

Article

Effect of Rice Planting on Nitrous Oxide (N₂O) Emission under Different Levels of Nitrogen Fertilization

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Abstract: Nitrogen (N) fertilization is one of the most effective practices to increase productivity, and has therefore had a fast global increase. Consequently, the effects of the application of N fertilizer on emissions of N₂O have been widely studied, but the effect of rice planting on N₂O emission was not adequately quantified. To evaluate the effect of rice cultivation on N₂O emissions, different levels of N were applied in a typical temperate rice field, and the N₂O fluxes were compared in rice-planted and non-planted soils. Seasonal N₂O fluxes responded differently with respect to N fertilization in the two different soil conditions. In non-planted soils, seasonal N₂O fluxes ranged within 0.31–0.34 kg N₂O ha⁻¹ under 0 kg N ha⁻¹ fertilization, and significantly increased by increasing N fertilization rates, with an average rate of 0.0024 kg N₂O kg⁻¹ N for 3 years. In rice-planted soils, seasonal N₂O fluxes were also increased by N fertilization but showed large negative N₂O fluxes, irrespective of the N fertilization level. This study confirms that the rice reacted as a reducer of N₂O emissions, not an emission source, in paddy fields, suggesting that N₂O fluxes should be estimated by the static chamber planted with rice to obtain a more precise field environment. The differences of N₂O fluxes between the rice-planted and non-planted soils might have been caused by the rice plant's rhizospheric activities, which may have influenced the N₂O consumption potential in the rice plants' rhizosphere. The N₂O consumption potential was significantly increased with increasing N fertilization rates and was highly correlated with rice biomass yields. Therefore, the decrease in N₂O fluxes by N fertilization in rice-planted soils might have been caused by a decreasing denitrification potential in paddy soils.

Keywords: static chamber method; nitrogen fertilization; N₂O emission; rice paddy



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1. Introduction

Nitrous oxide is a long-lived trace gas in the atmosphere and has a 298 times higher global warming potential (GWP) than equivalent amount of carbon dioxide (CO₂) [1]. Additionally, N₂O has become the most important substance contributing to ozone (O₃) layer depletion after chlorofluorocarbons [2]. The atmospheric concentration of N₂O has significantly increased from 270 ppbv at the pre-industrial era to 322.5 ppbv in the year 2009, with an average increase of 0.77 ppbv year⁻¹ for the period 2000–2009 [3], mainly due to agricultural activity.

In agricultural fields, the N₂O emissions are mainly produced by the chemical and organic N inputs [1]. Globally, agricultural sectors emitted almost 60% of the total anthropogenic N₂O emissions [4,5]. The N₂O emissions are predicted to increase nearly 35–60% by the end of 2030 to meet the increasing global food demand [5].

Soils react as both a source and a sink of N₂O gas [6], but the source activity largely dominates the sink one on the global scale. It is known that biological nitrification and

denitrification in soils cover around 70% of global N_2O emissions [6,7]. In particular, arable soils are estimated to produce approximately $2.8 \text{ Tg N}_2\text{O-N year}^{-1}$, which accounts for approximately 60% of total anthropogenic N_2O emissions [4,5]. Amongst agricultural practices, N fertilizer utilization is the most important activity related to direct or indirect N_2O emissions [8–10].

Rice is the most important staple food crop for over 3 billion people in the world [11]. Global rice consumption is projected to increase from 450 million tons in 2011 to about 490 million tons in 2020 and to around 650 million tons by 2050 [12]. Intensive farming practice with high fertilization is widely adopted to increase crop productivity, and therefore, the dependence on fertilizers has continually increased in the rice cropping industry [13,14]. However, the increase of N fertilizer application can stimulate greenhouse gas (GHG) emissions significantly, in particular N_2O . Thus, the effect of N fertilizer application on N_2O gas emission could be an important issue to find a soil management strategy to reduce the impact of its greenhouse gas emissions in agricultural fields.

Static chamber methods have been most commonly adapted for determining N_2O fluxes from agricultural fields. Plants inside chambers can create unique challenges and then influence N_2O emission characteristics significantly [15,16]. However, N_2O fluxes were measured under different planting conditions inside chambers. For example, many N_2O fluxes were developed using planted chambers, in particular in small biomass cropping fields (i.e., lawn, grass, etc.) [17,18], but a large number of N_2O fluxes were also measured using plant-excluded chambers, in big biomass plant cultivated fields like tomato, sunflower and rice [19–21]. Thus, the unified installation protocol of static chambers should be developed to reflect the field environment more precisely. In general, placing an opaque chamber cover over plants can reduce N_2O fluxes by blocking radiation and leading stomatal closure [22]. The magnitude of any reduced N_2O flux will depend on the plant species, the amount of biomass enclosed by the chamber, inorganic N forms in the soil and their amounts. If plants are enclosed in transparent chambers, there is clearly a conflict between the need to insulate the chamber to limit air temperature changes and a need to maintain solar radiation for plant functions.

