities are estimated to contribute approximately 15% of global GHG fluxes (International Fertilizer Association, 2018), with the emissions from agricultural and paddy soil accounting around 39% and 9% of the total anthropogenic GHG emissions, respectively (Tho and Umetsu, 2022; Tubiello et al., 2021). Nitrous oxide is considered one of the most significant contributors to global warming from cropping systems (Dung et al., 2023). According to EPA (2022), agricultural activities, including fertilizer application, manure, and residue management, contributed to 75% of the N_2O fluxes, with a global warming potential (GWP) approximately 298 times higher than CO_2 over a 100-years scale (EPA, 2022; Fagodiya et al., 2022; Tian et al., 2020). In the paddy soil systems, the main source of N_2O emissions via various pathways, including nitrification and denitrification (Li et al., 2021; Wu et al., 2023; Xiang et al., 2023). Due to the high spatial and temporal variability of

N_2O fluxes, the evaluation of soil management to mitigate the N_2O is a significant challenges in the agricultural system (Li et al., 2024). According to Chai et al. (2019), the application of organic N fertilizer is the main of N_2O emissions in the paddy rice. Numerous studies demonstrated that the fluxes of N_2O in the paddy fields significantly increased when the N fertilizer input exceeded 100 kg N ha^{-1} (Kim et al., 2019; Tang et al., 2018). Excessive N fertilizer application in the flooded soil condition promotes the denitrification process, leading to increased N_2O emissions (Hansen et al., 2014; Khalid et al., 2019). According to Tho and Umetsu (2022), increasing N fertilizer use resulted in increases of 4.56–7.11 g N_2O kg^{-1} each cropping season, and the GWP peaked in the treatments that received higher 100 kg N ha^{-1}. Numerous previous studies have implemented various soil and fertilizer management to mitigate the N_2O emission, such as the combination of N fertilizer with green manure, alternate wetting and drying irrigation, split N application, or the use of nitrification inhibitors (Arai, 2022; Ding et al., 2018; Dung et al., 2023; Zhou et al., 2017). Nevertheless, these studies have yet to demonstrate the effectiveness in reducing N_2O emission in the paddy rice cultivation systems. Therefore, it is essential for more effective approaches, with biological N fixation being recognized as a solution to increase N availability while mitigating N_2O emissions.

Biological N fixation (BNF) is recognized as the most important biological process on Earth (Weil and Brady, 2017). This reaction converts the atmospheric N_2 gas to the available forms for plants by archaea and bacteria, such as *Azotobacter*, *Rhizobium*, *Azospirium*, or *Bacillus*… (Ininbergs et al., 2011; Soumare et al., 2020). Currently, the studies focused on the non-nodulating N fixation systems, have been increasing in agriculture to improve the N availability in soil and reduce inorganic N fertilizers. In paddy rice cultivation systems, these bacteria offer a promosing approach to enhancing the soil fertility and increase N use efficiency. Previous studies have reported that the endophytic bacteria such as *Azospirium* spp., *Herbaspirillum* spp., *Gluconacetobacter* spp., have positive effect on improving the available soil N and increasing rice yield (Alquéres et al., 2013; Dung et al., 2021; Filgueiras et al., 2020; Hernández et al., 2023; Long et al., 2024; Rajani et al., 2023). Among these bacteria, *Gluconacetobacter diazotrophicus* – a gram-negative bacterium – a N-fixation bacterium is known to enhance the growth and development of plants (Ceballos-Aguirre et al., 2023; de Souza et al., 2016; Filgueiras et al., 2020; Mehnaz and Lazarovits, 2017; Meneses et al., 2017; Tufail et al., 2021). This bacterium was originally isolated from sugarcane (Chawla et al., 2014; Luna et al., 2012), and it has been detected in the rhizosphere of other plants (Jha et al., 2009; Tien et al., 2025). According to Reddy et al. (2017), *G. diazotrophiscus* can be responsible for supplying up to 50–70% of the N demand in the rice cultivation systems via BNF reaction. In the Mekong Delta (VMD) region, several studies reported that applying *G. diazotrophiscus* bacteria can maintain the growth and rice yield, and improve the soil properties, such as soil pH, EC, available soil N, Olsen-P under reduced N fertilization conditions (Dung et al., 2021; Long et al., 2024; Long et al., 2025). Nevertheless, the study which applying *G. diazotrophiscus* bacteria combined with N fertilization to the N_2O emissions in rice cultivation systems in the VMD region is lacking.

