Green Manuring with Oilseed Rape (Brassica napus L.) Mitigates Methane (CH\textsubscript{4}) and Nitrous Oxide (N\textsubscript{2}O) Emissions in a Rice-Ratooning System in Central China

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Abstract: The use of oilseed rape (OS, Brassica napus L.) as a winter green manure is crucial for enhancing soil fertility and reducing chemical N application in paddy fields. However, the impacts of replacing varying amounts of chemical N with OS on CH\textsubscript{4} and N\textsubscript{2}O emissions in paddy soils have not been well evaluated. In this study, GHG emissions, soil properties and OS decomposition in a rice-ratooning system with different OS-urea N replacement rates (0%, 25%, 50%, 75% and 100%) were investigated. Our results indicate that 84.7–90.7% of the initial C and 97.5–98.4% of the N were released during the 192-day decomposition process, and that the mineralization patterns of net C and net N in the OS residue were consistent with a single exponential decay model. The lowest CH\textsubscript{4} emissions (9.97 g m\textsuperscript{-2}) were observed at 0% OS, while the highest N\textsubscript{2}O emissions (0.40 g m\textsuperscript{-2}) were observed at this level of substitution. Conversely, the highest CH\textsubscript{4} emissions (20.71 g m\textsuperscript{-2}) and lowest N\textsubscript{2}O emissions (0.07 g m\textsuperscript{-2}) were observed at 100% OS. Compared to 0% substitution, 25% substitution significantly decreased GWP and GHGI without reducing rice grain yield. Environmental parameters such as soil redox, NH\textsubscript{4}\textsuperscript{+}-N and residual N and C were shown to be significantly associated with CH\textsubscript{4} emissions, whereas soil redox, NH\textsubscript{4}\textsuperscript{+}-N and residual C were the main drivers of N\textsubscript{2}O emissions. In conclusion, 25% substitution of OS was the most cost-effective measure for balancing greenhouse gas emission and rice yield.

Keywords: CH\textsubscript{4}; N\textsubscript{2}O; oilseed rape; substitution rate; C and N release

1. Introduction

With less than 9% of the world’s arable land, China provides food for 20% of the world’s population [1]. Its rice cultivation spans 30 million hectares, representing 18% of the world’s total area [2]. However, rice fields are acknowledged as a substantial contributor to atmospheric greenhouse gas (GHG) emissions [3], specifically methane (CH\textsubscript{4}) and nitrous oxide (N\textsubscript{2}O). Annual global methane emissions from rice production account for 8% (30 Tg CH\textsubscript{4}) of total global anthropogenic methane emissions [4], while yearly N\textsubscript{2}O emissions from cropland soils treated with fertiliser account for 16% of the world’s total anthropogenic N\textsubscript{2}O emissions (1.6 Tg N\textsubscript{2}O-N) [5]. China’s high crop production levels have been achieved through the increased use of fertilizers, which has led to concerns about its sustainability. According to reports, China’s total nitrogen fertilizer consumption accounts for around 24.5% of global consumption, with rice cultivation alone consuming 3899 kt, equivalent to 15.5% of domestic N fertilizer usage [6]. However, irrational use of nitrogen
fertilizers may actually trigger an increase in GHG emissions, like nitrous oxide (N\textsubscript{2}O)\cite{7,8}. Consequently, it is very important to obtain stable, high yield and low carbon paddy field by optimizing fertilization management.

There are a variety of factors that affect CH\textsubscript{4} and N\textsubscript{2}O production and emissions, such as rice varieties, soil characteristics and field management practices\cite{9,10}, especially fertilizer use\cite{11–13}. For instance, using inorganic N fertilizer in rice fields led to a rise in CH\textsubscript{4} emissions\cite{14}, resulting in greater C substrate availability for methanogenic bacteria and more rice biomass\cite{15}. In addition, inorganic N fertilizer also promoted N transformation processes like nitrification and denitrification, which are responsible for N\textsubscript{2}O production\cite{16}. By contrast, the application of inorganic fertilizers along with organic fertilizers has been shown to augment soil carbon, mitigate N\textsubscript{2}O emissions, and improve crop yields\cite{17}. However, a study discovered that the decay of organic fertilizers resulted in elevated levels of soil organic acids and CH\textsubscript{4} emissions\cite{18}. As a result, the outcomes regarding the impact of organic fertilizer compared to chemical fertilizer on greenhouse gases are inconclusive. Hence, a comprehensive investigation of nutrient management procedures is imperative.