To determine the effect of planted rice on N_2O fluxes, different levels of N fertilizer (urea) were added in a temperate rice cropping field, and N_2O emissions were characterized in soils with and without rice plants during the three-year rice cultivation period.

2. Materials and Methods

2.1. Preparation of Experimental Field for Rice Transplanting

Rice cropping studies were conducted at an agronomy field in Gyeongsang National University ($35^{\circ}06' \text{ N}$ and $128^{\circ}07' \text{ E}$), Jinju, Gyeongnam-province, South Korea, for 3 years (2014–2016). The selected soil was classified with the *Pyeongtaeg* series (fine-silty, mixed, mesic, Typic haplaquent with somewhat poor drainage). The soil before the test had moderately acidic pH (5.6 ± 0.2 , 1:5 with H_2O), low fertility with $8.9 \pm 0.6 \text{ g C kg}^{-1}$ of organic matter, $0.65 \pm 0.08 \text{ g N kg}^{-1}$ of total nitrogen and $73 \pm 4.2 \text{ mg P}_2\text{O}_5 \text{ kg}^{-1}$ of available phosphorus.

The study field was installed with 12 plots, and each plot was designed with 100 m^2 of size. Depending on the recommended fertilization levels ($\text{N-P-K} = 90\text{--}19.6\text{--}47.3 \text{ kg ha}^{-1}$) for rice cropping in Korea [23], four different levels (0, 45, 90 and 180 kg N ha^{-1}) of urea were applied with three replications and laid out with a randomized block design. However, the same doses of P and K were added in all different treatments. Fertilization was applied by rice growing stages such as basal fertilizer (1 day before transplanting, 50% of N, 100% of P, 70% of K), tillering fertilizer (14 days after transplanting, 20% of N), and panicle fertilizer (42 days after transplanting, 30% of N and K), respectively. The buffer zone (0.5 m width) was installed using a concrete barrier between each experimental site to prevent nutrient mixing effects.

Twenty-one-day-old seedlings (3–4 plants per hill) of rice (*Sindongjin-byeo* cultivar, Japonica) were transplanted by hand with a spacing of $15 \text{ cm} \times 30 \text{ cm}$ in the end of May for

each year. Irrigation water was automatically controlled with 5–7 cm of depth during the rice growing season and drained a month before harvesting. Rice was harvested manually in the mid of October, and its yield properties (gran and straw) were investigated by the Rural Development Administration (RDA) [24].

2.2. Gas Sample Correcting and Analysis

Nitrous oxide fluxes were estimated using a static-chamber method [25]. To evaluate the N₂O emission rates from the rice planted and without rice plant soils, six pairs of static chambers were fixed in each plot. In order to determine the N₂O fluxes in the rice-planted soils, three pairs of the transparent acrylic chamber (W. 62 cm × L. 62 cm × H. 112 cm) were installed in each plot after rice transplanting. The chambers accommodated eight rice plant hills inside each chamber. On the other hand, only three pairs of chambers were kept without plants to measure the N₂O fluxes from the bare soils. The chambers were equipped with a circulating fan to mix gases completely and a thermometer to determine the inner temperature during the sampling period. The flow of irrigated water was permitted through two holes at the chamber bottom. The chambers were only closed during the gas sampling period and always kept open during cropping season. The true height of each chamber was measured at every gas sample correction, since the air volume inside chamber might be different depending on the depth of the inner chamber in the soil and on the level of water flooding.

The gas samples were collected at 0, 15, 30 and 45 min after chamber close using 25 mL gastight syringes. To get the mean N₂O emission fluxes, gas samples were collected three times a day (8:30–9:00, 12:30–13:00 and 16:30–17:00). The sampled gases were promptly transferred into 20 mL vacuumed glass vials.

The N₂O gas concentrations were quantified using a gas chromatograph (GC–2010; Shimadzu) with an electron capture detector (ECD) and a Porapak NQ column (Q 80–100 mesh). The temperatures of the injector, detector and column were adjusted at 200, 300 and 35 °C, respectively. Hydrogen and helium gases were used as the burning and carrier gases, respectively.

