

Does replacing chemical fertilizer with ryegrass (*Lolium multiflorum* Lam.) mitigate CH₄ and N₂O emissions and reduce global warming potential from paddy soil?

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Research Article

Keywords: Ryegrass, CH₄, N₂O, Green manure substitution ratio, C and N release

Posted Date: July 20th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1784777/v1>

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Abstract

(Purpose)

The incorporation of ryegrass (*Lolium multiflorum* Lam.; RG), a winter grass manure, could partly replace chemical N and reduce N loss during the succeeding rice seasons, but little is known about its impact on greenhouse gas emission. This study investigated the effect of different RG-urea substitution ratios (0%, 25%, 50%, 75% and 100%) on C and N release, CH₄ and N₂O emissions in a paddy soil.

(Methods)

Gas samples for CH₄ and N₂O fluxes measurement were collected by using a closed chamber and determined by chromatograph method. C and N release from the incorporated RG residue were tested by a mesh bag method.

(Results)

C and N release from RG followed a single exponential decay model, with 95.5%-97.8% of the original C and 98.7%-99.3% of N released during 192 days. RG-urea substitution ratio increased CH₄ emission, but was negatively correlated with N₂O emission. In comparison with 0% substitution, global warming potential (GWP) and greenhouse gas intensity (GHGI) were not significantly different for the 25% and 50% RG substitutions, but were significantly higher for the 75% and 100% substitutions ($P < 0.05$). Soil redox, C and N remaining in litter residue were key characteristics explaining CH₄ emission, while NH₄⁺-N and NO₃⁻-N concentrations were correlated with the variation of N₂O emission.

(Conclusion)

The increased CH₄ emission by RG incorporation could be offset by the reduced N₂O emission when RG-urea substitution ratio was 50% or less.

Introduction

Methane (CH₄) and nitrous oxide (N₂O) are important greenhouse gases (GHGs) responsible for global climate change (Stocker 2014). Rice paddy soil is one of the important sources for CH₄ and N₂O production, and accounts for a significant proportion of global GHG emissions (Carter et al. 2014). Rice paddies are responsible for 48% of total anthropogenic CH₄ emission from agriculture (Carlson et al. 2016), while N₂O emission from paddy soils contributes 19%-25% of the of the global N₂O emissions (Van Groenigen et al. 2011). Therefore, it is crucial to mitigate GHG emissions from paddy soils in most rice production regions to maintain a sustainable grain food supply.

Green manure incorporation and chemical nitrogen (N) fertilizer applications are common soil management practices that may impact CH₄ and N₂O emissions. First, the release of organic carbon (C)

and inorganic N from these fertilizers determines the availability of substrates for CH₄ and N₂O releasing microorganisms, such as methanogenic bacteria (Liu et al. 2022) and methanogenic *Archaea* (Zhang et al. 2018), ammonia oxidizers (Gao et al. 2020) and denitrifiers (Huang et al. 2020). Several studies have reported increased CH₄ emission after organic amendments such as green manure (Haque et al. 2012), crop residue and animal manure incorporation (Bhattacharyya et al. 2012; Nguyen et al. 2020). N₂O emission peaks are usually observed immediately after intensive chemical N fertilizer application (Shaukat et al. 2019). Meanwhile, soil conditions that drive CH₄ and N₂O formation and consumption may be greatly changed following organic matter returning and fertilizer application. For example, during the early stage of organic matter decomposition, the reduced dissolved oxygen concentration and soil pH and the increased dissolved organic C availability in the paddy soil (Xu et al. 2017) provide a favorable condition for methanogens, thereby enhancing CH₄ production during the rice growing seasons (Zhou et al. 2020).

The rapidly rising soil NH₄⁺-N and NO₃⁻-N concentrations after chemical N fertilizer application benefit nitrification and denitrification, and increase the potential of N loss in the form of N₂O (Cecilio et al. 2022; Zhu et al. 2013). Replacing chemical N with green manures has potential to reduce N₂O emission because inorganic N release from the incorporated organic matter is much slower than from chemical fertilizers (Mehnaz et al. 2019; Zhu et al. 2014). However, acetate released during anaerobic decomposition of organic matter acts as a terminal electron acceptor for respiration and provides substrate for methanogens activities, resulting in increased CH₄ emissions (Amin et al. 2021).

The ratio of substitution of organic for chemical fertilizer is an important factor for determining C and N dynamics after fertilization (Lashermes et al. 2010) as well as the consequent changes in soil environment (Hou et al. 2022). In addition, the complex interaction between organic material and inorganic N under different organic-chemical N substitution ratio greatly affects CH₄ and N₂O emissions (Zou et al. 2009).

An important aspect concerning GHG production in paddy soil is the comparison of radiative forcing from different GHGs. The global warming potential (GWP) calculation integrates the radiative forcing of CH₄ and N₂O emissions into “CO₂-equivalents” (Robertson and Grace 2004). Greenhouse gas intensity (GHGI) is calculated by dividing GWP by grain yield and is used to relate GHG emission and food production (Shang et al. 2011).

Ryegrass (*Lolium multiflorum* Lam.; RG), a commonly used green manure in paddy soils, has the potential to partly replace chemical N fertilizers in most Asian countries. Increased rice yield and N use efficiency have been observed after ryegrass incorporation alone or in combination with urea (Asagi and Ueno 2009; Zhu et al. 2014). However, the impacts of RG-urea substitution ratio on CH₄ and N₂O emissions remains poorly understood. The objectives of this study were to: (1) estimate changes in CH₄ and N₂O emissions during rice growing seasons under different RG-urea substitution ratios; (2) quantify C

and N release from RG residue under different RG-urea substitution ratios; (3) measure the relationship between GHGs and soil properties after RG incorporation.

Materials And Methods

Experimental description

Two open glasshouse pot experiments were conducted at the experimental station of Yangtze University, Jingzhou, Hubei Province, China (30°20'N, 112°12'E) in 2021. The regional climate was subtropical with annual mean air temperature 16.5°C and precipitation 1200 mm. The paddy soil (0-15cm) was collected from a nearby farmer's paddy field followed by manual removal of stems, roots, stones, etc. The soil was then air-dried and sieved to pass 2 cm mesh. Soil properties were: sand 265 g kg⁻¹, clay 134 g kg⁻¹, silt 601 g kg⁻¹, pH 6.2, total N 1.4 g kg⁻¹, organic C content of 14.2 g kg⁻¹, available phosphorus 15.6 mg kg⁻¹ and available potassium 87.0 mg kg⁻¹. The fresh RG (*cv. Muteli*) aboveground litter had moisture content 79%, dry matter N content 4.94% and dry matter C content 38.1%.

Experiment 1: effects of RG-urea substitution ratio on CH₄ and N₂O emission

The experiment comprised five RG-urea substitution ratios: 0%, 25%, 50%, 75% and 100% with 3 replicates for each treatment. All the treatments received a total amount N at a rate of 100 mg N kg⁻¹ air-dried soil. The details for RG and urea application amount in each treatment were shown in Table 1. Plastic pots (20 cm in diameter and 15 cm in height), each containing 3 kg air-dried soil was used. The aboveground litter of RG was weighed, cut into 2–3 cm pieces and thoroughly mixed with soil in mid-April 2021. Superphosphate and potassium chloride were applied at 0.61 g kg⁻¹ and 0.21 g kg⁻¹ air-dried soil, respectively. On April 25, three rice seedlings (*cv. Tianliangyou 616*, 30 d old) were transplanted per pot. The seedlings were watered to 2 cm flooding depth and received no topdressing fertilizer during the whole growing season. The plants were harvested at 40 cm cutting height for the first maturity, leaving the stubbles for ratooning to get a second harvest.