Generally, the substitution rate of organic fertilizer is likely to influence the competition between crops and microbes for nutrients, particularly N and carbon (C). The rhizobial microorganisms’ utilization of organic matter is stimulated as a result, and this ultimately determines soil C and N dynamics\cite{19–21}. The breakdown of substrates, including green manure and rice straw, results in the production of acetate, a crucial component for the development of methanogenic bacteria, which helps methanogens to grow\cite{22}. Plus, organic fertilizers improve N uptake during rice growth and increase residual N content in the soil compared to chemical fertilizers, thereby reducing N losses\cite{23}. Notably, the elevated ratio of C to N found in green manure and straw results in slow N decomposition, which in turn has the potential to decrease the production of N\textsubscript{2}O\cite{24}. Therefore, it is imperative to comprehend how substituting chemical N fertilizers with organic matter affects soil-climate ecosystems via the quantification of substitution rates for carbon and N balance and cycling. However, much of the focus of previous research has been on a single substitution ratio for organic chemical nitrogen, and whether or not other substitution ratios have different effects has largely been left unexplored in greater depth.

The rice-ratooning system has gained recognition as an advantageous substitute for middle- and double-season rice in central China in recent years, owing to its reduced demand for additional labor in transplanting the second rice crop\cite{25}. Oilseed rape (OS, \textit{Brassica napus} L.) is widely used as a green manure for rice fields in southern China and has the potential to partially replace chemical nitrogen fertilizer. Therefore, experimenting with different ratios, the optimal OS substitution ratio was determined to achieve the greatest abatement effect and potential. This study focused on (1) monitoring changes in CH\textsubscript{4} and N\textsubscript{2}O emissions at different organic matter-urea substitution rates, and (2) after OS addition, key factors affecting GHG emissions were identified by analyzing soil physicochemical properties and monitoring carbon and nitrogen release from OS straw.

2. Materials and Methods

2.1. Study Area

Two simultaneous potting trials (Experiment 1 and 2) were conducted in 2023 in an open-air greenhouse (Open on all sides with a closed roof) at the Yangtze University Experimental Station in Jingzhou City, Hubei Province, China (30°20’ N, 112°12’ E, subtropical monsoon climate), the average temperature for the year was 16.5 °C and the rainfall was 1200 mm. The average daily temperature and rainfall during this experiment are shown in Figure 1. The soil utilized in the experiment was extracted from local paddy fields (0–15 cm) and allowed to naturally air-dry and sieve (2 cm). The soil has the following characteristics: sand 258 g kg\textsuperscript{-1}, clay 113 g kg\textsuperscript{-1}, silt 598 g kg\textsuperscript{-1}, pH 6.3, available phosphorus 14.6 mg kg\textsuperscript{-1}, available potassium 86.6 mg kg\textsuperscript{-1}, total nitrogen 1.6 g kg\textsuperscript{-1} and organic carbon 13.2 g kg\textsuperscript{-1}.
The applied fresh aboveground OS (cv. Huayouza 50) had an 82.3% moisture content, 49.4 g N kg$^{-1}$ and 445 g C kg$^{-1}$ dry matter.

Figure 1. Rainfall and average daily temperatures during the test period.

### 2.2. Experiment 1: CH$_4$ and N$_2$O Emissions with OS-Urea Substitution

Five OS-urea substitution ratios treatments: 0%, 25%, 50%, 75% and 100%, with three replications per treatment. The total N application rates in all the treatments were 100 mg N kg$^{-1}$ air-dried soil. The specific amounts of OS and urea in each treatment are detailed in Table 1. Aboveground parts of OS (in full bloom) were weighed on 12 April, cut into 1–2 cm segments and carefully mixed with soil. Potassium chloride and calcium superphosphate were applied at a rate of 0.63 g and 1.83 g, respectively, per pot of air-dried soil. During the experiment period, chemical fertilizer and fresh OS were applied on 25 April and 12 April, respectively. Each plastic pot was filled with 3 kg of air-dried soil and manually transplanted on 27 April with 3 rice seedlings per pot (30 d old, variety Tianliangyou 616). The rice was watered with tap water throughout the growing season, submerged to 2 cm above the soil surface, and no additional fertilizer was applied. The trial was conducted from 12 April until the end of the regrowth season harvest on 21 October, lasting for a total of 192 days.