2.3. Calculation of N₂O Fluxes

Nitrous oxide fluxes were estimated with the following equation [26]:

$$R = \rho \times (V/A) \times (\Delta c/\Delta t) \times (273/T) \quad (1)$$

where R: N₂O emission rate ($\mu\text{g m}^{-2} \text{h}^{-1}$), ρ : N₂O gas density (1.977 mg cm^{-3}) under a standardized state, V: volume of the closed chamber (m^3), A: surface area of the chamber (m^2), $\Delta c/\Delta t$: rate of N₂O increase in the closed chamber ($\text{mg m}^{-3} \text{d}^{-1}$), and T (absolute temperature): $273 + \text{mean temperature (}^\circ\text{C)}$ in the chamber.

The seasonal N₂O flux for the rice cropping period was integrated by the following equation [27]:

$$\text{Seasonal N}_2\text{O flux} = \sum_i^n (R_i \times D_i) \quad (2)$$

where R_i: N₂O emission rate ($\text{mg m}^{-2} \text{d}^{-1}$) in the i-th sampling interval, D_i: number of days in the i-th sampling interval, and n: gas sampling number.

2.4. Analysis of Air Temperature, Soil and Rice Yield Properties

The temperatures (soil and air) were automatically recorded by a thermometer installed at each place (air: 100–110 cm height from ground, soil: 5 cm depth in the soil) during cropping season. The Eh value (redox potential) of surface soils (5 cm depth) was monitored using the sensor PRN-41, from DKK-TOA Corporation, with the platinum Eh electrode (EP-201, Fujiwara, 24 cm) during gas sampling.

Rice yield properties were measured by the Korean standard methods at the maturing stage [24]. The root biomass was arithmetically estimated as 10% of the total aboveground biomass productivity [28].

2.5. Statistical Analysis

A two-way ANOVA was conducted with SAS package (version 9.3, SAS Institute) to compare rice yield properties among N application rates, experiment years and the interaction of these two factors. The mean values were compared with least significant difference (LSD) tests at the 0.05 level of probability. The response of the quadratic model was estimated using Sigma plot software. Linear regression and correlation analyses were conducted to evaluate relationships between response variables.

3. Results

3.1. Changes of Soil Temperature and Eh Value during Rice Cultivation

During the field investigations for 3 years, typical climate environments of the selected site were observed. Soil temperature was a little bit higher in 2016 than in other years. In 2016, the air and soil temperature were approximately 1.5 °C and 1.8 °C higher than in 2014–2015 (Figure 1).