We hypothesized that *G. diazotrophiscus* can penetrate and colonize with the rice roots and establish an endophytic symbiotic environment when inoculated it onto the rice seeds during the soaking. This study aimed to determine the effect of *G. diazotrophicus* combined with N fertilizer reduction on the emission of N_2O, the GWP, and the greenhouse gas intensity (GHGI) in the intensive rice cultivation in the VMD region.

MATERIALS AND METHODS

Experimental site and soil properties

The experimental site was conducted in two rice cropping seasons: Winter-Spring (WS) 2023–2024 and Summer-Autumn (SA) 2024 in Mong Tho A commune, Kien Giang province (10°02′27.6″N, 105°09′17.7″E). According to FAO-WRB (2014), the soil is classified as Eutric Gleysols. The soil texture was classified as clay, with silt and clay contents of 39.1% and 60.4%, respectively. The topsoil is slightly acidic (pH 5.88), and electrical conductivity varied around 1.24 mS cm^{-1}. Soil organic matter (7.95%) and available N (73.9 mg NH_4^+-N kg^{-1}) were considered at a high level for the paddy field.

Field experimental design and management

The field experiment was laid out in a split-plot design with two factors: the NFB method (main plot) and N fertilizer rates (subplot). NFB methods included: Rice seeds were inoculated with *G. diazotrophicus* (G_d) and without inoculated with *G. diazotrophicus* (G_o). Nitrogen fertilizer rates were: 0 (N_0), 50 (N_{50}), 75 (N_{75}), and 100 kg N ha^{-1} (N_{100}).

The rice seed inoculation method was described by Long et al. (2024): Rice seeds were soaked in water for 24 hours, incubated for 24 hours, then soaked in *G. diazotrophicus* bacteria solution (10^8 CFU mL^{-1}) for 1 hour, and incubated for 4–6 hours before sowing.

Each plot had a size of 30 m^{2,} which was separated by the bunds. Rice variety Dai Thom 8, which has a growing time range of approximately 95 days, was used in this study. All phosphorus fertilizer (60 kg P_2O_5 ha^{-1}) was applied at the beginning of experiment, N fertilizer was top-dressed at 10, 20, and 45 days after sowing (DAS), and K fertilizer (30 kg K_2O ha^{-1}) was applied at 20 and 40 DAS.

Nitrous oxide sampling and analysis

The N_2O samples were taken at 0, 15, and 30 minutes from 8:00 to 11:00 am, using the closed chamber (250 L). Nitrous oxide samples were collected weekly from 7 to 84 DAS. A 60 mL PVC syringe was used to collect the gas sample from the chamber, which was then transferred into 20-mL glass vials.

The N_2O samples were analyzed using a gas chromatograph machine equipped with an electron capture detector (Model Shimazu, 2014, Japan) at 350 °C. The daily N_2O emissions were determined using the equation according to Dung et al. (2023):

$$F_{N_2O} = \frac{G \times V \times M \times 60 \times 24}{22.4 \times \frac{273+T}{273} \times S \times 1000} \quad (1)$$

where: F_{N2O} is the daily N_2O emissions (mg m^{-2} day^{-1}); G is the amount of N_2O emission per minute (ppm min^{-1}); M is the molecular mass of N_2O (g mol^{-1}); V is the chamber volume (L); S is the area of chamber base (m^2); T is the temperature inside the chamber (°C); 60 is min per hour; 24 is hours per day; 1000 is the converting from L to m^3; 22.4 is the molar volume of an ideal gas at a standard temperature and pressure; and 273 is the temperature in standard condition (°K).