#### Table 1. Contribution of OS or urea applied to carbon and nitrogen per treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N Applied from Urea (g pot$^{-1}$)</th>
<th>N Applied from OS (g pot$^{-1}$)</th>
<th>C Applied from OS (g pot$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% OS</td>
<td>0.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>25% OS</td>
<td>0.225</td>
<td>0.075</td>
<td>0.67</td>
</tr>
<tr>
<td>50% OS</td>
<td>0.15</td>
<td>0.15</td>
<td>1.35</td>
</tr>
<tr>
<td>75% OS</td>
<td>0.075</td>
<td>0.225</td>
<td>2.03</td>
</tr>
<tr>
<td>100% OS</td>
<td>0</td>
<td>0.3</td>
<td>2.71</td>
</tr>
</tbody>
</table>

### 2.3. Experiment 2: Release of C and N during OS Humification

In this experiment, the dynamics of C and N release during OS decomposition were monitored using the buried bag method and elemental analyzer [23]. The collected aboveground parts were put into nylon mesh bags (length and width) of 10 cm × 10 cm in size and 0.5 mm mesh size, and then buried in pots, and the fertilization method and management were the same as that in Experiment 1. There was a total of 39 nylon mesh bags for each treatment, and 3 bags were taken each time as 3 replications.
2.4. Sample Collection and Measurement

In Experiment 1, static box gas chromatography was used for the collection and analysis of CH$_4$ and N$_2$O emissions [26]. The static gas collection tank consists of a sealed PVP tube chamber (height 110 cm, outer diameter 25 cm) with a hose for the inlet and outlet of the gas, an electric fan on top of the inside of the chamber for mixing the internal air, a three-way valve fitted to the hose connection outside of the PVP tube, and a base made of PVP (height 10 cm, outer diameter 30 cm). Each time the gas is collected, the potted plant growing rice is first placed on the base, then the gas collection box is covered and water is injected into the base to a height of 5 cm to act as a seal; after the collection is completed, the box is taken out and set aside. Gas collection is typically performed between 9:00–11:00 a.m. at intervals of 4 to 12 days, with most cases occurring at 7-day intervals. Before each sample, the air within the chambers was thoroughly mixed with a syringe, and a sample of the headspace gas in each chamber was collected (0, 10, 20, 30 min after sealing). Using a syringe (100 mL) connected to a three-way valve, draw a gas sample from the gas collection tank into a 0.5 L vacuum bag. A gas chromatograph (Agilent 7890 B, Agilent Technologies Inc., Santa Clara, CA, USA) was used to set the flame ionization detector (FID) temperature at 200 °C to analyze the concentration of CH$_4$ and the electron capture detector (ECD) temperature at 330 °C to determine the concentration of N$_2$O [27]. Meanwhile, redox state (Eh), pH and soil temperature were measured using an ORP meter (Leici TR-901, INESA Scientific Instrument Co., Ltd., Shanghai, China), a pH meter (Leici PHS-25, INESA Scientific Instrument Co., Ltd., Shanghai, China) and a digital thermometer model 2455, respectively, measured on the same day as the gas collection. Rice grains in three pots per treatment were collected at the maturity of the first season (15 August) and at the maturity of the regeneration season (21 October) to calculate yields, and yields per pot were converted to yields per square meter per unit area. Rice grain moisture was maintained at about 14%.

In Experiment 2, three nylon mesh bags (3 replicates) for each treatment were retrieved after 14, 24, 29, 34, 42, 48, 55, 69, 83, 111, 139, 167 and 192 days to determine the C and N release dynamic in OS residues. On the day of sampling, the nylon mesh bags were removed to wash the soil with tap water, retaining only the OS residue, and placed in an oven at 80 °C for 72 h. Dried samples were weighed, ground, and sieved (100 µm). Thereafter, 5 mg of the sample was weighed and wrapped in aluminum foil cups for the determination of C and N content by elemental analysis (Costech ECS4010, Coster Tecnologie Speciali S.p.A. Calceranica al Lago (TN), Italy). The soil was collected as samples from three randomly selected points in the pot and mixed well with each other. Samples were taken with a small-diameter potting soil auger made of stainless steel (19 mm in diameter and 300 mm in length) to a depth of the soil surface to the bottom of the pot. After extraction of nitrate (NO$_3^-$-N) and ammonium (NH$_4^+$-N) from the soil with 2 mol L$^{-1}$ KCl, the concentrations were measured with a continuous flow analyzer (SEAL AA500, SEAL Analytical GmbH, Werkstrasse 5, D-22844, Norderstedt, Germany).