Table 1
The amount of C and N contributions for each treatment by RG or urea application

Treatment	N applied from urea (g pot ⁻¹)	N applied from RG (g pot ⁻¹)	C applied from RG (g pot ⁻¹)
0% RG	0.3	0	0
25% RG	0.225	0.075	0.58
50% RG	0.15	0.15	1.16
75% RG	0.075	0.225	1.74
100% RG	0	0	2.31

Experiment 2: C and N release during RG decomposition

In experiment 2, the mesh bag method (Zhu et al. 2014) was used to measure C and N release dynamics from the incorporated RG litter. Treatments were the same as in experiment 1. RG aboveground litter was cut and placed in 0.5mm mesh size nylon bags (10 cm × 10 cm) and buried in the soil with 39 replications for each treatment.

Sampling and analysis

In Experiment 1, gas samples for CH₄ and N₂O fluxes measurement were collected using a static chamber (Zhang et al. 2020) at 3–7 d intervals after RG incorporation until the harvest of the ratoon crop. The pot with plant was placed in a closed PVP pipe chamber (height 110 cm, diameter 25 cm). An electric fan at the top of the chamber was used to mix the air in the chamber. The gas samples were collected from the chamber at 0, 10 and 20 min between 9:00 and 11:00 am and transferred to 0.5 L sample bags. CH₄ and N₂O concentrations were determined by gas chromatography (Agilent 7890 B, USA) with a flame ionization detector (FID) at 200°C and an electron capture detector (ECD) at 330°C, respectively. Soil pH and redox state (Eh) were simultaneously measured by pH meter (Leici PHS-25, China) and ORP meter (Leici TR-901, China), respectively.

In Experiment 2, RG residue in nylon mesh bags was collected 14, 24, 29, 34, 42, 48, 55, 69, 83, 111, 139, 167 and 192 days after incorporation (n = 3). The residue was carefully washed with tap water, oven-dried at 80°C to constant weight, weighed, ground and sieved (100 mesh). C and N contents in RG residue were determined by an elemental analyzer (Costech ECS4010, Italy). Soil samples were collected at three random locations for each pot and mixed thoroughly for ammonium (NH₄⁺-N) and nitrate (NO₃⁻-N) concentrations measurements by using a UV spectrophotometer (Hash DR6000, China) after extraction with 2 mol L⁻¹ KCl.

Calculations

The CH₄ and N₂O fluxes were calculated in detail as follows:

$$F = p \times h \times dc/dt \times 273 / (273 + T)$$

where F is CH₄ flux (mg m⁻² h⁻¹) or N₂O flux (μg m⁻² h⁻¹); p is density of CH₄ or N₂O under the standard temperature and pressure (CH₄ 0.714 kg/m³, N₂O 1.964 kg/m³); h is the height of the chamber (cm); dc/dt is the change of CH₄ or N₂O concentration (mg m⁻³) over time (h); T is the air temperature inside the chamber (°C).

The seasonal CH₄ or N₂O emission was calculated as follows:

$$\text{SeasonalCH}_4\text{orN}_2\text{Ocumulativeemissions} = \sum_{i=1}^n \frac{F_i + F_{i-1}}{2} \times (D_{i+1} - D_i)$$

where F is the CH_4 ($\text{mg m}^{-2} \text{h}^{-1}$) or N_2O ($\mu\text{g m}^{-2} \text{h}^{-1}$) flux, i is the sampling time, $D_{i+1}-D_i$ is the number of days between two adjacent sampling time (h), and n is the total number of sampling intervals.

Global warming potential (GWP) was calculated as the sum of N_2O ($\text{GWP}_{\text{N}_2\text{O}}$) and CH_4 (GWP_{CH_4}) as IPCC (2021):

$$\text{GWP}(\text{gCO}_2 - \text{eqm}^{-2}) = 29.8 \times \text{CH}_4 (\text{gm}^{-2}) + 273 \times \text{N}_2\text{O} (\text{gm}^{-2})$$

The greenhouse gas intensity (GHGI) was calculated as follows:

$$\text{GHGI}(\text{gGWPpergofyield}) = \text{GWP}(\text{gCO}_2 - \text{eqm}^{-2}) / \text{grainyield} (\text{gm}^{-2})$$

Percentage of C and N remaining in RG residues were calculated as described by Zhu et al. (2014):

$$Y_c(\%) = \frac{Y_t}{Y_i} \times 100$$

where Y_c is the C or N remaining percent of RG residue, Y_t is the C or N content of RG residues at each sampling time, Y_i is the original C or N content in RG.

An exponential decay model was used to describe C and N release from RG litter over time as follows:

$$YR = \exp^{-kt} \times 100$$

where YR is C or N remaining in RG residue at time t and k is the C (k_C) or N (k_N) release rate.

Statistical analysis

Analysis of variance (ANOVA) for RG substitution ratio on CH_4 and N_2O emission fluxes, GWP, GHGI, grain yield and soil properties was conducted with SPSS Statistics 19.0 (IBM Co., NY, USA). The multiple comparisons significant differences were set at $P < 0.05$. Pearson correlation analysis was used to examine the correlation between greenhouse gas emissions and soil properties. Canoco 5 (Beijing Huanzhong Ruichi Technology Co., LTD, China) was used to make the redundancy analysis (RDA) between GHG emissions and soil properties.

Result

CH_4 and N_2O emissions

A major peak was observed at 38 d after urea and RG application for CH_4 emissions (Fig. 1a), and at 17 d for N_2O emissions (Fig. 2a). Several minor peaks were also detected for CH_4 and N_2O emissions during the whole observing seasons. The highest CH_4 peaks were found in the 100% RG treatment (41.3 mg m^{-2}

h^{-1}), followed by 75% RG, 50% RG, 25% RG and 0% RG (Fig. 1a). By contrast, N_2O peaks were observed to be highest in 0% RG ($784.9 \mu\text{g m}^{-2} \text{h}^{-1}$) and decreased with the rising RG-urea substitution ratio (Fig. 2a).

Most of the CH_4 (54.3%-63.7%) was emitted before the tillering stage and 73.7%-82.1% of the CH_4 emission took place before the first season maturity. Regression analysis showed that there was a significant quadratic relationship between RG-urea substitution ratio and total CH_4 emission ($R^2 = 0.99$, $p < 0.01$; Fig. 1b). When it came to N_2O , 65.4%-79.6% of the seasonal emissions occurred before the tillering stage and 91.5%-98.3% of the total emissions were contributed before the first season maturity. A significant quadratic relationship was identified between RG-urea substitution ratio and total N_2O emission (Fig. 2b; $R^2 = 0.99$, $p < 0.01$).