The cumulative N_2O emissions were calculated over 95 days in both rice crop seasons. The GWP was determined by the equation:

$$GWP = Total\ N_2O \times 298 \quad (2)$$

where: GWP is the global warming potential of N_2O converted into CO_2 on a 100-year scale; 298 is the index of N_2O converted into CO_2 equivalent (Fagodiya et al., 2022).

The GHGI was calculated by the total N_2O emissions over the grain yield in each rice cropping season.

Data statistical

The effect of NFB (G) and N fertilizer rates (N) and their interaction on the total N_2O emissions, GWP, and GHGI are determined by analysis of variance using the R 4.4.0 software package (Core Team, 2020). Treatments showing with significant effects were futher compared using Tukey's HSD test at 5% level.

RESULTS

Weather

Mong Tho A commune is one of the largest triple-crop rice cultivation area in Kien Giang province. The study site is characterized by a tropical monsoon climate with a distinct dry season (November–April) and a wet season (April–November). In addition, the experimental site is minimally affected by saltwater intrusion during the dry season. The average temperatures in WS 2023–2024 and SA 2024 were around 27.8 °C and 29.6 °C, respectively (Figure 1). There was no rain recorded in WS 2023–2024 and the total precipitation was recoded at 842.4 mm in SA 2024.

Daily N_2O flux in WS 2023-2024 and SA 2024 crops

Daily variations of daytime N_2O emission in the WS 2023–2024 and SA 2024 cropping seasons are shown in Figure 2 and Figure 3, respectively. In the WS 2023–2024 crop, the average of

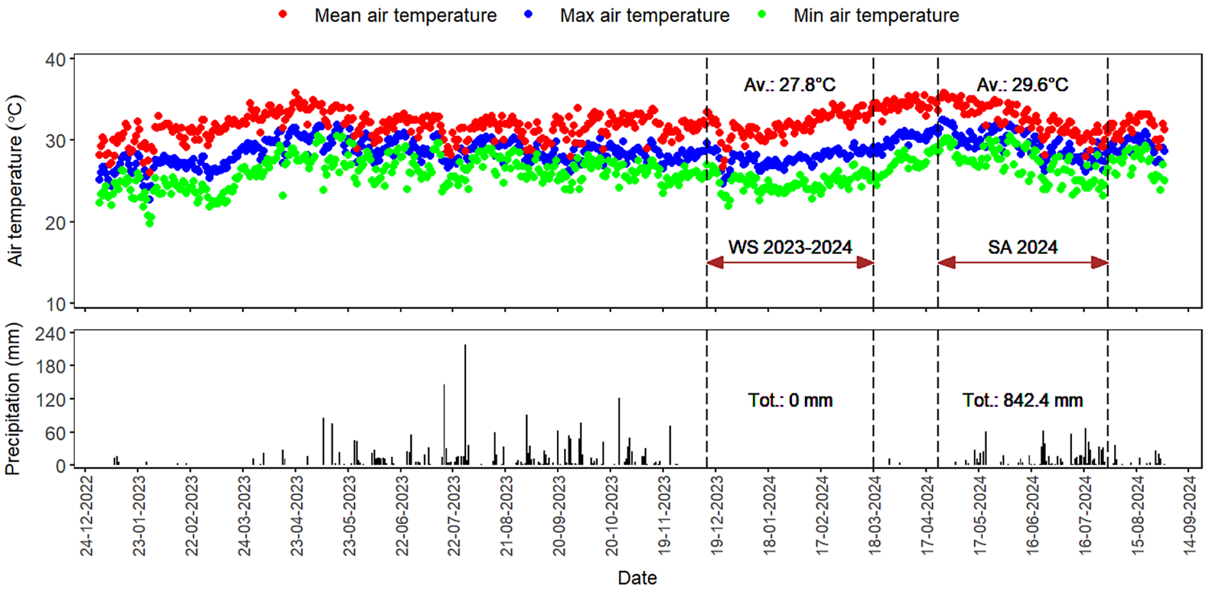

Figure 1. The temperature and rainfall of the study site from 01/2023 to 08/2024
Source: The Station of Meteorology and Hydrology of Kien Giang Province, Vietnam)

N_2O flux ranged from 4.77 to 5.90 mg N_2O m^{-2} day^{-1} (Figure 2). In general, the emission of N_2O increased after sowing and reached the maximum at 21 DAS in all treatments (20.8–33.1 mg N_2O m^{-2} day^{-1}). After that, the N_2O flux was reduced and maintained until the 84 DAS.