2.5. Data Calculation Formula

The emission fluxes of CH$_4$ and N$_2$O are calculated in the following way: (1).

$$ F = \frac{p \times h \times dc/dt \times 273}{273 + T} $$

In the above equation, F represents CH$_4$ flux (mg m$^{-2}$ h$^{-1}$) or N$_2$O flux (µg m$^{-2}$ h$^{-1}$); p is CH$_4$/N$_2$O density at standard pressure and temperature (CH$_4$ 0.714 kg m$^{-3}$, N$_2$O 1.964 kg m$^{-3}$); h is the height of the inside of the gas collection tank (cm); dc/dt is the rate of change in the CH$_4$ or N$_2$O concentration (mg m$^{-3}$ h$^{-1}$); T is the temperature (°C) inside the static collection box.

The formula for calculating seasonal C or N cumulative emissions is as follows Equation (2).

$$ \text{Seasonal CH}_4 \text{ or N}_2\text{O cumulative emissions} = \sum_{i=1}^{n} \frac{F_i + F_{i-1}}{2} \times (D_{i+1} - D_i) \times 24 $$ (2)
where \( F \) is the \( \text{CH}_4 (\text{N}_2\text{O}) \) emission flux in milligrams \( \text{mg m}^{-2} \text{h}^{-1} \) (\( \mu\text{g m}^{-2} \text{h}^{-1} \)), \( i \) is the consecutive sampling interval, \( D_{i+1} - D_i \) is the number of days between two consecutive samples (d), \( n \) is the total number of sampling intervals and 24 is a conversion factor for a 24-h day.

The global warming potential (GWP) of \( \text{CH}_4 \) and \( \text{N}_2\text{O} \) was calculated in \( \text{CO}_2 \) equivalent (\( \text{CO}_2\text{-eq} \)) over a time horizon of 100 years as in Equation (3) [28].

\[
\text{GWP} \left( \text{g CO}_2\text{-eq m}^{-2} \right) = 29.8 \times \text{CH}_4 \left( \text{g m}^{-2} \right) + 273 \times \text{N}_2\text{O} \left( \text{g m}^{-2} \right)
\]  

(3)

The greenhouse gas intensity (GHGI) was quantified from Equation (4)

\[
\text{GHGI} \left( \text{g GWP per g of yield} \right) = \frac{\text{GWP} \left( \text{g CO}_2\text{-eq m}^{-2} \right) / \text{rice yield} \left( \text{g m}^{-2} \right)}{100}
\]  

(4)

The formula from Zhu et al. [23] was used to calculate the percentage of residual C and N in OS residues.

\[
Y(\%) = \frac{Y_t}{Y_i} \times 100
\]  

(5)

where \( Y \) is the amount of C or N remaining in the OS residue (in percent), \( Y_t \) is the amount of C or N in the OS residue at different points in time, and \( Y_i \) represents the initial amount of C or N when the OS was not applied to the soil.

To further explore the release of C and N from OS residues, changes in C and N over time scales were described by exponential decay models.

\[
\text{YE} = \exp^{-kt} \times 100
\]  

(6)

\( \text{YE} \) is the C or N remaining after the decomposition of the operating system at time \( t \), and \( k \) denotes the release rate of C (\( k_C \)) or N (\( k_N \)).

2.6. Statistical Analysis

Least significant difference analyses, correlation analyses, and analyses of variance (ANOVA) were conducted using IBM SPSS Statistics 26 (New York, NY, USA) on the effects of OS substitution rates on \( \text{CH}_4 \) and \( \text{N}_2\text{O} \) emissions, GWP, yields, GHG indices, and soil properties, and SigmaPlot 12.0 (Systat Software Inc., San Jose, CA, USA) was used to fit an exponential recession model to fit exponential recession models. The differences between the treatments were found to be statistically significant at the \( p < 0.05 \) level. In order to ascertain the relationship between various soil properties and \( \text{CH}_4 \) and \( \text{N}_2\text{O} \) emissions, a structural equation model (SEM) was constructed using Stata/SE 15.1 (Stata Corp LLC Inc., College Station, TX, USA).

