

## Article

# The Study of Chicken Manure and Steel Slag Amelioration to Mitigate Greenhouse Gas Emission in Rice Cultivation

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**Abstract:** Organic matter, fertilizers, and soil amendments are essential for sustainable agricultural practices to guarantee soil productivity. However, these materials can increase the emission of greenhouse gases (GHGs) such as CH<sub>4</sub> and N<sub>2</sub>O. Thus, technologies for reducing GHG emissions in concert with the increase in rice production from rice fields are needed. The objectives of this study were to determine the best chicken manure (CM) and steel slag (SS) combination to mitigate CH<sub>4</sub>, N<sub>2</sub>O, and CO<sub>2</sub> emissions in an incubation experiment, to identify the best CM:SS ameliorant mixture to mitigate CH<sub>4</sub> and N<sub>2</sub>O, and to evaluate dry biomass and grain yield in a pot experiment. A randomized block design was established with four treatments, namely conventional (chemical fertilizer only) and three combinations of different ratios of CM and SS (1:1, 1:1.5, and 1:2.5), with five replications in a pot experiment. CM:SS (1:2.5) was identified as the best treatment for mitigating CH<sub>4</sub>, N<sub>2</sub>O, and CO<sub>2</sub> in the incubation experiment. However, CM:SS (1:1.5) was the best CM and SS ameliorant for mitigating CH<sub>4</sub> and N<sub>2</sub>O in the pot experiment. The global warming potential of CH<sub>4</sub> and N<sub>2</sub>O revealed that CM:SS (1:1.5) had the lowest value. None of the combinations of CM and SS significantly increased dry biomass and grain yield.

**Keywords:** methane; nitrous oxide; incubation experiment; pot experiment; rice paddy yield



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## 1. Introduction

Agriculture contributes an estimated 10–12% of global greenhouse gas (GHG) emissions, mainly as nitrous oxide (N<sub>2</sub>O) (46%), followed by methane (CH<sub>4</sub>) (45%), and carbon dioxide (CO<sub>2</sub>) (9%) [1]. Rice (*Oryza sativa* L.) is the dominant staple food for more than half of the world's population, and its production is critical for global food security. Rice cultivation is a significant source of CH<sub>4</sub> (a significant GHG) emissions, accounting for 11% of global anthropogenic CH<sub>4</sub> emissions. Rice cultivation under submerged conditions enhances CH<sub>4</sub> emissions owing to increase soil-reduced conditions conducive to methanogenesis [2]. Agricultural production in the world must continue to meet the basic needs of society. As the population increases, the demand for rice also increases, which encourages intensive and extensive rice cultivation. Attempts to increase rice production have led to an increase in CH<sub>4</sub> production. However, efforts are required to reduce CH<sub>4</sub> emissions without reducing agricultural crop production.

The application of organic materials, fertilizers, and soil amendments is essential for sustainable agricultural practices to guarantee soil productivity. However, practices can increase GHG emissions, such as CH<sub>4</sub> and N<sub>2</sub>O emissions. Water management in the agricultural sector significantly affects GHG emissions. In submerged rice cultivation, CH<sub>4</sub>

is formed from the anaerobic decomposition of organic matter in the rhizosphere of rice in the presence of methanogenic microbes, such as *Methanosarcina* and *Methanobacterium* bacteria [3]. The process of methanogenesis occurs optimally when the redox potential (Eh) is below 150 mV, pH ranges from 6 to 8, soil temperature ranges from 30 to 40 °C, and degraded organic materials, such as root exudate and fresh residue, are readily available [4].

In recent years, the application of industrial byproducts (e.g., slags from different metal and process-based industries) as amendments in paddy fields for rice cultivation has become increasingly popular for improving soil quality, enhancing crop productivity, and mitigating GHG emissions [5,6]. Furthermore, iron slag addition has proven to be effective at reducing CH<sub>4</sub> emissions from paddy fields [5,7]. Singla and Inubushi [8] also conducted an experiment on steel slag (SS) using two different types of slag fertilizers in paddy soil. The application of SS at a rate of 2 t·ha<sup>-1</sup> reduced CH<sub>4</sub> emissions by 27.54% compared to the control. Ali et al. [9] used SS at three study sites, namely the Republic of Korea, Bangladesh, and Japan. The addition of SS with urea to the soil was found to reduce N<sub>2</sub>O in the Republic of Korea, Bangladesh, and Japan by 5.74, 14.18, and 17.65%, respectively, compared to the control (NPK). SS was also found to provide adequate silicate ions necessary for higher crop productivity, especially rice [10].