In the SA 2024 rice season, the N_2O emission ranged from 1.71 to 2.33 mg N_2O m^{-2} day^{-1} (Figure 3). The results showed that the daily N_2O emission during SA 2024 did not vary significantly between the treatments with/without *G. diazotrophicus* bacteria application and among the different N fertilizer rate treatments.

Total N_2O emissions

The total N_2O emissions varied across N fertilizer rates and cropping seasons (Figure 4). In the WS 2023–2024 season, N_2O emissions increased with higher N inputs, reaching the highest values at 100 kg N ha^{-1} (N_{100}). Both treatments, G_o (control) and G_d (*G. diazotrophicus* inoculation), exhibited similar trends, with G_d generally showing slightly higher emissions at each N level, although differences were not statistically significant due to overlapping error bars. This study also showed that there was no significant interaction between *G. diazotrophicus* inoculation and N fertilizer reduction on total N_2O emissions ($p>0.05$). In contrast, the SA 2024 season recorded markedly lower N_2O emissions across all N rates compared to WS 2023–2024. Emissions ranged between approximately 1.5 and 2.5 kg N_2O ha^{-1}, with minor differences between G_o and G_d treatments. Overall, the data suggest that N application rate strongly influences N_2O emissions, particularly during the WS season, while microbial inoculation had minimal impact under the conditions tested. In addition, the results also indicated that the total N_2O emission in SA 2024 was 2.05 kg ha^{-1}, significantly lower than that in WS 2023–2024 season (5.12 kg ha^{-1}).

The GWP and GHGI

The GWP exhibited clear differences between cropping seasons and N fertilizer rates (Figure 5). During the WS 2023–2024 season, GWP values were substantially higher than in the SA 2024 season across all N treatments. In WS 2023–2024, GWP increased slightly with higher N input rates, with the highest emissions observed at N_{100}. Both G_o (without *G. diazotrophicus* inoculated) and G_d (*G. diazotrophicus* inoculated) treatments showed comparable GWP values, with G_d generally having marginally higher means, though the error bars indicate non-significant differences. Statistical analysis indicated that the combining of *G. diazotrophicus* with reduced N fertilizer did not show a significant interactive effect on GWP in both cropping seasons ($p>0.05$). In the SA 2024 season, GWP values were significantly lower, ranging from approximately 600 to 800 kg CO_{2eq} ha^{-1}. Unlike the dry season, the effect of increasing