3. Results

3.1. Fluxes of \( \text{CH}_4 \) and \( \text{N}_2\text{O} \)

The \( \text{CH}_4 \) and \( \text{N}_2\text{O} \) fluxes with different OS-urea substitution ratios showed the largest peaks at 31 and 17 days after OS incorporation, respectively (Figures 2a and 3a). A minor peak was also detected in the regeneration season. The highest monitored \( \text{CH}_4 \) emissions (45.9 mg m\(^{-2}\) h\(^{-1}\)) occurred in 100% of the OS treatments and increased with increasing OS urea replacement rates (Figure 2a). By contrast, the 0% OS (784.9 µg m\(^{-2}\) h\(^{-1}\)) and 100% OS (108.2 µg m\(^{-2}\) h\(^{-1}\)) had the largest and the smallest \( \text{N}_2\text{O} \) peaks, respectively. And the increasing OS-urea, the substitution ratio was reduced by the \( \text{N}_2\text{O} \) peaks (Figure 3a).
Figure 2. Emission fluxes (a) and cumulative emissions (b) of CH$_4$ at five OS urea substitution rates. The tillering stage, filling stage, first maturity stage and regeneration stage represent the first season of rice from transplanting to tillering, from tillering to filling, from filling to first season maturity and from first season maturity to regrowth season maturity, respectively, hereafter. The bar perpendicular to the x-axis represents the standard error (SE), below. Different lowercase letters indicate significant differences between treatments ($p < 0.05$), below. Quadratic linear regression between OS-urea substitution rate and cumulative CH$_4$ emissions was significant ($p < 0.01$). The red curve is the fitted curve of the equation.
Figure 3. Emission fluxes (a) and cumulative emissions (b) of N$_2$O at five OS-urea substitution rates. Quadratic linear regression between OS-urea substitution rate and cumulative N$_2$O emissions was significant ($p < 0.01$). The red curve is the fitted curve of the equation.

Seasonal emissions of CH$_4$ and N$_2$O throughout the experiment were categorized into five stages according to the growth stage of rice: before transplanting (0–17 d), tillering stage (18–63 d), filling stage (64–98 d), first season maturity stage (99–125 d) and regeneration season maturity stage (125–192 d). The CH$_4$ emission before the tillering stage and before the first season maturity stage accounted for 59.6–73.5% and 74.2–82.7%, respectively. There is a quadratic and significant relationship between OS urea substitution rate and CH$_4$ emissions with the equation $y = 12.66x^2 - 2.06x + 10.08$ ($R^2 = 0.99$, $p < 0.01$; Figure 2b). The
N$_2$O emissions from the experiment start to the tillering stage accounted for 70.9–86.7% of the total N$_2$O emissions, and the N$_2$O emissions from the experiment start to the first mature stage accounted for 95.5–98.7% of total N$_2$O emissions. The regression analysis shows a significant quadratic correlation between the rate of substitution of OS-urea and the total emissions of N$_2$O ($R^2 = 0.99, p < 0.01$; Figure 3b). Overall, CH$_4$ emissions increased progressively as oilseed rape substitution increased, while N$_2$O emissions decreased progressively as oilseed rape substitution increased.

3.2. Yield, GWP and GHGI

As shown in Table 2, the GWP due to CH$_4$ were significantly higher than those due to 0% OS in all treatments with OS addition, but the GWP due to N$_2$O were highest in the 0% OS treatment and significantly higher than those in the 50% OS, 75% OS and 100% OS treatments ($p < 0.05$). The results of the GWP totals showed that total GWP was significantly lower ($p < 0.05$) for the 25% OS and 50% OS treatments than for the 0% OS treatment, but 75% OS and 100% OS had significantly more. The results of the study showed that rice grain yield ranged from 444.3 g m$^{-2}$ to 796.3 g m$^{-2}$ under different OS-urea substitution rate treatments (Table 2). There was no significant variation in yield with 25% OS compared to 0% OS. By contrast, rice yield under 50% OS, 75%OS, 100% OS treatments was significantly lower than 0% OS treatment. GHGI was significantly increased by 17.3%, 76.9% and 175.0% for 50% OS, 75% OS and 100% OS treatments, respectively, compared to 0% OS treatment ($p < 0.05$), but significantly decreased by 5.77% for 25% OS treatment ($p < 0.05$).