In this study, we conducted an incubation experiment to determine the best combination of chicken manure (CM) and SS to mitigate CH<sub>4</sub> and N<sub>2</sub>O emissions, as well as a pot experiment to evaluate dry biomass and grain yield.

## 2. Materials and Methods

This study consists of two parts: an incubation experiment and a pot experiment.

### 2.1. Incubation Experiment

#### 2.1.1. Experimental Design and Set Up for Gas Analysis

Soil samples were collected from paddy soil at the experimental farm of the Agriculture Faculty, Ehime University (Matsuyama, Japan). The soil sample was collected from 0 to 20 cm depth, air-dried at room temperature, and passed through a 2 mm stainless steel sieve. The soil had the following properties: pH (4.7), total carbon (C, 1.52%), total nitrogen (N, 0.15%), available P<sub>2</sub>O<sub>5</sub> (60 mg·kg<sup>-1</sup>), and light clay texture (54.7% sand, 17.0% silt, 28.3% clay). CM had the following properties: pH (8.8), total C (29.9%), total N (3.77%), total P (50,400 mg·kg<sup>-1</sup>), and SS had the following properties: pH (11.5), Fe<sub>2</sub>O<sub>3</sub> (8.5%), SiO<sub>2</sub> (34.4%), and CaO (38.8%). Eight treatments with five replicates were used as follows: CM, CM:SS (1:1), CM:SS (1:1.5), CM:SS (1:2.5), SO (soil only), SS (1), SS (1.5), and SS (2.5). All treatments were applied to 15 g of dry soil. In the CM treatment, CM was applied at a dose of 250 mg. For CM:SS treatments, CM was applied with the same amount as CM treatment, and SS was applied based on the ratio according to the weight of CM. For the SO and SS treatments, no CM was added. CM:SS (1:1), CM:SS (1:1.5), CM:SS (1:2.5), SS (1.0), SS (1.5), and SS (2.5) were applied at 250, 375, 625, 250, 375, and 625 mg, respectively. GHG production from the CM of each CM:SS treatment was also calculated by eliminating the GHG originating from the soil by subtracting the CM:SS treatment from the SS treatment. Before incubation, CM and SS were added to the soil and mixed thoroughly into a 50 mL tube. Deionized water was added to 15 mL (saturated condition) and tightly closed with a rubber stopper consisting of a three-way valve on top of the headspace. Thereafter, the headspace in the tube was replaced with nitrogen gas (N<sub>2</sub>) for 20 s to maintain anaerobic conditions and then inserted into the incubator at 25 °C under dark conditions.

#### 2.1.2. GHG Measurements

Gas samples were collected and measured after 1, 2, 3, 5, 7, 10, 14, 21, 28, and 42 days of incubation. Before collecting the gas samples, the tubes were mixed to release gas from the soil to the headspace. Two syringes were used to collect the gas; the first syringe was filled with 20 mL of N<sub>2</sub> and the other in an empty condition. A 20 mL volume of N<sub>2</sub> gas was injected into the headspace and mixed using another syringe. Thereafter,

20 mL of gas sample was collected from the headspace and injected into a vacuum vial bottle. The concentrations of CH<sub>4</sub> and N<sub>2</sub>O were measured using a gas chromatography instrument equipped with a flame ionization detector and an electron capture detector (GC-14A, Shimadzu, Kyoto, Japan), while CO<sub>2</sub> concentrations were measured with a thermal capture detector (GC-8A, Shimadzu, Kyoto, Japan). The following equation was used to calculate the gas fluxes (F) of CH<sub>4</sub>, N<sub>2</sub>O, and CO<sub>2</sub>:

$$F = \rho \times V/M \times dC/dt \times [273/(273 + T)] \times \alpha \quad (1)$$

where  $\rho$  is the density of CH<sub>4</sub>, N<sub>2</sub>O, and CO<sub>2</sub> at standard temperature and pressure (0.717 g L<sup>-1</sup>, 1.97 g L<sup>-1</sup>, and 1.98 g L<sup>-1</sup>, respectively),  $V$  is the volume of the incubation tube (L),  $M$  is the mass of soil (g),  $dC/dt$  is the slope of the linear regression for gas concentration gradient through time,  $T$  is the incubation temperature (°C), and  $\alpha$  is the conversion factor of CH<sub>4</sub> to C (12/16), N<sub>2</sub>O to N (28/44), and CO<sub>2</sub> to C (12/44). Using the trapezoidal rule, the cumulative CH<sub>4</sub>, N<sub>2</sub>O, and CO<sub>2</sub> emissions were calculated as the sum of the area bounded by the rate.