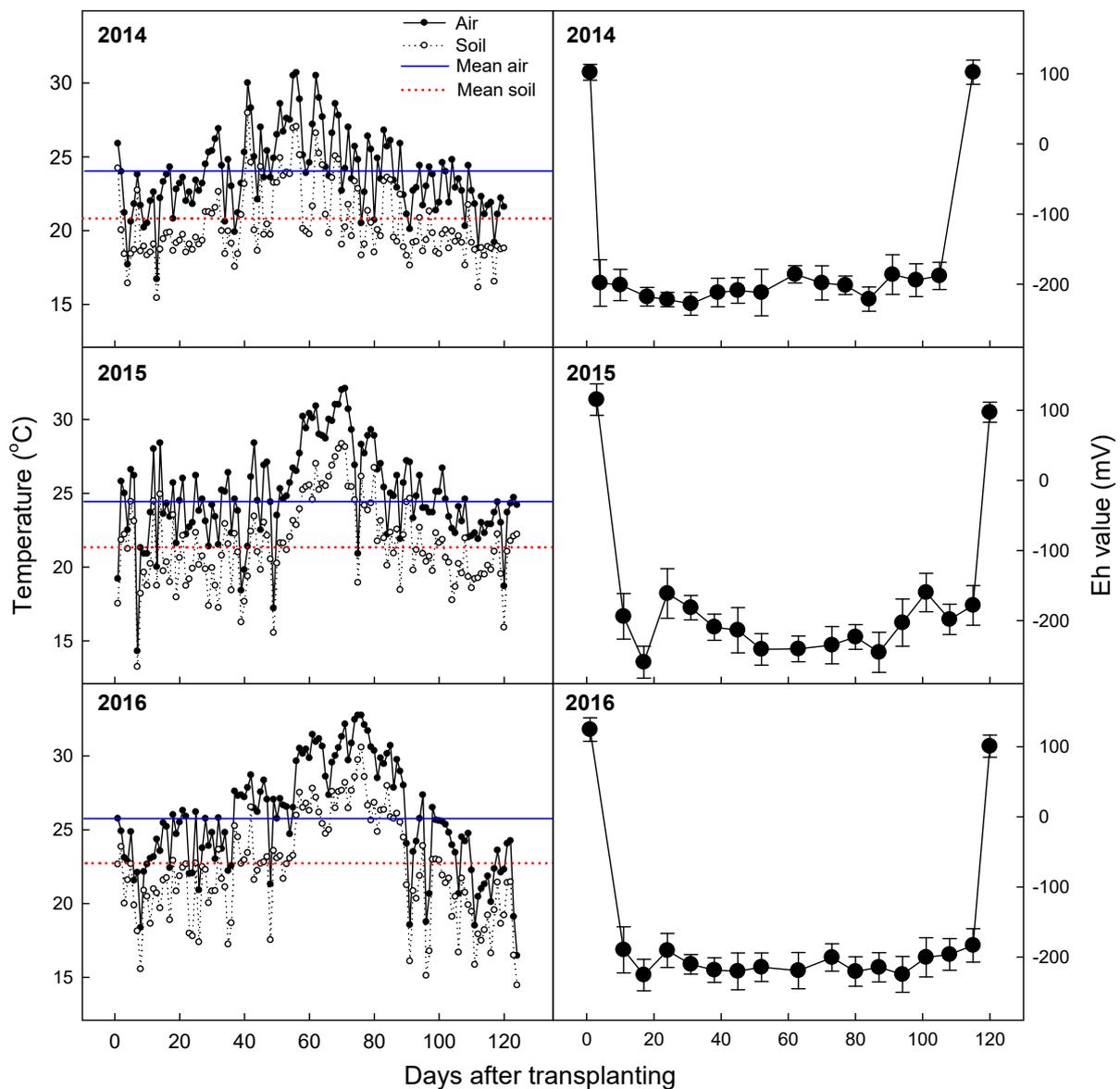


Figure 1. Changes in air and soil temperatures and soil Eh values during the rice cultivation periods.

The Eh values did not significantly differ among treatments throughout the three-year experiment period (Figure 1). The soil Eh values sharply dropped from over 100 mV in every experimental plot with flooding for rice cultivation. Within one to two weeks after rice transplanting, the Eh values dropped to less than -210 mV. This extremely reduced soil condition was maintained throughout the flooded rice cropping period, and the Eh values ranged from -210 to -250 mV in this period. After the drainage for harvesting, soil Eh values speedily increased.

3.2. Rice yield Properties

Rice yield and growth characteristics were similarly changed to the different levels of N application among the experimental years (Table 1). Rice total biomass and straw yield were significantly increased with increasing N fertilizer application, but rice grain yields were increased by a quadratic response. The maximum grain productivity might be attained at 110 – 120 kg N ha⁻¹ of urea application with approximately 30% yield increase over no-N application (control treatment), and thereafter grain yields clearly decreased with N fertilization increases. The increased panicle number per hill mainly influenced the grain yield increase among rice yield components (Table 1).

Table 1. The properties of rice growth and yield characteristics at the harvesting stage under different levels of nitrogen application.

Year	N Levels (kg N ha ⁻¹)	Yield (Mg ha ⁻¹)	Straw (Mg ha ⁻¹)	Root (Mg ha ⁻¹)	Height (cm)	Tiller Number Per Hill
2014	0	4.60 ± 0.51 b ^a	5.93 ± 0.19 c	1.05 ± 0.05 c	87 ± 0.9 d	11 ± 0.3 c
	45	5.21 ± 0.27 ab	7.07 ± 0.14 b	1.23 ± 0.03 b	94 ± 0.6 c	14 ± 1.2 b
	90	5.90 ± 0.05 a	8.26 ± 0.49 a	1.42 ± 0.05 a	105 ± 0.8 b	14 ± 1.0 b
	180	5.41 ± 0.33 ab	9.06 ± 0.31 a	1.45 ± 0.01 a	108 ± 0.6 a	18 ± 0.4 a
2015	0	4.98 ± 0.23 b	6.14 ± 0.18 c	1.11 ± 0.03 c	92 ± 0.6 c	12 ± 0.7 b
	45	5.42 ± 0.99 ab	7.30 ± 0.12 b	1.30 ± 0.10 b	96 ± 0.9 b	15 ± 1.0 ab
	90	6.74 ± 0.24 a	8.39 ± 0.26 b	1.51 ± 0.05 a	97 ± 0.9 b	16 ± 16.1 a
	180	5.84 ± 0.39 ab	9.32 ± 0.55 a	1.52 ± 0.04 a	104 ± 1.2 a	17 ± 1.4 a
2016	0	5.31 ± 0.39 b	6.32 ± 0.38 c	1.16 ± 0.01 b	95 ± 2.4 b	11 ± 0.5 b
	45	6.03 ± 0.43 ab	7.66 ± 0.14 b	1.37 ± 0.03 ab	96 ± 2.0 b	13 ± 2.9 ab
	90	6.92 ± 1.12 a	8.56 ± 0.45 b	1.55 ± 0.09 a	98 ± 2.7 b	14 ± 2.9 ab
	180	6.32 ± 1.71 ab	9.58 ± 0.42 a	1.59 ± 0.20 a	107 ± 1.1 a	17 ± 0.42 a
Statistical analysis ^b						
Year (A)		*	**	NS	**	NS
N application (B)		**	***	***	***	***
A × B		NS	NS	NS	***	NS