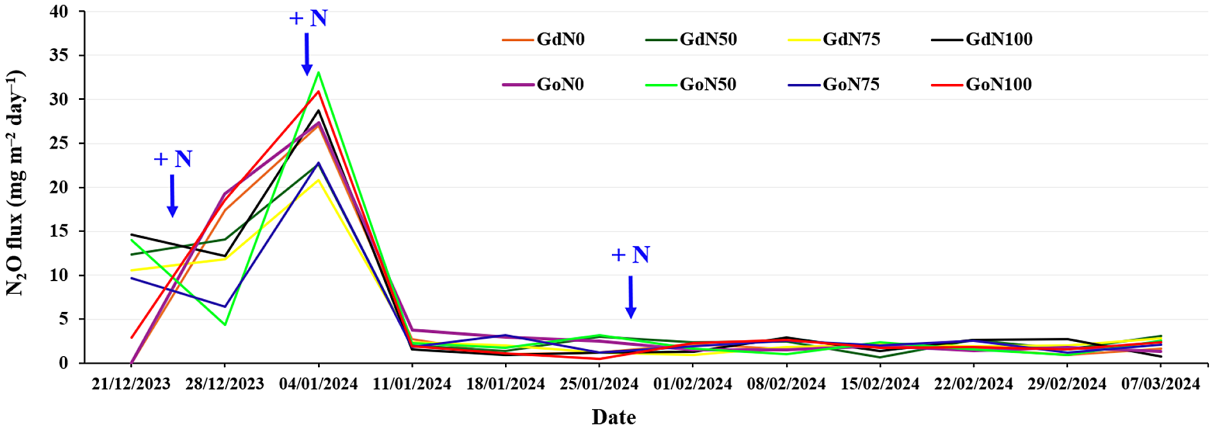

Figure 2. The N_2O flux under rice seed inoculation methods and N fertilizer rates in WS 2023–2024 cropping season (Arrows labeled "+N" indicate the time of N fertilizer application)

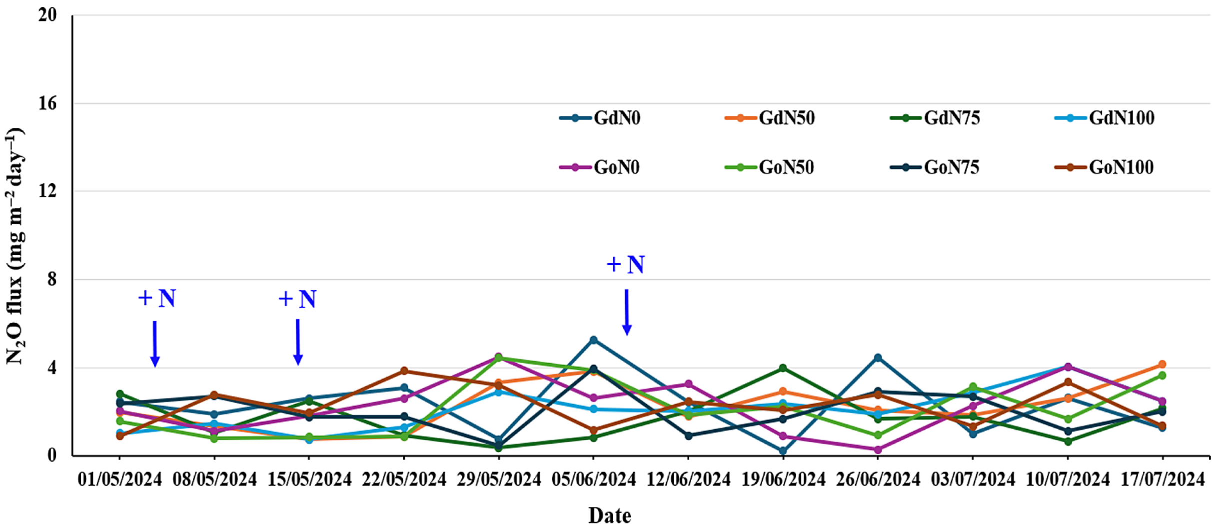

Figure 3. The N_2O flux under rice seed inoculation methods and N fertilizer rates in SA 2024 cropping season (Arrows labeled "+N" indicate the time of N fertilizer application)