Global warming potential and greenhouse gas intensity

The GWP for seasonal CH_4 and N_2O emissions showed a significant quadratic relationship with RG-urea substitution ratio (Fig. 3a; $R^2 = 0.99$, $p < 0.05$). The values for GWP were lowest in the 25% and 50% RG treatments (6.3% and 6.5% lower than 0% RG, respectively), which were significantly lower than those in 75% and 100% RG. As shown in Table 2, contribution of CH_4 emission to the GWP (71.7%-96.7%) was much higher than that from N_2O emission (3.3%-28.3%). CH_4 and N_2O emissions from the first 45 d of the experiment accounted for 47.5%-56.4% of the total GWP while comparable total GWP (43.6%-52.5%) were detected in the relatively longer following 46–125 d (Fig. 3b).

Table 2

Total global warming potential (GWP), CH₄ and N₂O-induced GWP, grain yield, greenhouse gas intensity (GHGI) as affected by ryegrass (RG)-urea substitution

Treatment	CH ₄ -induced GWP ^a		N ₂ O -induced GWP ^b		Total GWP (g CO ₂ -eq m ⁻²)	Grain yield (g m ⁻²)	GHGI (kg CO ₂ -eq kg ⁻¹ yield)
	GWP (g CO ₂ -eq m ⁻²)	Account for total GWP ^c (%)	GWP (g CO ₂ -eq m ⁻²)	Account for total GWP (%)			
0% RG	277.1 d ^d	71.7	109.4 a	28.3	386.5 c	796.3 a	0.49 c
25% RG	291.6 d	80.5	70.5 b	19.5	362.1 c	773.7 a	0.47 c
50% RG	323.0 c	89.3	38.5 c	10.7	361.5 c	747.2 a	0.48 c
75% RG	455.0 b	93.7	30.8 c	6.3	485.8 b	639.2 b	0.76 b
100% RG	595.6 a	96.7	20.6 c	3.3	616.2 a	594.9 b	1.04 a
^a Global warming potential calculated from CH ₄ emission							
^b Global warming potential calculated from N ₂ O emission							
^c The percentage of CH ₄ or N ₂ O induced GWP to the total GWP							
^d Different letters within the same column mean significant differences at $p < 0.05$ level.							

Rice grain yield in the 25% and 50% RG treatments was not significantly different from that in the 0% RG treatment, but was significantly higher than that in the 75% and 100% RG treatments (Table 2; $p < 0.05$). Similar as GWP, GHGI was higher in the 75% and 100% RG treatments as compared to the 0%, 25% and 50% RG treatments (Table 2).

C and N release from RG residue

The majority of C (83.2%-86.9%) and N (97.4%-97.5%) from the incorporated RG residue was released during the first 29 d after start of the experiment (Figs. 4a and b). At the end of experiment, less than 4.5% of C and 1.3% of N remaining remained in RG residue. The decay exponential model successfully described C ($R^2 = 0.92-0.94$, $p < 0.05$) and N ($R^2 = 0.98-0.99$, $p < 0.05$) release dynamics after RG incorporation in the paddy soil (Table 3). The values for k_N were generally higher than for k_C (0.0877–

0.1368 vs 0.0689-0.0800), indicating N release from RG residue was faster than C release. The values for k_N increased with higher RG-urea substitution ratio while the opposite was true for k_C (Table 3).

Table 3
The rate of C (k_C) and N releases (k_N) from ryegrass (RG) residue under different RG-urea substitution ratios, estimated using a single exponential model

Treatment	K_C	r^2	K_N	r^2
25% RG	0.0800	0.9177	0.0877	0.9791
50% RG	0.0697	0.9400	0.1104	0.9948
75% RG	0.0706	0.9255	0.1368	0.9978
100% RG	0.0689	0.9251	0.1254	0.9975

Importance of soil properties for CH₄ and N₂O emissions

Soil NH₄⁺-N concentrations were much higher than NO₃⁻-N concentrations during the experiment (Figs. 5a and b). Both NH₄⁺-N and NO₃⁻-N increased steadily and peaked 14–29 d after RG and urea application. NH₄⁺-N and NO₃⁻-N appeared to be highest in 0% RG and lowest in 100% RG treatment. Redox state (Eh value) decreased gradually within 30–40 d after the start of the experiment and increased steadily thereafter. Lower Eh values tended to be observed in higher RG-urea substitution ratios (Fig. 5c). Soil pH was reduced during the first two months especially for the 25%, 50% and 100% RG treatments (Fig. 5d).

Distance-based RDA showed that the first two components accounted for 99.5% of the variation in CH₄ and N₂O emissions (Figs. 6a and c). C remaining ($F = 49.8$, $p = 0.002$), N remaining ($F = 7.8$, $p = 0.014$) and soil Eh ($F = 6.9$, $p = 0.032$) had a significant impact on CH₄ emission when NH₄⁺-N ($F = 15.4$, $p = 0.004$) and NO₃⁻-N ($F = 6.6$, $p = 0.034$) showed a significant impact on N₂O emission (Table 4). Variance decomposition showed that 90.3% of the variance of CH₄ emission could be explained by C and N remaining in RG residue, soil Eh and other indicators (Fig. 6b), while 84% of the variance of N₂O emission was explained by soil NH₄⁺-N, NO₃⁻-N and C remaining in RG residue (Fig. 6d).

Table 4
Results of ANOVA RDA model

Soil factors	CH ₄		N ₂ O	
	F	<i>p</i>	F	<i>p</i>
Eh	6.9	0.032 ^{*a}	3.6	0.092
pH	0.8	0.402	0.3	0.568
NH ₄ ⁺ -N	1.8	0.214	15.4	0.004 ^{**}
NO ₃ ⁻ -N	0.6	0.402	6.6	0.034 [*]
C remaining	49.8	0.002 ^{**b}	5.1	0.056
N remaining	7.8	0.014 [*]	1.5	0.276
^a Significant differences at <i>p</i> < 0.05 level				
^b Significant differences at <i>p</i> < 0.01 level				

Discussion

Effects of RG-urea substitution ratio on CH₄ emission

Incorporation of organic amendments such as green manure and crop straw in an important soil practice to increase biological C fixing in agricultural fields, but the enhanced active C input may stimulate CH₄ production in paddy soils (Fu et al. 2018). This was confirmed by the rapidly increasing CH₄ emission after RG incorporation, especially in the treatments with high RG-urea substitution ratios (Fig. 1). Dramatically increased CH₄ emissions have also been reported in flooded paddy soils with fresh green manure incorporation (Lee et al. 2010; Liu et al. 2019). In this study, major CH₄ emissions took place at the time of rapid C release from RG residue, suggesting that C input by RG incorporation was an important source for CH₄ production (Fig. 1a, Fig. 4a). RG-urea substitution ratio affected the rate of green manure decomposition, which consequently determined the availability of soil C substrates for methanogens (Dalal et al. 2008; Xu et al. 2017).

Soil condition is another factor affecting the activity of methanogens and methane-oxidizing bacteria which are responsible for CH₄ production and consumption in the soil. In the present study, Effects of soil condition changes by RG-urea substitution ratio on GHG emission could be better measured in a pot study than in field experiments, because some undesirable interferences such as rainfall and leaching could be well prevented. In the present study, rapid decomposition of RG litter may have reduced dissolved oxygen concentration in the flooded paddy soil, resulting in a decrease of soil Eh (Fig. 5c). A

low Eh provides a suitable condition for methanogens and reduces CH₄ oxidation in the soil (Muhammad et al. 2020). Significantly higher CH₄ emissions in low redox soils have also been reported by Fan et al. (2020) and Xu et al. (2017). About 5% of the variance in CH₄ emission was explained by changes in soil Eh while 0.6% was explained by pH (Fig. 6b). Decreased soil pH by RG incorporation (Fig. 5d) was attributed to the formation of small organic acids such as acetic acid and butanoic acid during the process of RG fermentation. This lower pH and increased acetic acid availability stimulates the activity of methanogens (Baumann et al. 2009; Qualls 2005).