^a Mean values followed by different letters in the same column indicate a significance difference among treatments within a single year at $p < 0.05$. ^b NS means not significant F-values for $p < 0.05$, *, **, and *** indicate significant difference at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

Differing with the changes of grain productivity, rice root and straw biomass yields were clearly increased by the increase in the level of N fertilizer (Table 1). For instance, total biomass productivities were approximately 1200 kg ha⁻¹ in no-N application, and clearly increased by increasing the level of N fertilization (approximately 1600–1700 kg ha⁻¹ at 180 kg N ha⁻¹), mainly due to an increase of rice straw biomass productivity. Comparing with the other parameters, straw biomass productivity was more substantially increased by N fertilizer application, occupied approximately 50% of the total biomass in the control and then increased proportionately to around 55–60% with 180 kg N ha⁻¹ of urea fertilization. The rice root biomass productivity showed a trend similar to that of the rice straw yield with increasing N fertilization.

3.3. Changes of N₂O Emission Rates

A distinct pattern of N₂O flux was recorded for submerged paddy-field rice-planted soil and for non-planted soil (Figure 2). Irrespective of treatments and rice cropping years, N₂O emission increased up to the initial 20 days after rice transplanting. However, N₂O emission sharply decreased thereafter in rice-planted soils, and no real N₂O flux was detected in those soils throughout the rice cropping (Figure 2). The positive N₂O flux from rice-planted soils was recorded after 120 days of rice cultivation in these soils, i.e., after removing flooded water prior to rice harvesting.