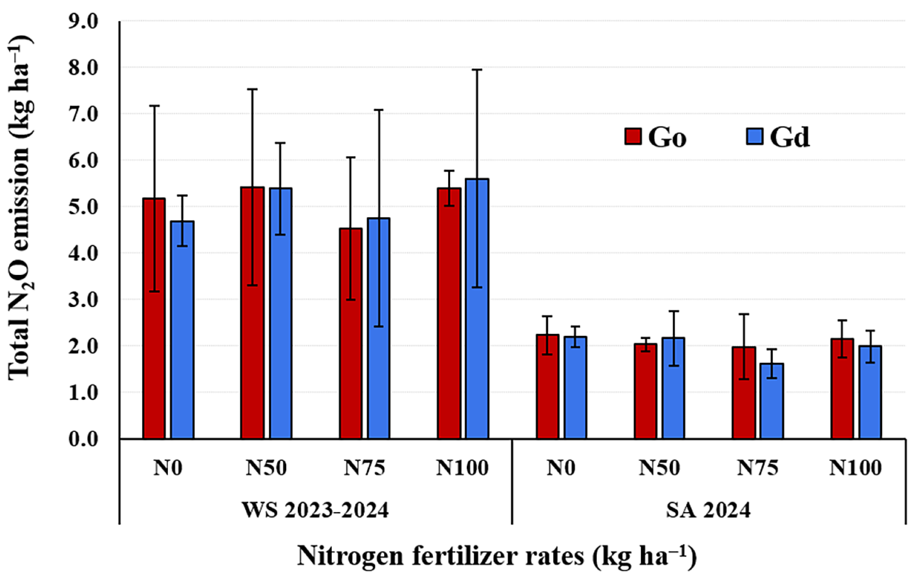

Figure 4. The total of N_2O emission in WS 2023–2024 and SA 2024 cropping seasons

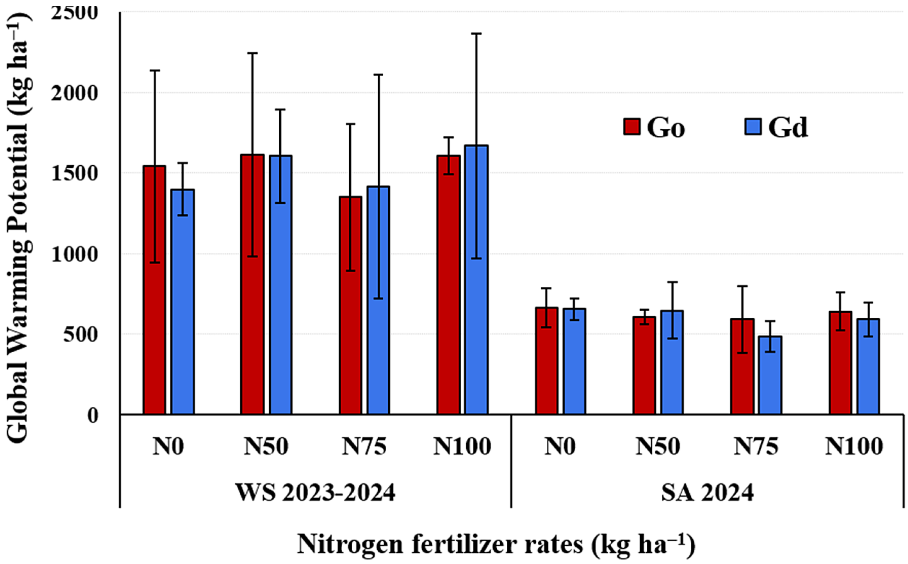

Figure 5. The GWP in WS 2023-2024 and SA 2024 cropping seasons

N rates on GWP in SA 2024 was less pronounced, and differences between G_o and G_d were minimal. Generally, the results showed that the total GWP in WS 2023–2024 season was 1525 kg CO_{2eq} ha^{-1}, significantly higher than in SA 2024 season (611 kg CO_{2eq} ha^{-1}). These results indicate that GWP is more strongly influenced by seasonal conditions than by inoculation or N rates alone.

The Greenhouse Gas Intensity (GHGI) varied notably across N fertilizer rates and between cropping seasons (Figure 6). In the WS 2023–2024 season, GHGI values ranged from approximately 0.2 to 0.35 kg CO_{2eq} kg^{-1}, with the highest values recorded at N_{50} for the G_d treatment. Overall, G_d-inoculated treatments tended to show slightly higher GHGI than G_o controls, especially at lower N levels (N_0 and N_{50}). However, large error bars suggest high variability, and differences may not be statistically significant. Similarly, no significant interaction between *G. diazotrophicus* inoculation and N fertilizer rates was detected for GHGI in both cropping seasons ($p>0.05$). In contrast, GHGI values in the SA 2024 season ranged between 0.1 and 0.2 kg CO_{2eq} kg^{-1}, with minimal variation across N rates and treatments. Both G_o and G_d treatments displayed similar trends, and GHGI appeared relatively stable across all fertilization levels. The results of study indicated that GHHI index in WS 2023–2024 season (0.28) was significantly higher than in SA 2024 cropping season (0.13). These results highlight the influence of seasonal variation on GHGI and suggest that *G. diazotrophicus* inoculation may slightly increase GHGI under dry season conditions.