Effects of RG-urea substitution ratio on N₂O emission

Green manure applied alone or in conjunction with chemical fertilizers has been reported to improve total N use efficiency and decrease N losses into the environment (Gao et al. 2020; Liang et al. 2022; Zhu et al. 2012). However, the results of observations on N₂O emission, a form of gaseous N loss in paddy fields, are inconsistent. Some reported significant N₂O emissions after fertilization with chemical N (Shaukat et al. 2019; Xu et al. 2022). Others found negligible N₂O emissions over the entire rice growing season (Chen et al. 2021; Cowan et al. 2021). In the current study, a major N₂O peak was detected following urea application while several minor peaks were afterwards (Fig. 2a). Similar as with Zhang et al. (2020), both the major peak and seasonal N₂O emissions were significantly decreased with the increase of RG-urea substitution ratio, in spite that the soils received a same amount of total N input (Fig. 2). Soil mineral N concentrations, especially NH₄⁺-N content, were significantly decreased with RG-urea substitution ratio in the peaking stage (Fig. 5a). This may result in a reduced N available for nitrification or coupled nitrification-denitrification which are the main mechanisms for N₂O production in agricultural soils (Gao et al. 2020; Nie et al. 2021). In addition, the lower redox condition which may reduce the activity of ammonia oxidizers (Tao et al. 2018). These results supported the beneficial effect of replacing chemical N with green manure on N₂O mitigation. To trace N₂O production from different N sources in a paddy ecosystem, ¹⁵N labeled green manure or chemical fertilizer should be used in further studies.

Global warming potential and greenhouse gas intensity

The assessment of GWP is important because of the dramatically different effects of RG substitution on CH₄ and N₂O emissions in this study. The majority of GWP (71.7%-96.7%) were caused by CH₄ emission, confirming that CH₄ is the dominant GHG responsible for radiative forcing in the studied paddy soil (Peyron et al. 2016; Wang et al. 2016). Nevertheless, GWP in 25% and 50% RG-urea substitution ratios were slightly decreased when compared with 0% RG (361.5-362.1 g CO₂-eq m⁻² vs 386.5 g CO₂-eq m⁻²) while CH₄-induced GWP increased with RG-urea substitution ratio (Table 2). This confirms a positive trade-off between CH₄ emission and N₂O emission for the 25% and 50% RG-urea substitution treatments (Shang et al. 2011). However, RG-urea substitution ratios higher than 50% tended to increase GWP due to the rising CH₄ emission. Nearly half of the GWP was observed during the first 45 d after fertilization (Fig. 3b), suggesting that GHG mitigation strategies in paddy soil with organic matter incorporation

should be focused on the early stages of organic material decomposition and rice growth. Shang et al. (2011) suggest incorporating organic matter when the soil is drained and before rice is planted in order to minimize CH₄ and N₂O emissions. Because rice grain yields were not significantly decreased by 25% and 50% RG-urea substitution ratios, their GHGI values were comparable with 0% RG (Table 2). The increased GHGI under 75% and 100% RG-urea substitution could both be attributed to the promoted GWP and the reduced grain yields.

Conclusions

This study demonstrated that RG-urea substitution ratio played an important role in CH₄ and N₂O emissions. CH₄ emissions were positively related with RG-urea substitution ratio, while the opposite was true for N₂O emissions. Compared with 0% RG, the increased CH₄ emissions in the 25% RG and 50% RG substitution ratios were offset by decreased N₂O emissions, leading to comparable GWP and GHGI values. The RG-urea substitution ratio affected C and N release from RG residues, which further affected CH₄ and N₂O emissions in the paddy soil. Soil Eh, C and N remaining were key characteristics correlated with CH₄ emission while NH₄⁺-N, NO₃⁻-N and C remaining were main factors on N₂O emissions. As CH₄ was the main contributor to GWP in paddy soils, further studies should be taken to reduce CH₄ fluxes, especially in the early stage of organic matter returning.

Abbreviations

RG, ryegrass; GWP, global warming potential; GHGI, greenhouse gas intensity.

Declarations

Acknowledgements

The authors thank Dr. Paul Dijkstra from Northern Arizona University for his revision and comments on the manuscript. Thanks for the sponsorship and support of the National Natural Science Foundation of China (No. 31870424) and the Young Elite Scientists Sponsorship Program by CAST (2016QNRC001).

Author Contributions

Bo Zhu and Zhangyong Liu designed the experiments. Wei Yang, Lai Yao, Mengzhen Zhu, Xueru Ji, Chengwei Li and Shaoqiu Li performed the experiments and data collection. Wei Yang and Bin Wang analyzed the data and Bo Zhu wrote the first draft of the manuscript.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

References

1. Amin FR, Khalid H, El-Mashad HM, Chen C, Liu G, Zhang R (2021) Functions of bacteria and archaea participating in the bioconversion of organic waste for methane production. *Sci Total Environ* 763:143007. <https://doi.org/10.1016/j.scitotenv.2020.143007>
2. Asagi N, Ueno H (2009) Nitrogen dynamics in paddy soil applied with various ¹⁵N-labelled green manures. *Plant Soil* 322:251–262. <https://doi.org/10.1007/s11104-009-9913-4>
3. Baumann K, Marschner P, Smernik RJ, Baldock JA (2009) Residue chemistry and microbial community structure during decomposition of eucalypt, wheat and vetch residues. *Soil Biol Biochem* 41:1966–1975. <https://doi.org/10.1016/j.soilbio.2009.06.022>
4. Bhattacharyya P, Roy KS, Neogi S, Adhya TK, Rao KS, Manna MC (2012) Effects of rice straw and nitrogen fertilization on greenhouse gas emissions and carbon storage in tropical flooded soil planted with rice. *Soil Tillage Res* 124:119–130. <https://doi.org/10.1016/j.still.2012.05..015>
5. Carlson KM, Gerber JS, Mueller ND, Herrero M, MacDonald GK, Brauman KA, Havlik P, O Connell CS, Johnson JA, Saatchi S, West PC (2016) Greenhouse gas emissions intensity of global croplands. *Nat Clim Change* 7:63–68. <https://doi.org/10.1038/nclimate3158>
6. Carter MS, Sørensen P, Petersen SO, Ma X, Ambus P (2014) Effects of green manure storage and incorporation methods on nitrogen release and N₂O emissions after soil application. *Biol Fert Soils* 50:1233–1246. <https://doi.org/10.1007/s00374-014-0936-5>
7. Cecilio FA, Nascimento CS, Pereira BJ, Nascimento CS (2022) Nitrogen fertilization impacts greenhouse gas emissions, carbon footprint, and agronomic responses of beet intercropped with arugula. *J Environ Manag* 307:114568. <https://doi.org/10.1016/j.jenvman.2022.114568>
8. Chen D, Zhou Y, Xu C, Lu X, Liu Y, Yu S, Feng Y (2021) Water-washed hydrochar in rice paddy soil reduces N₂O and CH₄ emissions: A whole growth period investigation. *Environ Pollut* 274:116573. <https://doi.org/10.1016/j.envpol.2021.116573>
9. Cowan N, Bhatia A, Drewer J, Jain N, Singh R, Tomer R, Kumar V, Kumar O, Prasanna R, Ramakrishnan B, Kumar D, Bandyopadhyay SK, Sutton M, Pathak H (2021) Experimental comparison of continuous and intermittent flooding of rice in relation to methane, nitrous oxide and ammonia emissions and the implications for nitrogen use efficiency and yield. *Agr Ecosyst Environ* 319:107571. <https://doi.org/10.1016/j.agee.2021.107571>
10. Dalal RC, Allen DE, Livesley SJ, Richards G, Eckard R, Livesley S, Arndt S, Livesley S, Arndt S, Eckard R (2008) Magnitude and biophysical regulators of methane emission and consumption in the Australian agricultural, forest, and submerged landscapes: a review. *Plant Soil* 309:43–76. <https://doi.org/10.1016/10.1007/s11104-007-9446-7>