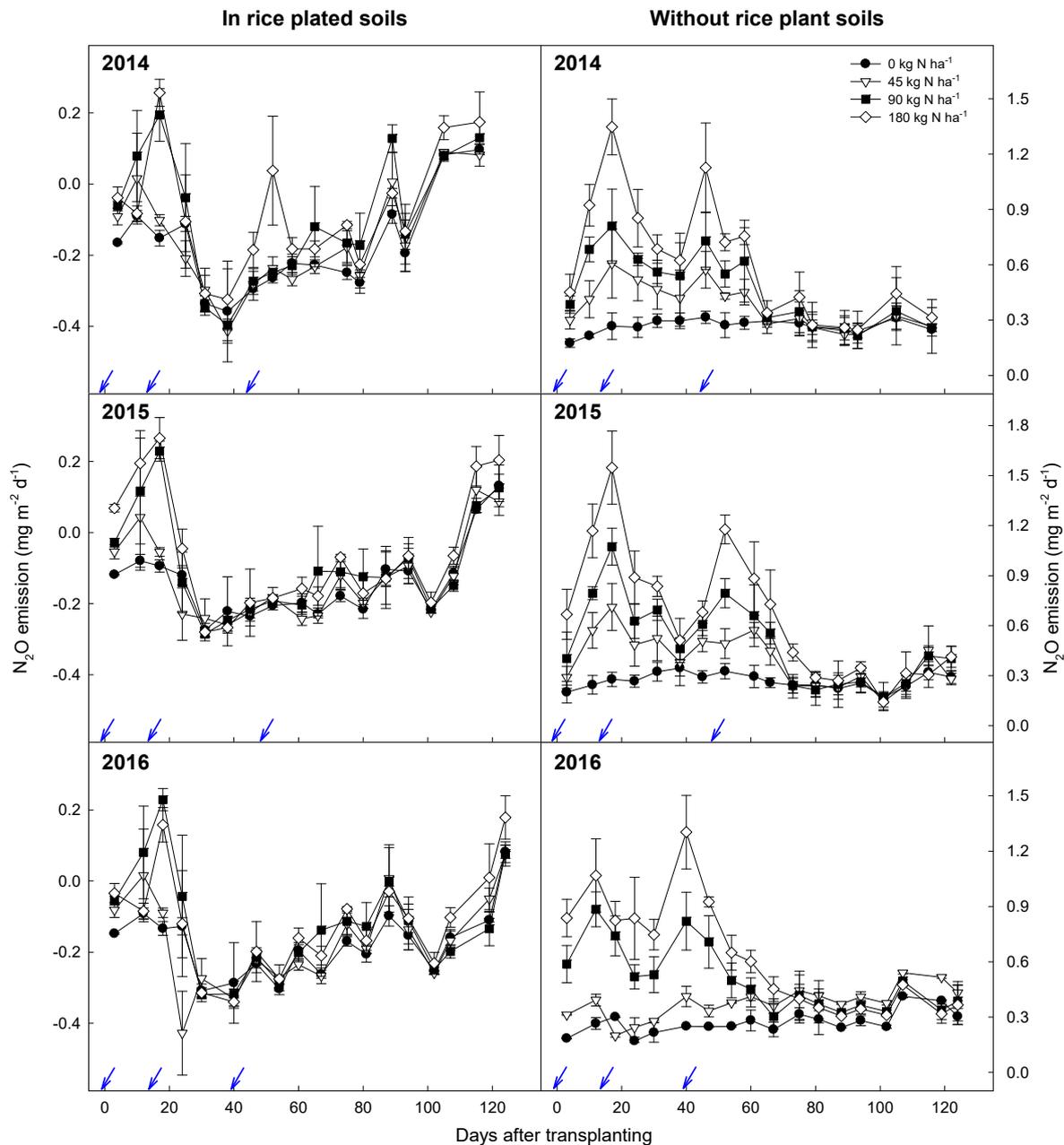


Figure 2. Changes in N₂O emission rates in rice-planted and non-planted soils under different levels of N application (↘ indicates N fertilizer application time).

In the case of non-planted soils, the flux of N₂O emission up to 60 days was proportional to the rates of N application, and two peaks of N₂O emission fluxes were recorded right after N fertilizer application during rice cultivation (Figure 2). The first peak of N₂O

emission was recorded within 15–20 days of rice cultivation, while the second peak was observed within 40–60 days after rice transplanting. The N_2O emission peaks were significantly controlled by increasing levels of N fertilization. The highest peak was observed at 180 kg N ha^{-1} in all years. After 70–80 days of rice growth, N_2O emission was not significantly different among the treatments.

3.4. Changes of Seasonal N_2O Fluxes

Seasonal N_2O fluxes significantly varied depending on whether the N_2O gas concentration was measured in rice-planted on non-planted soils (Figure 3). Seasonal N_2O flux in rice-planted soils had shown negative values, suggesting that N_2O may have been converted into other N forms. Though the numerical values of the negative N_2O fluxes were increased with increasing N application rates, the study did not show any treatment-wise variation or seasonal N_2O emission from the treatments, which varied from -233 to $-155 \text{ g N}_2\text{O ha}^{-1}$.

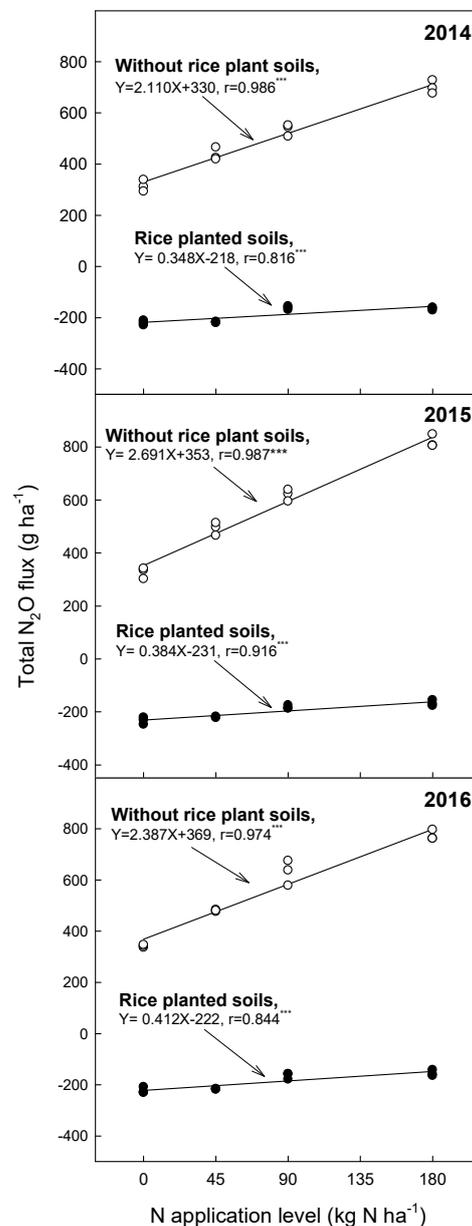


Figure 3. Seasonal N_2O fluxes from submerged rice paddy soil as affected by the rate of N application and the presence of plants. *** indicate significant difference at $p < 0.001$.