Table 2. Effects of different OS-urea substitution rates on total GWP, CH$_4$ and N$_2$O-induced global warming potential, rice yield and GHGI.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CH$_4$-Induced GWP</th>
<th>N$_2$O-Induced GWP</th>
<th>Total GWP</th>
<th>Grain Yield</th>
<th>GHGI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GWP (g CO$_2$-eq m$^{-2}$)</td>
<td>Account for Total GWP (%)</td>
<td>GWP (g CO$_2$-eq m$^{-2}$)</td>
<td>Account for Total GWP (%)</td>
<td>(g CO$_2$-eq m$^{-2}$)</td>
</tr>
<tr>
<td>0% OS</td>
<td>297.1 e</td>
<td>73.3</td>
<td>108.1 a</td>
<td>26.7</td>
<td>405.2 b</td>
</tr>
<tr>
<td>25% OS</td>
<td>312.3 d</td>
<td>84.5</td>
<td>57.3 a</td>
<td>15.5</td>
<td>369.7 d</td>
</tr>
<tr>
<td>50% OS</td>
<td>362.4 c</td>
<td>92.1</td>
<td>31.2 b</td>
<td>7.9</td>
<td>393.5 c</td>
</tr>
<tr>
<td>75% OS</td>
<td>465.0 b</td>
<td>95.8</td>
<td>20.6 c</td>
<td>4.2</td>
<td>485.6 b</td>
</tr>
<tr>
<td>100% OS</td>
<td>617.2 a</td>
<td>97.2</td>
<td>17.8 d</td>
<td>2.8</td>
<td>635.0 a</td>
</tr>
</tbody>
</table>

In the same column, different lowercase letters indicate significant differences between OS-urea replacement rate treatments ($p < 0.05$).

Figure 4a shows a substantial quadratic relationship between the OS-urea substitution ratio and the net GWP of seasonal CH$_4$ and N$_2$O emissions (Figure 4a, $R^2 = 0.99, p < 0.01$). GWPs were lowest for 25% OS (9.4% below 0% OS) and highest for 100% OS (55.6% above 0% OS). In addition, 73.3–97.2% of the total GWP was derived from CH$_4$ emission (Table 2). As illustrated in Figure 4b, the contribution of CH$_4$ and N$_2$O emission to the GWP reached 48.9–60.4% in the first 38 d while 39.6–51.1% was emitted from 38 d to 192 d. Similar to GWP, GHGI was lowest in 25% OS treatment (3.9% lower than 0% OS). Overall, 25% substitution significantly decreased GWP and GHGI without reducing rice grain yield, as compared to 0% substitution.

3.3. C and N Released during Decomposition of OS

Carbon release (68.6–81.7%) and nitrogen release (92.7–97%) from oilseed rape residues were mainly concentrated within 69 and 29 days after application to the soil, respectively (Figure 5a,b). After 192 days, C remaining in OS residue (9.3–15.3%) was much higher than N remaining in OS residue (1.6–2.5%). A single decay index model effectively described the release dynamics of C ($r^2 = 0.68–0.84, p < 0.05$) versus N ($r^2 = 0.96–0.99, p < 0.05$) following the addition of OS to rice soil (Table 3).
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Figure 5. Percentage of carbon (a) and nitrogen (b) residues in oilseed rape residues at different OS-urea substitution ratios from fertilizer application to day 192.
Table 3. C, N release rates (kC, kN) of OS residues at different OS-urea substitution rates (single-index model).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>kC</th>
<th>r²</th>
<th>kN</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>25% OS</td>
<td>0.0272</td>
<td>0.8313</td>
<td>0.0706</td>
<td>0.9574</td>
</tr>
<tr>
<td>50% OS</td>
<td>0.0303</td>
<td>0.8380</td>
<td>0.0667</td>
<td>0.9563</td>
</tr>
<tr>
<td>75% OS</td>
<td>0.0232</td>
<td>0.6834</td>
<td>0.0832</td>
<td>0.9855</td>
</tr>
<tr>
<td>100% OS</td>
<td>0.0202</td>
<td>0.7946</td>
<td>0.0689</td>
<td>0.9747</td>
</tr>
</tbody>
</table>

The values for kC (0.0202–0.0303) were much lower than for kN (0.0667–0.0832), illustrating C release from OS residue was slower than N release. The highest C release rate was found in the 50% OS treatment and had the lowest N release rate, while the 100% OS treatment had the lowest kC value (0.0202) and the 75% OS treatment had the highest kN value (0.0832) (Table 3). The OS residues released 84.7% to 90.7% C and 97.5% to 98.4% N over 192 days, with the mineralization of C and N being consistent with a single exponential decay model.

3.4. Effect of OS Decomposition on Soil Properties and the Relationship between the Concentrations of Different N and C Forms Produced

The concentrations of NO$_3^-$-N and NH$_4^+$-N in the soil were relatively high during the pre-experimental period, with NH$_4^+$-N concentrations being considerably higher than NO$_3^-$-N. (Figure 6a,b). NO$_3^-$-N and NH$_4^+$-N peaked at 14–29 days after the addition of OS to paddy soil. The highest NH$_4^+$-N content was found in the 0% OS treatment, while the opposite was true for the 100% OS treatment. Redox state (Eh value) increased steadily throughout the experiment, with relatively low Eh values for higher OS substitution rates (Figure 6c). Soil pH showed acidic during 40–130 days and relatively low with OS incorporation (Figure 6d).