DISCUSSION

This study indicated that seasonal conditions and N fertilizer inputs were the dominant control on N_2O emissions in the intensive rice system, while microbial inoculation with *G. diazotrophicus* did not exhibit a statistically significant interaction with N fertilizer rates. In addition, the soil redox potential strongly influenced by water saturation, soil moisture, and temperature, may have reduced microbial activity, as nitrification under microaerophilic or denitrification under anaerobic conditions. A marked seasonal variation in N_2O fluxes and cumulative emissions was observed, with substantially higher values recorded during the WS 2023–2024 season compared to the SA 2024 season. This trend is consistent with previous studies indicating that dry-season rice cultivation, characterized by greater soil aeration due to reduced water saturation, can enhance nitrification-denitrification cycles and increase N_2O release (Cosentino et al., 2013; Linquist et al., 2012; Ponti et al., 2020). In contrast, the SA season coincided with higher precipitation and elevated waterlogged conditions, which likely suppressed N_2O emissions due to the predominance of anaerobic pathways favoring complete denitrification to N_2 gas (Akiyama et al., 2010; Berendt et al., 2023; Chapuis-Lardy et al., 2007). Temperature may have also played a role, with slightly higher average temperatures in SA 2024 (29.6 °C) potentially accelerating microbial turnover but also promoting methanogenic rather than nitrifying pathways under anaerobic soil conditions.

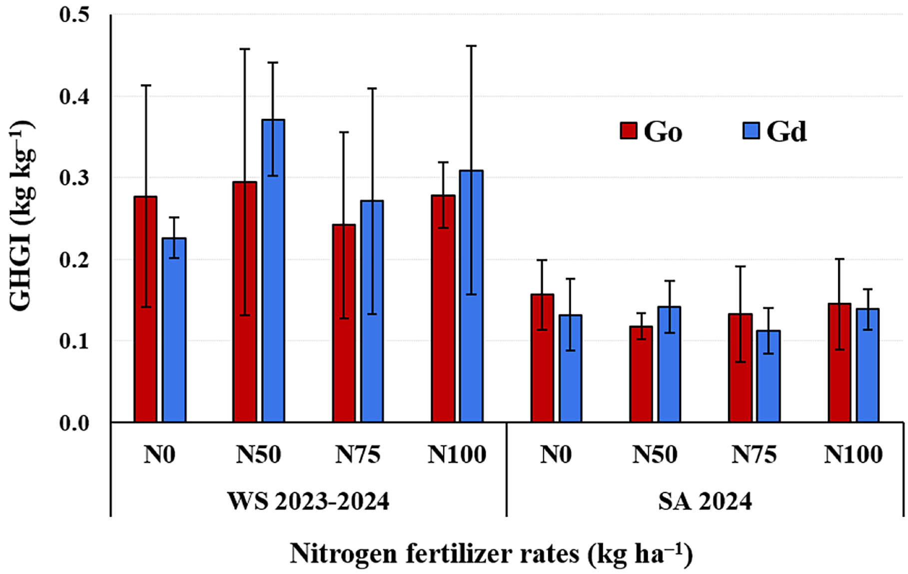

Figure 6. The GHGI in WS 2023-2024 and SA 2024 cropping seasons

The interplay of soil moisture and temperature thus appears to exert a dominant control over N_2O flux, overshadowing the effects of inoculation or N rates during this season.