### 2.1.3. Soil Analysis for the Incubation Experiment

To investigate the changes in soil chemical properties, we prepared additional incubation tubes for SO, CM:SS (1:1), CM:SS (1:1), CM:SS (1:1.5), and CM:SS (1:2.5). These tubes were replicated three times and incubated for 40 d in the dark at 25 °C. Soil samples were collected at 5, 10, 15, 20, 30, and 40 days after incubation (DAI). Soil pH was determined from soil-water suspensions (1:5 *v/v*) using a pH meter (B-212, HORIBA, Kyoto, Japan). Soil ammonium-N (NH<sub>4</sub><sup>+</sup>-N) and nitrate-N (NO<sub>3</sub><sup>-</sup>-N) were extracted with 2 M KCl, and their concentrations were determined by calorimetric methods using the indophenol blue method and the vanadium chloride nitrate reduction method. Ferrous iron (Fe<sup>2+</sup>) content was determined by the colorimetric method using the phenanthroline method in the extraction by acetic-acid buffer at pH 5.5.

## 2.2. Pot Experiment

### 2.2.1. Treatments and Management Practices

The pot experiment was conducted from June to September 2020 in Matsuyama, Ehime Prefecture, Japan. Rice plants (*Oryza sativa* L. cv. Koshihikari) were used in the experiment. The experiment was arranged in a randomized block design. Four treatments with five replicates were used as follows: conventional (Conv), CM:SS (1:1), CM:SS (1:1.5), and CM:SS (1:2.5). In conventional chemical fertilizers, N, P<sub>2</sub>O<sub>5</sub>, and K<sub>2</sub>O concentrations of 14, 14, and 14%, respectively, were applied. In the CM:SS treatments, granulated material of CM and SS mixture in 1:1, 1:1.5, and 1:2.5 weight base was applied. Because SS has high pH, the N and C concentrations in the utilized granular materials of 1:1, 1:1.5, and 1:2.5 were 1.70, 1.42, and 1.00%, and 16.4, 13.0, and 9.38%, respectively.

Rice was cultivated in 1/5000 as Wagner pots with a size of 0.02 m<sup>2</sup>. Each pot received 3.5 kg of dry soil, which is a quite low-fertility soil. The soil had the following properties: pH (7.1), total C (0.02%), total N (0.01%), available P<sub>2</sub>O<sub>5</sub> (47 mg kg<sup>-1</sup>) with sandy loam texture (81.1% sand, 7.1% silt, 11.8% clay). Each chemical fertilizer and granulated material of basal fertilizer was applied and mixed with soil and deionized water on 17 June 2020. The basal N fertilizer application rate was 30 gN m<sup>-2</sup> for all treatments. Because the amount of basal N fertilizer application rate was the same in all treatments, the total application rate of granulated materials and C in CM:SS (1:1), CM:SS (1:1.5), CM:SS (1:2.5) was 35.29 g m<sup>-2</sup>, 42.25 g m<sup>-2</sup>, 60 g m<sup>-2</sup> and 5.78 gC m<sup>-2</sup>, 5.51 gC m<sup>-2</sup>, and 5.63 gC m<sup>-2</sup>, respectively. Three rice seedlings were planted per pot. The pots were irrigated daily and kept under anaerobic conditions with deionized water. Supplemental NPK fertilizer was applied 30 days after transplanting (DAT) in CM:SS (1:1), CM:SS (1:1.5), and CM:SS (1:2.5) at a rate of 10 gN m<sup>-2</sup> because rice growth in these treatments was quite poor. Rice plants and grains were harvested on 19 September.

### 2.2.2. CH<sub>4</sub> and N<sub>2</sub>O Flux Measurements in the Pot Experiment

Fluxes of CH<sub>4</sub> and N<sub>2</sub>O were measured using the closed-chamber technique. The chamber was made of acrylic equipped with a fan, thermometer, and sample collecting tube. There were two sizes of the chamber: short and tall. The short chamber had a diameter of 16 cm and height of 16 cm and was used for the early growth of paddy from 4 DAT until 21 DAT. The long chamber had a diameter of 16 cm and a height of 85 cm, and was used from 28 DAT until 93 DAT. Gas fluxes were measured weekly from 21 June to 18 September 2020 (1 d before harvest). The collected gas samples were then inserted into vacuum-sealed vial bottles with a butyl rubber stopper. The gas samples were collected at 0, 10, and 20 min from the time the chambers were deployed. Concentrations of CH<sub>4</sub> and N<sub>2</sub>O were analyzed with the same analyzers explained above.