11. Fan LC, Dippold MA, Ge TD, Wu JS, Thiel V, Kuzyakov Y, Dorodnikov M (2020) Anaerobic oxidation of methane in paddy soil: Role of electron acceptors and fertilization in mitigating CH₄ fluxes. *Soil Biol Biochem* 141:107685. <https://doi.org/10.1016/j.soilbio.2019.107685>
12. Fu B, Conrad R, Blaser M (2018) Potential contribution of acetogenesis to anaerobic degradation in methanogenic rice field soils. *Soil Biol Biochem* 119:1–10. <https://doi.org/10.1016/j.soilbio.2017..10.034>
13. Gao SJ, Zhou GP, Robert MR, Cao WD (2020) Green manuring inhibits nitrification in a typical paddy soil by changing the contributions of ammonia-oxidizing archaea and bacteria. *Appl Soil Ecol* 156:103698. <https://doi.org/10.1016/j.apsoil.2020.103698>
14. Haque MM, Kim SY, Pramanik P, Kim GY, Kim PJ (2012) Optimum application level of winter cover crop biomass as green manure under considering methane emission and rice productivity in paddy soil. *Biol Fert Soils* 49:487–493. <https://doi.org/10.1007/s00374-012-0766-2>
15. Hou PF, Xue LX, Wang J, Petropoulos E, Deng XZ, Qiao J, Xue LH, Yang LZ (2022) Continuous milk vetch amendment in rice-fallow rotation improves soil fertility and maintains rice yield without increasing CH₄ emissions: Evidence from a long-term experiment. *Agr Ecosyst Environ* 325:107774. <https://doi.org/10.1016/j.agee.2021.107774>
16. Huang R, Wang Y, Gao X, Liu J, Wang Z, Gao M (2020) Nitrous oxide emission and the related denitrifier community: A short-term response to organic manure substituting chemical fertilizer. *Ecotox Environ Safe* 192:110291. <https://doi.org/10.1016/j.ecoenv.2020.110291>
17. Intergovernmental Panel on Climatic Change (2021) Climate change 2021: The physical science basis: Working group I contribution to the sixth assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press Cambridge New York
18. Lashermes G, Nicolardot B, Parnaudeau V, Thuriès L, Chaussod R, Guillotin ML, Linères M, Mary B, Metzger L, Morvan T, Tricaud A, Villette C, Houot S (2010) Typology of exogenous organic matters based on chemical and biochemical composition to predict potential nitrogen mineralization. *Bioresource Technol* 101:157–164. <https://doi.org/10.1016/j.biortech.2009.08.025>
19. Lee CH, Park KD, Jung KY, Ali MA, Lee D, Gutierrez J, Kim PJ (2010) Effect of Chinese milk vetch (*Astragalus sinicus* L.) as a green manure on rice productivity and methane emission in paddy soil. *Agr Ecosyst Environ* 138:343–347. <https://doi.org/10.1016/j.agee.2010.05.011>
20. Liang H, Li S, Zhang L, Xu CX, Lv YH, Gao SJ, Cao WD (2022) Long-term green manuring enhances crop N uptake and reduces N losses in rice production system. *Soil Tillage Res* 220:105369. <https://doi.org/10.1016/j.still.2022.105369>
21. Liu X, Shen LD, Yang WT, Tian MH, Jin JH, Yang YL, Liu JQ, Hu ZH, Wu HS (2022) Effect of elevated atmospheric CO₂ concentration on the activity, abundance and community composition of aerobic methanotrophs in paddy soils. *Appl Soil Ecol* 170:104301. <https://doi.org/10.1016/j.apsoil.2021.104301>
22. Liu Y, Tang HY, Muhammad A, Zhong C, Li P, Zhang P, Yang BJ, Huang GQ (2019) Rice yield and greenhouse gas emissions affected by Chinese milk vetch and rice straw retention with reduced

- nitrogen fertilization. *Agron J* 111:3028–3038. <https://doi.org/10.2134/agronj2019.03.0145>
23. Mehnaz KR, Corneo PE, Keitel C, Dijkstra FA (2019) Carbon and phosphorus addition effects on microbial carbon use efficiency, soil organic matter priming, gross nitrogen mineralization and nitrous oxide emission from soil. *Soil Biol Biochem* 134:175–186. <https://doi.org/10.1016/j.soilbio.2019.04.00>
24. Muhammad Q, Huang J, Waqas A, Li DC, Liu SJ, Zhang L, Cai AD, Liu LS, Xu YM, Gao JS, Zhang HM (2020) Yield sustainability, soil organic carbon sequestration and nutrients balance under long-term combined application of manure and inorganic fertilizers in acidic paddy soil. *Soil Tillage Res* 198:104569. <https://doi.org/10.1016/j.still.2019.104569>
25. Nguyen BT, Trinh NN, Bach QV (2020) Methane emissions and associated microbial activities from paddy salt-affected soil as influenced by biochar and cow manure addition. *Appl Soil Ecol* 152:103531. <https://doi.org/10.1016/j.apsoil.2020.103531>
26. Nie JW, Zhou Y, Yang W, Li SQ, Li HX, Wu JW, Li CW, Yan XY, Zhu R, Zhu B, Liu ZY (2021) Effect of fertilization regimes and seasonal change on nosZ-denitrifying bacterial community in a double-rice paddy field. *J Soil Sci Plant Nut* 22(1):324–333. <https://doi.org/10.1007/s42729-021-00651-5>
27. Peyron M, Bertora C, Pelissetti S, Said-Pullicino D, Celi L, Miniotti E, Romani M, Sacco D (2016) Greenhouse gas emissions as affected by different water management practices in temperate rice paddies. *Agr Ecosyst Environ* 232:17–28. <https://doi.org/10.1016/j.agee.2016.07.021>
28. Qualls RG (2005) Biodegradability of fractions of dissolved organic carbon leached from decomposing leaf litter. *Environ Sci Technol* 39:1616–1622. <https://doi.org/10.1021/es049090o>
29. Robertson GP, Grace PR (2004) Greenhouse gas fluxes in tropical and temperate agriculture: The need for a full-cost accounting of global warming potentials. *Environ Dev Sustain* 6:51–63. <https://doi.org/10.1023/B:ENVI.0000003629.32997e>
30. Shang QY, Yang XX, Gao CM, Wu PP, Liu JJ, Xu YC, Shen QR, Zou JW, Guo SW (2011) Net annual global warming potential and greenhouse gas intensity in Chinese double rice-cropping system: a 3-year field measurement in long-term fertilizer experiments. *Global Change Biol* 17:2196–2210. <https://doi.org/10.1111/j.1365-2486.2010.02374.x>
31. Shaukat M, Samoy-Pascual K, Maas E, Ahmad A (2019) Simultaneous effects of biochar and nitrogen fertilization on nitrous oxide and methane emissions from paddy rice. *J Environ Manage* 248:109242. <https://doi.org/10.1016/j.jenvman.2019.07.013>
32. Stocker T (2014) Climate change 2013: The physical science basis: working group I contribution to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press New York. <https://doi.org/10.1017/CBO9781107415324>
33. Tao R, Wakelin SA, Liang Y, Hu B, Chu G (2018) Nitrous oxide emission and denitrifier communities in drip-irrigated calcareous soil as affected by chemical and organic fertilizers. *Sci Total Environ* 612:739–749. <https://doi.org/10.1016/j.scitotenv.2017.08.258>
34. Van Groenigen KJ, Osenberg CW, Hungate BA (2011) Increased soil emissions of potent greenhouse gases under increased atmospheric CO₂. *Nature* 475:214–216. <https://doi.org/10.1038/nature10176>