As anticipated, increasing N fertilizer application led to elevated N_2O emissions in both seasons, particularly during WS 2023–2024. This is in line with the well-established positive relationship between synthetic N inputs and N_2O release, attributed to increased substrate availability for nitrifiers and denitrifiers (Butterbach-Bahl et al., 2013; Fudjoe et al., 2023; Li et al., 2021; Li et al., 2023; Qiu et al., 2023). Notably, the response was more pronounced in the WS season, reinforcing the notion that emission potentials are context-dependent and may be amplified under drier, more oxic soil conditions. However, the diminishing marginal increase in emissions from N_{75} to N_{100} suggests a possible threshold effect or saturation point beyond which additional N inputs do not proportionally raise emissions. This has important implications for optimizing N use efficiency and minimizing environmental losses in rice production systems.

Contrary to expectations, inoculation with *G. diazotrophicus* had minimal and non-significant effects on N_2O emissions, GWP, and GHGI across both cropping seasons and N treatments. While *G. diazotrophicus* is known for its biological N fixation capabilities and potential to partially substitute chemical N inputs (Ceballos-Aguirre et al., 2023; Dung et al., 2021; Filgueiras et al., 2020; Long et al., 2025; Tufail et al., 2021), its direct impact on N_2O dynamics under field conditions appears limited in this context. One possible explanation is that the additional N fixed by the bacteria was either insufficient to significantly alter the soil N pool or was released in a form and at a rate that did not favor enhanced N_2O production. Alternatively, the microbial inoculum may not have established effectively in the soil microbiome due to abiotic or biotic constraints, highlighting the importance of field-scale validation of microbial amendments under varied agroecosystems.

Greenhouse gas intensity, which normalizes GHG emissions per unit of yield, revealed notable differences between seasons and treatments. Higher GHGI in WS 2023–2024, particularly under lower N inputs (N_0 and N_{50}), suggests a trade-off between emission reduction and productivity. Interestingly, Gd-inoculated treatments at low N rates showed slightly higher GHGI, possibly due to comparable emissions but reduced yield gains, underscoring the importance of balancing microbial inoculation strategies with appropriate nutrient management. The lower GHGI values in SA 2024 reinforce the environmental advantage of wet-season rice cultivation from a climate perspective. However, yield penalties under excessive rainfall or nutrient limitations must be addressed to ensure food security without compromising mitigation efforts.

Collectively, the results underscore that the timing and rate of N application remain critical levers for mitigating N_2O emissions in rice production. While microbial inoculants like *G.*

diazotrophicus show promise for reducing dependence on synthetic fertilizers, their role in direct N_2O mitigation may be limited unless accompanied by improved agronomic integration or synergistic use with other bio-based inputs. From a practical standpoint, adopting site- and season-specific N management, coupled with the use of low-emission practices such as alternate wetting and drying, could offer a more robust approach to reduce the climate footprint of rice farming in the VMD region and similar agroecological zones.

CONCLUSIONS

This study demonstrated that seasonal variation and N fertilizer rates significantly influenced N_2O emissions, GWP, and GHGI in paddy rice in Kien Giang province, Vietnam. However, the inoculation of rice seeds with *G. diazotrophicus* showed no statistically significant reductions in N_2O emissions, GWP, or GHGI compared to the non-inoculated control.The results also indicated that N_2O emissions, GWP, and GHGI in WS 2023-2024 season significantly higher than in SA 2024 season.The application of N fertilizer strongly correlated with increased N_2O emissions and GWP, with the highest values observed at the farmer's practice rate of 100 kg N ha^{-1} (N_{100}). Notably, both GWP and GHGI were substantially lower in the SA 2024 season, indicating that seasonal climatic factors play a more dominant role than microbial inoculation or moderate changes in N rates in shaping greenhouse gas outcomes. In addition, there was no significant interaction between *G. diazotrophicus* inoculation and N fertilizer rates on N_2O emissions, GWP, and GHGI in both cropping seasons. These findings underscore the importance of optimizing nitrogen fertilizer application in accordance with seasonal conditions to minimize the environmental footprint of rice cultivation. Long-term field studies are needed to evaluate repeated *G. diazotrophicus* inoculation under different N fertilizer rates, determining its potential interactions with biological N inputs, and adaptive management options for improving productivity and reducing greenhouse gas intensity in paddy rice systems.

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