The following equation was used to calculate the gas fluxes ( $F$ ) of CH<sub>4</sub> (mgC m<sup>-2</sup> h<sup>-1</sup>) and N<sub>2</sub>O (μgN m<sup>-2</sup> h<sup>-1</sup>) according to Toma et al. [11]:

$$F = \rho \times V/A \times dC/dt \times [273/(273 + T)] \times \alpha \quad (2)$$

where  $\rho$  is the density of CH<sub>4</sub> and N<sub>2</sub>O, as described above;  $V$  is the volume of the chamber (m<sup>3</sup>);  $A$  is the area of the chamber (m<sup>2</sup>);  $dC/dt$  is the slope of the linear regression for the gas concentration gradient through time,  $T$  is the temperature inside the chamber (°C), and  $\alpha$  is the conversion factor explained above. Each gas flux was calculated by linear regression, and the cumulative fluxes were determined using the trapezoidal method according to Toma et al. [11]. We converted the pot scale flux to area-scale flux by using the pot's base area (0.02 m<sup>2</sup>) and then converted it to hectares (ha).

### 2.2.3. Global Warming Potential (GWP)

To estimate GWP, CO<sub>2</sub> is typically taken as the reference gas, and a change in the emission of CH<sub>4</sub> or N<sub>2</sub>O is converted into "CO<sub>2</sub>- equivalents". The GWP for CH<sub>4</sub> is 34 (based on a 100-year time horizon and a GWP for CO<sub>2</sub> of 1), while that for N<sub>2</sub>O is 298. The GWP of the combined emissions of CH<sub>4</sub> and N<sub>2</sub>O was calculated using the following equations:

$$\text{GWP}_{\text{CH}_4} (\text{kg CO}_{\text{eq}} \text{ ha}^{-1}) = \text{CH}_4 \text{ flux (kg C ha}^{-1}) \times 16/12 \times 34 \quad (3)$$

$$\text{GWP}_{\text{N}_2\text{O}} (\text{kg CO}_{\text{eq}} \text{ ha}^{-1}) = \text{N}_2\text{O flux (kg N ha}^{-1}) \times 44/28 \times 298 \quad (4)$$

### 2.2.4. Measurement of Plant Growth Parameters

The plant growth parameters measured in the pot experiment were plant height (cm), chlorophyll content, and the number of tillers. These growth parameters were measured weekly in each pot from 7 DAT to 92 DAT. The chlorophyll content was measured using the SPAD 502-Plus chlorophyll meter (Konica Minolta, Inc., Osaka, Japan).

### 2.2.5. Rice Biomass and Grain Yield

The rice plants were harvested and separated into aboveground (AG) and belowground (BG) parts. The AG parts were collected and divided into stems, leaves, and panicles, while the BG parts were comprised of the roots. All samples were oven-dried at 70.0 °C for 24 h, weighed, and the dry biomass was calculated. Grain yield (g pot<sup>-1</sup>) was obtained by measuring the total weight of the grains per pot. Grain yield was divided into fresh and dry harvest weights (g pot<sup>-1</sup>).

### 2.2.6. Ancillary Measurement

Soil water (5 cm depth) was collected weekly using a soil moisture sampler (DIK-301, Daiki Rika Kogyo, Saitama, Japan). The pH, NO<sub>3</sub><sup>-</sup>-N, and NH<sub>4</sub><sup>+</sup>-N concentrations were measured in soil water. The soil water collected in the syringe tube was filtered using a syringe filter (<0.2 μm), and the pH, NO<sub>3</sub><sup>-</sup>-N, and NH<sub>4</sub><sup>+</sup>-N concentrations were measured using the same method described above.

Soil redox potential (Eh) was measured at 5 cm soil depth with a platinum electrode (EP-201, Fujiwara, Tokyo, Japan) and a portable soil Eh meter (PRN-41, Fujiwara, Tokyo, Japan) and maintained throughout the cultivation period. Two pots for each treatment were analyzed to measure Eh. The first measurement was conducted at 1 DAT (1 day after installation).

During the study period, soil temperatures at a 5 cm depth were measured continuously every 10 min in two pots by thermistors equipped with a data logger (RTR 502, T&D Corporation, Nagano, Japan).