Figure 6. Soil NH$_4^+$-N (a), NO$_3^-$-N (b), Eh (c) and pH (d) changes of different OS-urea substitution ratios.
Soil properties affect CH$_4$ and N$_2$O emissions based on the structural equation model (SEM, Figure 7). NH$_4^+$-N ($r = -0.27$, $p < 0.01$) and C remaining ($r = -0.71$, $p < 0.001$) were significantly negatively affected by Eh. A significant positive correlation existed between residual N and pH ($p < 0.01$). Additionally, soil N remaining ($r = -0.77$, $p < 0.001$) and Eh ($r = -0.26$, $p < 0.01$) were significantly negatively linked with CH$_4$ emission, whereas C remaining ($r = 0.66$, $p < 0.01$) and NH$_4^+$-N ($r = 0.22$, $p < 0.05$) were positively linked with CH$_4$ emission. N$_2$O emissions were significantly negatively correlated with C remaining ($r = -0.7$, $p < 0.01$) and Eh ($r = -0.6$, $p < 0.001$) and significantly positively correlated with NH$_4^+$-N ($r = 0.32$, $p < 0.01$). The results indicated that soil N remaining, C remaining, NH$_4^+$-N and Eh were factors influencing CH$_4$ emission, whereas C remaining, NH$_4^+$-N and Eh were important factors controlling N$_2$O emission.

![Figure 7](image_url)  
*Figure 7.* The effects of each soil indicator on CH$_4$ and N$_2$O emissions were analyzed by constructing a structural equation model (SEM). The results of model fitting parameters: $\chi^2$/df = 1.94, $p = 0.88$, RMSEA (Root Mean Square Error of Approximation) = 0.094, NFI (Normed Fit Index) = 0.94, CFI (Comparative Fit Index) = 0.97, GFI (Goodness Fit Index) = 0.92. Soil properties: Eh, pH, C remaining, N remaining, NH$_4^+$-N and NO$_3^-$-N. The black and red lines indicate negative correlations, positive correlations and the value of the standardized path coefficient represents the degree of influence between the two indicators, respectively. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

4. Discussion

4.1. Influence of OS-Urea Substitution Ratio on CH$_4$ Emission

The addition of organic matter (green manure, animal excrement, straw, etc.) leads to an increase in the production and emission of CH$_4$ in rice soils [29,30]. It was proven that OS incorporation increased CH$_4$ emissions, particularly the higher the OS-urea substitution ratio, the more obvious the effect (Figure 2). Similarly, previous studies demonstrated that fresh green manure as an alternative to chemical fertilization can significantly increase CH$_4$ emission in flooded paddy soils [31]. The rapid phase of C release from oilseed rape residues was found to be the time of concentrated CH$_4$ emission, indicating that CH$_4$ emission was influenced by C in oilseed rape residues. (Figures 2a and 5a). The ratio of OS-urea substitution significantly affects the decomposition of green manure, where C substrates are utilized by methanogens to produce CH$_4$ [32,33].

Organic supplements can improve the quality of depleted soils, but they also impact the activities of methane-oxidizing and methanogenic bacteria, thus influencing the production and consumption of CH$_4$. The rapid decomposition of OS consumed a large amount of oxygen (O$_2$) in the soil and water layer, resulting in a decrease in Eh (Figure 7). Lower Eh provides a suitable anaerobic environment for methanogenic activities in paddy soil [34]. At the same time, the community structure of methanogenic bacteria in the soil changed
due to the decrease in pH value [35]. Higher CH₄ emissions at lower pH and Eh were also found by Baumann et al. [36] and Fan et al. [37]. Therefore, the significant increase in methane was due to the large amount of OS-organic materials input.