35. Wang W, Lai DYF, Wang C, Tong C, Zeng C (2016) Effects of inorganic amendments, rice cultivars and cultivation methods on greenhouse gas emissions and rice productivity in a subtropical paddy field. *Ecol Eng* 95:770–778. <https://doi.org/10.1016/j.ecoleng.2016.07.014>
36. Xu HS, Zhu B, Liu JN, Li DY, Yang YD, Zhang K, Jiang Y, Hu YG, Zeng ZH (2017) Azolla planting reduces methane emission and nitrogen fertilizer application in double rice cropping system in southern China. *Agron Sustain Dev* 37:1–9. <https://doi.org/10.1007/s13593-017-0440-z>
37. Xu PS, Li ZT, Wang JY, Zou JW(2022) Fertilizer-induced nitrous oxide emissions from global orchards and its estimate of China. *Agr Ecosyst Environ* 328:107854. <https://doi.org/10.1016/j.agee.2022.107854>
38. Zhang GB, Yang YT, Huang Q, Ma J, Yu HY, Song KF, Dong YJ, Lv SH, Xu H (2020) Reducing yield-scaled global warming potential and water use by rice plastic film mulching in a winter flooded paddy field. *Eur J Agron* 114:126007. <https://doi.org/10.1016/j.eja.2020.126007>
39. Zhang WZ, Sheng R, Zhang MM, Xiong GY, Hou HJ, Li SL, Wei WX (2018) Effects of continuous manure application on methanogenic and methanotrophic communities and methane production potentials in rice paddy soil. *Agr Ecosyst Environ* 258:121–128. <https://doi.org/10.1016/j.agee.201802.018>
40. Zhou GP, Gao SJ, Xu CX, Zeng NH, Robert MR, Cao WD (2020) Co-incorporation of Chinese milk vetch (*Astragalus sinicus* L.) and rice (*Oryza sativa* L.) straw minimizes CH₄ emissions by changing the methanogenic and methanotrophic communities in a paddy soil. *Eur J Soil Sci* 71:924–939. <https://doi.org/10.1111/ejss.12930>
41. Zhu B, Yi LX, Guo LM, Chen G, Hu YG, Tang HM, Xiao CF, Xiao XP, Yang GL, Acharya SN, Zeng ZH (2012) Performance of two winter cover crops and their impacts on soil properties and two subsequent rice crops in Dongting Lake Plain, Hunan, China. *Soil Tillage Res* 124:95–101. <https://doi.org/10.1016/j.still.2012.05.007>
42. Zhu B, Yi LX, Hu YG, Zeng ZH, Lin CW, Tang HM, Yang GL, Xiao XP (2014) Nitrogen release from incorporated ¹⁵N-labelled Chinese milk vetch (*Astragalus sinicus* L.) residue and its dynamics in a double rice cropping system. *Plant Soil* 374:331–344. <https://doi.org/10.1007/s11104-013-1808-8>
43. Zhu X, Burge M, Doane TA, Horwath WR (2013) Ammonia oxidation pathways and nitrifier denitrification are significant sources of N₂O and NO under low oxygen availability. *Proc Natl Acad Sci USA* 110:6328–6333. <https://doi.org/10.1073/pnas.1219993110>
44. Zou WJ, Huang Y, Qin MY, Liu S, Shen Q, Pan G, Lu Y, Liu Q (2009) Changes in fertilizer-induced direct N₂O emissions from paddy fields during rice-growing season in China between 1950s and 1960s. *Global Change Biol* 15(21):229–242. <https://doi.org/10.1111/j.1365-2486.2008.01775.x>

Figures

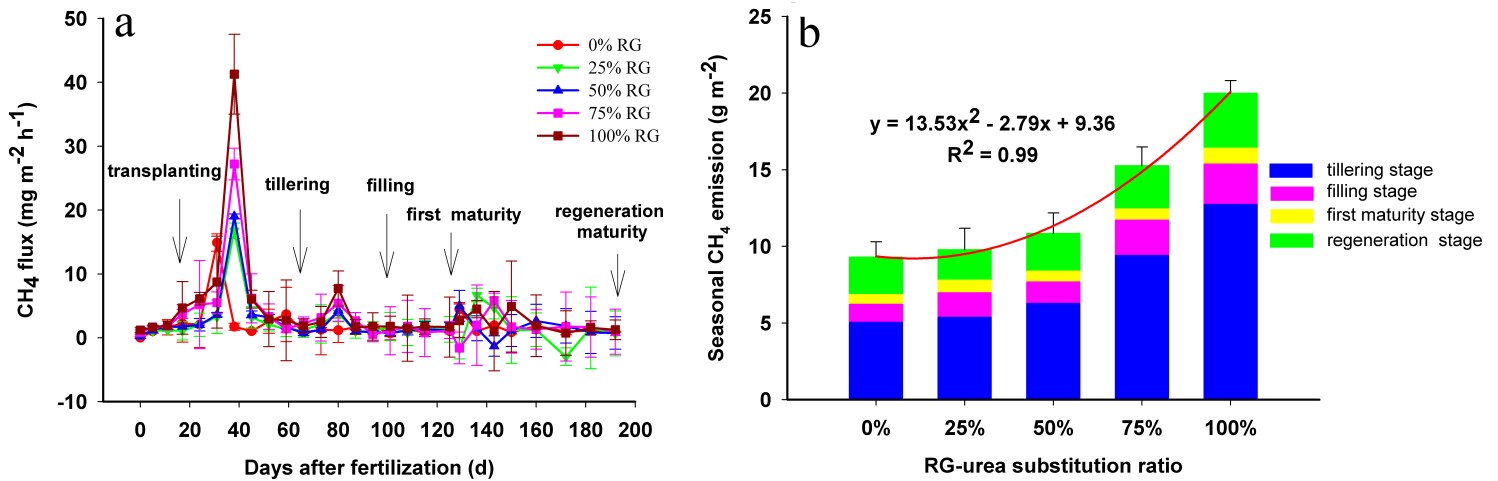


Figure 1

CH₄ emission flux (a) and cumulative CH₄ emissions (b) of different RG-urea substitution ratios: 0% RG, 25% RG, 50% RG, 75% RG, 100% RG. Tillering stage represents cumulative CH₄ emissions from the start of experiment to rice tillering stage, filling stage represents cumulative CH₄ emissions from tillering stage to rice filling stage, first maturity represents cumulative CH₄ emissions from filling stage to first season rice maturity, second maturity represents cumulative CH₄ emissions from first season harvest to the second season maturity. Bars indicate the SE (standard error). The quadratic linear regression relationship between CH₄ cumulative emission and RG-urea substitution ratio was significant ($P < 0.01$).