However, the seasonal N₂O flux showed large positive values in non-planted soils, and clearly increased with multiplicative N application (Figure 3). In the control treatment, the seasonal N₂O flux differed at 315–342 g N₂O ha⁻¹ and proportionately increased with increasing N application up to 700–820 g N₂O ha⁻¹ at 180 kg N ha⁻¹.

The N₂O flux gaps between rice-planted and non-planted soils were significantly increased with increasing N application level. In 2014, the N₂O flux gaps in the control (0 kg N ha⁻¹) treatment amounted to 0.53 kg N₂O ha⁻¹, and to 0.86 kg N₂O ha⁻¹ in 200% (180 kg N ha⁻¹) treatment. These trends were similar in all years, and decreasing N₂O fluxes were highly correlated to rice biomass properties such as straw, root, tiller number and rice height (Table 2).

Table 2. Correlation between rice growth or yield properties and decreased N₂O fluxes.

Properties	Year		
	2014	2015	2016
Grain	0.547 *	0.482	0.351
Straw	0.899 ***	0.946 ***	0.984 ***
Root	0.856 ***	0.865 ***	0.824 ***
Tiller number	0.898 ***	0.952 ***	0.870 ***
Rice height	0.937 ***	0.850 ***	0.787 **

*, **, and *** indicate significant difference at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

4. Discussion

Nitrous oxide is a gaseous intermediate in the reaction sequence of denitrification and a by-product of nitrification that leaks from microbial cells into the soil and atmosphere [29–31]. In these two pathways, N₂O in aerobic upland soil is generally formed following nitrification, while denitrification is the dominant pathway under an anaerobic soil environment [32]. However, one of the main controlling factors in this reaction is the availability of soil N [33]. Irrespective of chamber installation conditions, seasonal N₂O fluxes significantly increased with increasing N fertilizer application (Figure 3). The application of N fertilizer increased the concentration of nitrate (NO₃⁻-N), the precursor of N₂O, in the soil (Figure S1) and then increased N₂O emissions [4,34].

The static chamber method has been the most commonly used for measuring N₂O fluxes from agricultural fields, since this technique is relatively inexpensive, versatile in the field and easy to adopt. However, the existence and non-existence of plants inside the chambers can change the air and soil environments inside the chamber, particularly soil inorganic N, and influence N₂O fluxes significantly [15,16]. We confirmed a big difference of seasonal N₂O fluxes between rice-planted and non-planted chambers under the same N fertilization levels (Figure 3). The seasonal N₂O fluxes in the non-planted chambers showed positive values within 315–820 g N₂O ha⁻¹ under 0–180 kg N ha⁻¹ of urea application, and were significantly increased by increasing N fertilization with the average rate of 2.11–2.69 g N₂O kg⁻¹ N for 3 years. These values were significantly lower than IPCC default values from the flooded rice paddy field (4.71g N₂O kg⁻¹ N) [35]. In comparison, the seasonal N₂O fluxes in rice-planted chambers were proportionally increased by N application with the rate of 0.348–0.412 g N₂O kg⁻¹ N, but showed negative values within minus 155–233 g N₂O ha⁻¹ with 0–180 kg N ha⁻¹ of urea application. Several studies also showed a similar result, i.e., a negative N₂O flux value in the rice-planted chambers [36–38]. This means that the level of N₂O flux was consumed during rice cultivation, and rice fields acted as a sink of N₂O, not a source of N₂O emission. Since the suppression of N₂O flux occurred in the soil including rice plants, it could be hypothesized that the phenomenon happened in rice rhizosphere, and the roots of rice plants may have played an important role in the process. The application of N fertilizers improved plant physiology, i.e., it increased both the above-ground and root biomass of rice plants (Table 1) and the plants' N uptake. The comparison revealed a significantly positive correlation between root biomass and reduction in N₂O flux (Table 2). For further illustration, a three-dimensional (3D)

scattered plot was generated by considering doses of N fertilization as the x-axis and the root biomass as the y-axis. The points representing the decrease in annual N_2O flux followed a linear relation in the 3D plot (Figure 4).