4.2. Influence of OS-Urea Substitution Ratio on N₂O Emission

N₂O emission increases with the increase of chemical nitrogen fertilizer (urea), with results that are consistent with those of previous authors [38–40]. However, some researchers have shown that green manure helps improve N use efficiency and reduces N₂O emissions [40,41] due to the ability of appropriate organic substitution ratios to influence soil physicochemical properties and ammonia-oxidising bacteria (AOB) community structure, thereby reducing the AOB contribution of N₂O [42]. In this study, N₂O emission reached different peaks after applying different amounts of urea (Figure 3a), and the N₂O emission peaks and seasonal N₂O emissions had lower values at higher OS-urea substitution ratios (Figure 3). Nitrification and denitrification are the major contributors to N₂O production [43]. The process of NH₄⁺-N to NO₃⁻-N through nitrification leads to the production of N₂O intermediates [44]. The results indicate that low OS-urea substitution ratio supplied greater NH₄⁺-N than high OS-urea substitution ratio, contributing to higher N₂O emission (Figure 6a). In addition, the amount of residual C after OS decomposition showed a significant negative correlation with the association of N₂O emissions ($p < 0.01$, Figure 7). It may be that OS can increase soil C source and organic C content, providing a suitable environment for denitrifying anaerobic microorganisms, resulting in N₂O emission [44].

4.3. GWP and GHGI

In the study, GWP and GHGI were used to estimate the global climatic impacts of CH₄ and N₂O emissions under different OS-urea substitution ratios. Approximately 73.3–97.2% of GWP comes from CH₄ emission, suggesting that CH₄ is a major GHG [45,46] (Figure 4a). Meanwhile, 48.9–60.4% of GWP occurred in the first 38 d after OS incorporation, suggesting that the critical period for reducing GHG emissions was during the pre-growth period of rice or the early stage of OS decomposition (Figure 4b). The 25% (decreased by 9.4%) and 50% OS (decreased by 3.6%) treatments significantly decreased GWP compared to the 0% OS (408.2 g CO₂-eq m⁻²) treatment, mainly due to lower N₂O emissions [47]. However, higher OS-urea substitution ratios have higher GWP because the replacement of chemical N fertilizer with OS reduces N₂O emissions but significantly increases CH₄ emissions. Although 25% OS increased CH₄ emissions, the significantly lower N₂O emissions more than offset the CH₄ increase, thus offsetting the GWP caused by CH₄, resulting in a significant 8.76% lower total GWP for 25% OS (369.7 g CO₂-eq m⁻²) compared to the 0% OS treatment (405.2 g CO₂-eq m⁻²), while the 25% OS yield did not change significantly from 0% OS treatment, resulting in lower GIGH (Table 2). Therefore, in order to maintain grain yield while allowing for GHG reductions, 25% OS treatment is an effective alternative strategy. The OS-urea alternative did not positively affect rice yield under the same pure nitrogen application conditions. Because the green manure oilseed rape, although rich in nutrients, is a slow-acting source of fertilizer compared with urea, a fast-acting fertilizer with high nitrogen content, the release of nutrients is slower. And to a certain extent, it is possible to meet the needs of crop growth in the early stage, but with the gradual reduction of easily decomposable substances in the decomposition of straw, the remaining difficult to degrade substances decompose slowly, and the release of nutrients is low [48]. Coupled with the reduction in the amount of urea applied, the fast-acting nitrogen that can be supplied can not promptly meet the needs of subsequent growth of rice, thus causing rice yield reduction. In particular, the greater the substitution rate, the more obvious the negative impact on yield. In this study, only 25% of the OS substitution rate did not significantly reduce yield (Table 2). Therefore, in subsequent related studies, measures can be taken to offset the yield reduction caused by the slow release of a small amount of straw as well as the reduction of urea dosage, such as increasing the amount of straw input. At the same time, more attention needs to be paid to GHG emission reduction during the
pre-straw decomposition period (0–38 d), because more CH$_4$ is emitted during this period (Figure 4b), and the contribution of CH$_4$ emission to GHG is significantly higher than that of N$_2$O emission (Figure 4a).

5. Conclusions

OS-urea substitution rate during rice cultivation has a role in influencing CH$_4$ and N$_2$O emissions from soil. CH$_4$ emissions were strongly positively correlated with the OS-urea replacement ratio, while N$_2$O emissions showed the opposite trend. The 25% OS treatment had the lowest total GWP and GHGI and the 100% OS had the highest GWP and GHGI, but the 25% OS treatment reduced GHG emissions while safeguarding rice yields compared to the conventional 0% OS treatment with fertilizer alone. The OS-urea substitution rate led to changes in C and N content in paddy soils, and CH$_4$ and N$_2$O emissions were further affected. Soil NH$_4^{+}$-N, Eh, N and C remaining were the key variables linked to CH$_4$ emissions, while Eh, NH$_4^{+}$-N and C remaining were the significant factors affecting N$_2$O emissions.


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