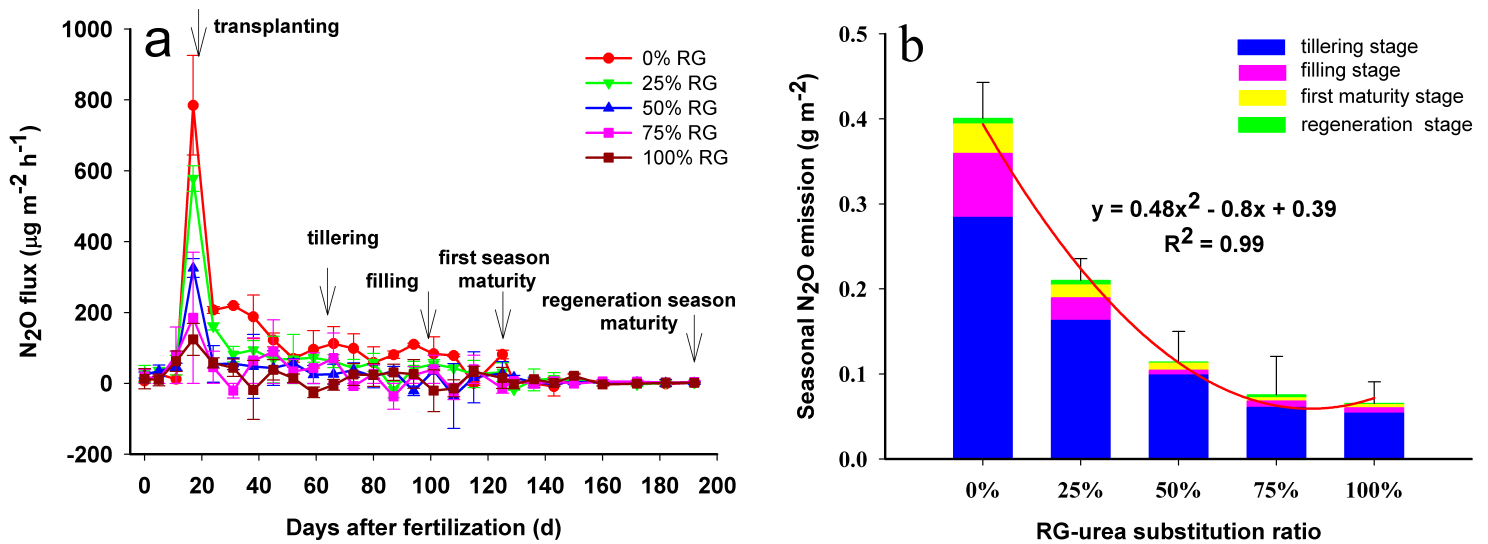


Figure 2

N₂O emission flux (a) and cumulative N₂O emissions (b) of different RG-urea substitution ratios: 0% RG, 25% RG, 50% RG, 75% RG, 100% RG. Tillering stage represents cumulative N₂O emissions from the start of experiment to rice tillering stage, filling stage represents cumulative N₂O emissions from tillering stage to rice filling stage, first maturity represents cumulative N₂O emissions from filling stage to first season

rice maturity, second maturity represents cumulative N₂O emissions from first season harvest to the second season maturity. Bars indicate the SE (standard error). The quadratic linear regression relationship between N₂O cumulative emission and RG-urea substitution ratio was significant ($P < 0.01$).

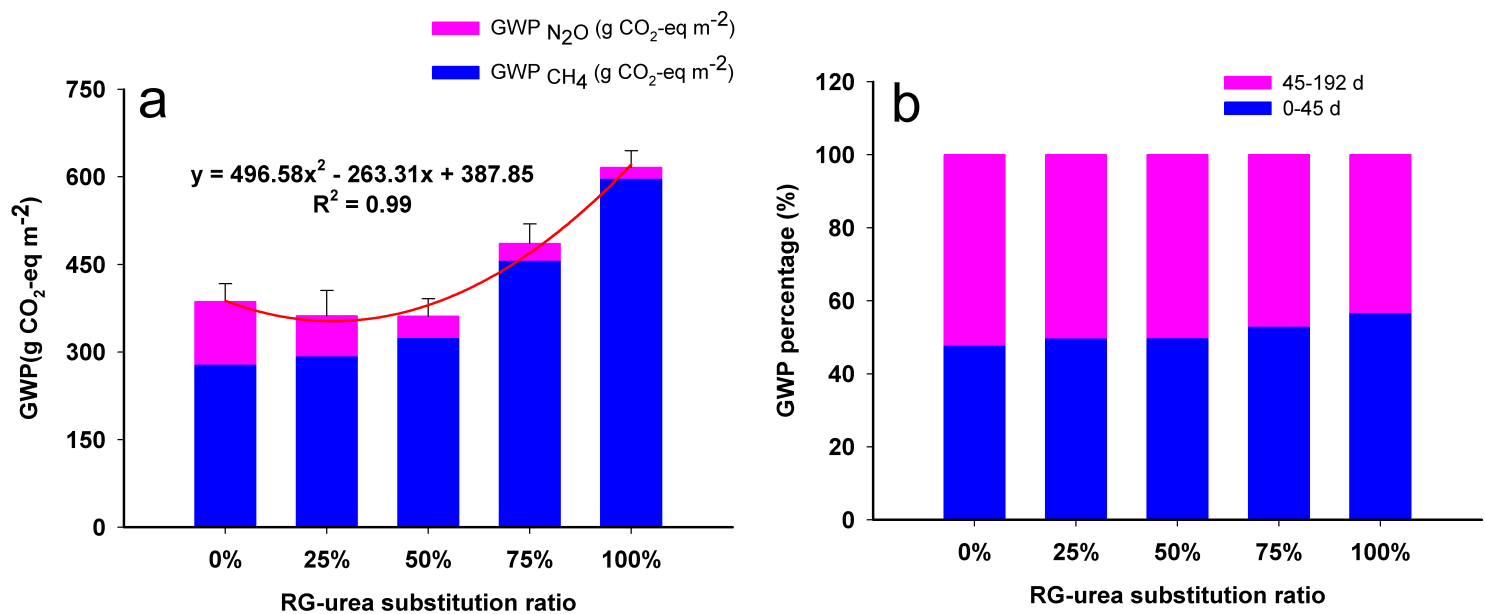


Figure 3

Global warming potential (GWP, a) of different RG-urea substitution ratios: 0% RG, 25% RG, 50% RG, 75% RG and 100% RG. GWP_{CH₄} represents GWP caused by CH₄ emission, GWP_{N₂O} represents GWP caused by N₂O emission. Bars indicate the SE (standard error). The quadratic linear regression relationship between GWP and RG-urea substitution ratio was significant ($P < 0.01$). Percentage of GWP (b) before and after major CH₄ peaks. 0-45d represents GWP calculated by cumulated CH₄ and N₂O emissions from the start of fertilization to 45 days, 45-192d represents GWP calculated by cumulated CH₄ and N₂O emissions from 45 days to 192 days.