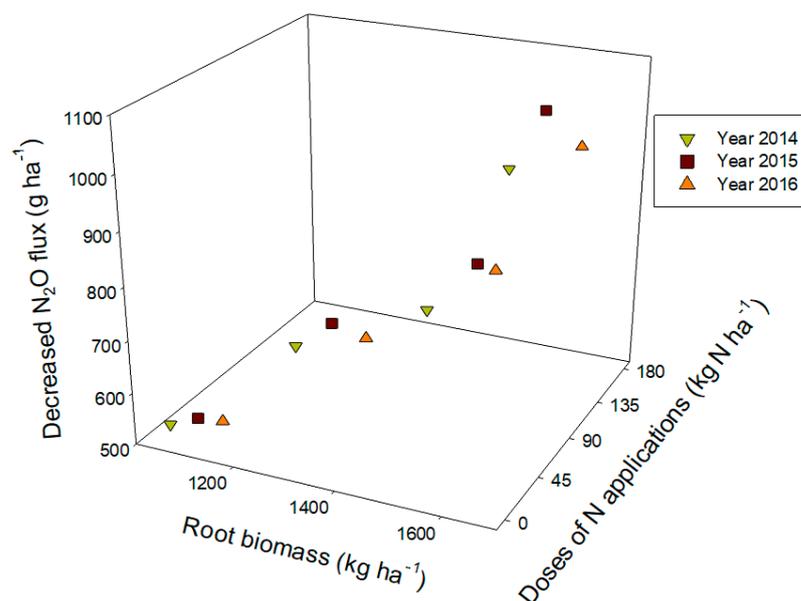


Figure 4. Relationship of the rates of N application and root biomass of rice plants with decreased N_2O flux by rice plants during rice cultivation.

It is well known that plants can significantly affect N_2O fluxes from soils [15,16]. Therefore, planting conditions inside the chambers may clearly differentiate the N_2O fluxes in soils. Plants assimilate huge amounts of N during the growing stage, reduce labile N concentration in soils and then suppress N_2O emission [39]. Plant leaves could also emit N_2O during N assimilation [16,40], but the relative significance of plant-derived N_2O production is not well understood. The magnitude of any reduced N_2O flux by planting will depend on plant species, the amount of biomass enclosed by the chamber, inorganic N forms in the soil and their amounts. Thus, researchers need to be aware of these issues when designing experiments specifically to look at the plants' effects on N_2O fluxes.

Rice has a bunched root system and air transmission through hollow aerenchyma to make the rhizosphere of rice plants partially aerobic [41]. We previously confirmed that the higher root biomass of different rice plants is responsible for increased aeration in their rhizosphere, and that, in turn, was attributed to the enhanced CH_4 oxidation in the soil [42]. Therefore, it could be inferred that the increased root biomass of higher doses of N-treated rice plants was possibly increased by a more aerobic environment in their rhizosphere, which leads to more oxidation of N_2O generated in the soil. Based on these observations, it could be concluded that an increased root biomass due to higher rates of N fertilization antagonistically influences N_2O formation by enhancing the aeration in the rhizosphere of rice plants.

5. Conclusions

Nitrogen fertilization significantly increased N_2O emissions in rice paddy soil during cropping season. Seasonal N_2O fluxes responded differently to N fertilization in soils with and without planted rice. In non-planted soils, seasonal N_2O fluxes showed positive values and were proportionally increased by increasing N fertilization, with an average rate of 2.11–2.69 g N_2O kg⁻¹ N. In rice-planted soils, seasonal N_2O fluxes were significantly increased by N fertilization, with the rate of 0.348–0.412 g N_2O kg⁻¹ N, but showed negative values ranging from minus 155 to minus 233 g N_2O ha⁻¹ at 0–180 kg N ha⁻¹ of urea application, indicating that rice cropping fields reacted as a N_2O sink, and not

as the source. Therefore, N₂O fluxes should be measured using the rice planted static chamber to obtain a more exact agricultural field environment. The difference in N₂O fluxes between rice-planted and non-planted soils might have been caused by rice rhizospheric activities, resulting in an increase of the N₂O consumption potentials of the rice plants' rhizosphere. This N₂O consumption potential clearly increased with the increase of the level of N fertilizer application, and positively correlated with root, straw and total biomass productivities. The decrease of N₂O fluxes at high levels of N fertilization in rice-planted soils might be caused by the decreasing denitrification potential in paddy fields, but further study is needed to figure out the specific mechanism.

Supplementary Materials: The following are available online at <https://www.mdpi.com/2073-4395/11/2/217/s1>, Figure S1: Changes in NO₃⁻-N concentration in rice-planted and non-planted soils under different levels of N fertilizer application.

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