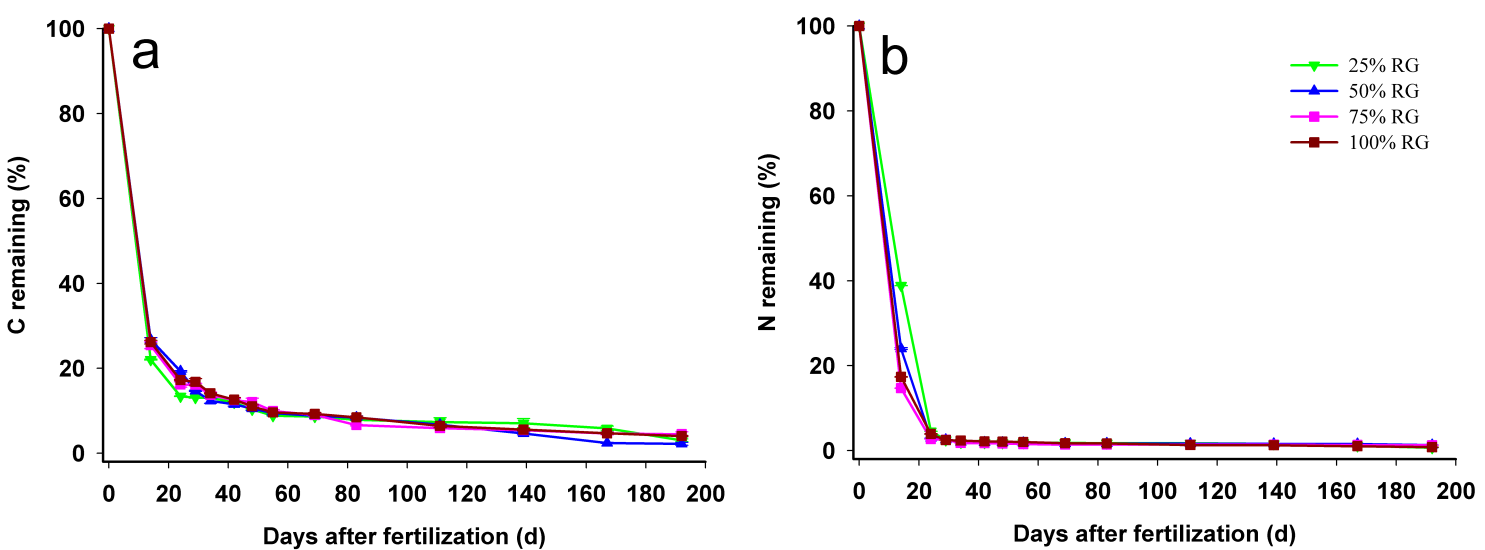


Figure 4

Percentage of C (a) and N (b) remaining in ryegrass residue during 192 days after fertilization under different RG-urea substitution ratios: 25% RG, 50% RG, 75% RG and 100% RG.

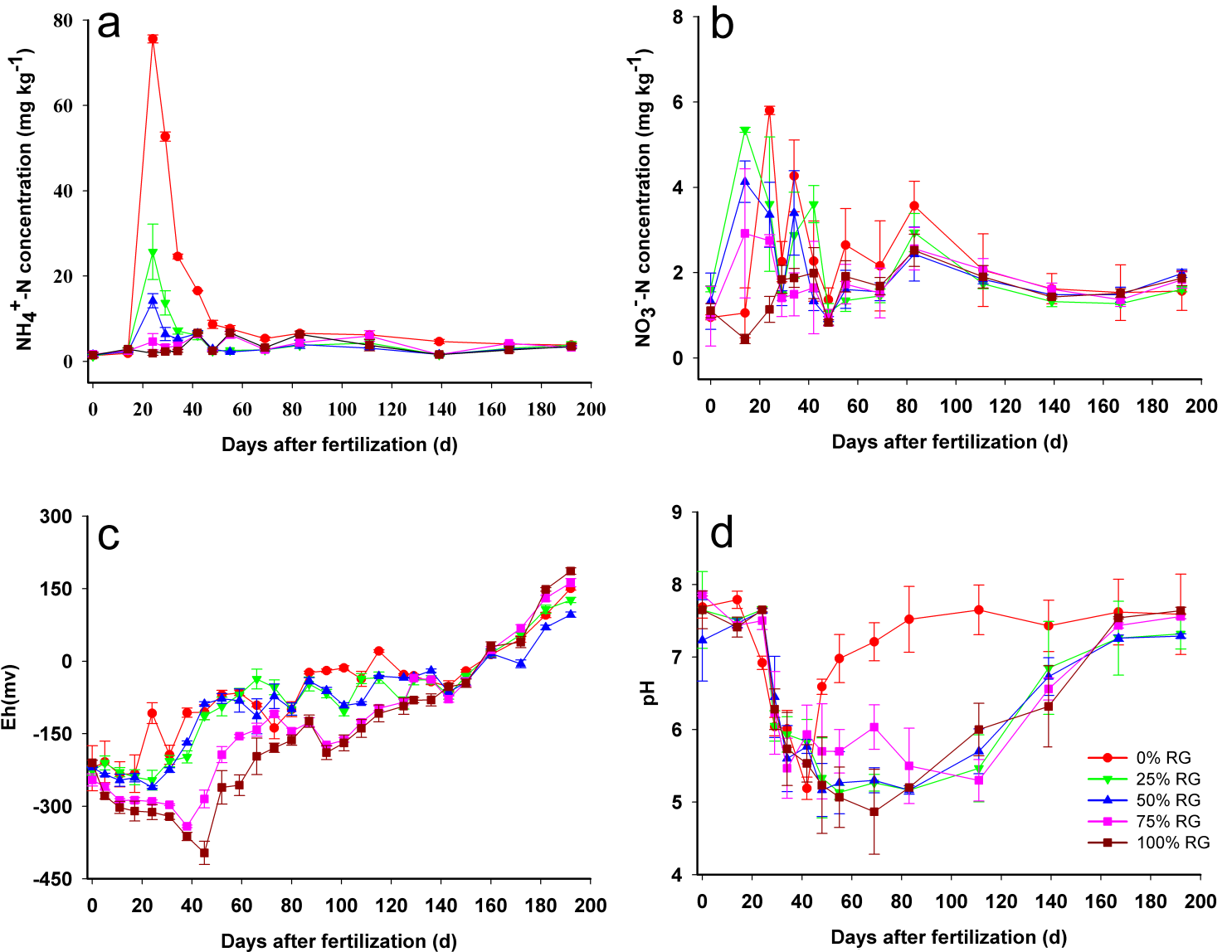


Figure 5

Soil NH₄⁺-N (a), NO₃⁻-N (b), Eh (c) and pH (d) changes of different RG-urea substitution ratios: 0% RG, 25% RG, 50% RG, 75% RG and 100% RG. Bars indicate the SE (standard error).

Figure 6

Distance-based redundancy analysis (dbRDA) of composition of CH₄ (a), N₂O (c) and soil properties. Variance decomposition, with the percentages of CH₄ (b) and N₂O (d) variance explained by soil

properties. Soil properties include $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, Eh, pH, C and N remaining in ryegrass residue.