### 2.2.7. Data Analysis and Statistics

Statistical analysis was performed to determine the effects of the treatments on the experimental parameters. The significance of treatments was tested by one-way analysis of variance (ANOVA) and Tukey's HSD test at a probability lower than 5% ( $p < 0.05$ ) was applied for the differences in mean values. All statistical analyses were performed using SPSS Statistics version 20 (IBM, New York, NY, USA).

## 3. Results

### 3.1. Incubation Experiment

The cumulative CH<sub>4</sub>, N<sub>2</sub>O, and CO<sub>2</sub> emissions are listed in Table 1. The rate of CH<sub>4</sub> production was found to significantly decrease with increasing levels of SS amendment in the incubation experiment. The lowest cumulative CH<sub>4</sub> emission was shown in CM:SS (1:2.5) (0.01 mgC kg<sup>-1</sup> period<sup>-1</sup>) and was statistically significant with other CM:SS treatments. CM:SS (1:1), CM:SS (1:1.5), and CM:SS (1:2.5) reduced CH<sub>4</sub> emissions by 18.8%, 28.2%, 56.4%, 98.5%, and 99.7%, respectively, compared to CM. The highest cumulative N<sub>2</sub>O emission was released by SS (1) (0.1 µgN kg<sup>-1</sup> period<sup>-1</sup>). However, the lowest cumulative N<sub>2</sub>O emission was released by CM:SS (1:1) (−0.80 µgN kg<sup>-1</sup> period<sup>-1</sup>). The lowest cumulative CO<sub>2</sub> emission was released by CM:SS (1:2.5) (−0.01 mgC kg<sup>-1</sup> period<sup>-1</sup>). However, CM:SS (1:1) (4.47 mgC kg<sup>-1</sup> period<sup>-1</sup>) had the highest cumulative CO<sub>2</sub>, but this was not statistically significant relative to SO, SS (1.5), and SS (2.5).

**Table 1.** Cumulative emissions in each treatment during the incubation experiment (Mean ± Standard Error).

Treatments	Cumulative Emissions		
	CH <sub>4</sub> (mgC kg <sup>-1</sup> Period <sup>-1</sup> )	N <sub>2</sub> O (µgN kg <sup>-1</sup> Period <sup>-1</sup> )	CO <sub>2</sub> (mgC kg <sup>-1</sup> Period <sup>-1</sup> )
CM	2.35 ± 0.03 d	−0.30 ± 0.10 a	3.66 ± 0.96 bc
CM:SS (1:1)	1.69 ± 0.07 c	−0.81 ± 0.16 a	4.47 ± 0.43 c
CM:SS (1:1.5)	1.03 ± 0.26 b	−0.72 ± 0.82 a	3.51 ± 0.35 bc
CM:SS (1:2.5)	0.01 ± 0.00 a	−0.29 ± 0.08 a	−0.01 ± 0.15 a
SO	1.38 ± 0.09 bc	−0.27 ± 0.24 a	2.04 ± 0.73 ab
SS (1)	0.43 ± 0.11 a	0.10 ± 0.22 a	2.70 ± 0.08 bc
SS (1.5)	0.03 ± 0.01 a	−0.94 ± 0.10 a	0.07 ± 0.16 a
SS (2.5)	0.07 ± 0.02 a	−0.18 ± 0.04 a	−0.04 ± 0.02 a

CM: chicken manure, SS: steel slag, SO: soil only. CM:SS means the weight ratio given between chicken manure and steel slag. SS (1, 1.5, 2.5) represents the weight ratio given of steel slag without CM. All values are expressed as mean. Different letters within the same column among the treatments indicate a significant difference ( $p < 0.05$ ).

The GHG production from CM for each treatment is shown in Table 2. The highest CH<sub>4</sub> production from CM was shown in CM:SS (1:1) (1.26 mgC kg<sup>-1</sup> period<sup>-1</sup>) but was not statistically significant relative to CM and CM:SS (1:1.5). However, the lowest CH<sub>4</sub> production from CM was observed in CM:SS (1:2.5) (−0.06 mgC kg<sup>-1</sup> period<sup>-1</sup>) and was statistically significant relative to the other treatments. The highest N<sub>2</sub>O production from CM was shown in CM:SS (1:1.5) (0.21 µgN kg<sup>-1</sup> period<sup>-1</sup>); however, this was not statistically significant relative to other treatments. The lowest N<sub>2</sub>O production from CM was observed in CM:SS (1:1) (−0.91 µgN kg<sup>-1</sup> period<sup>-1</sup>), but this was not statistically significant relative to other treatments. The highest CO<sub>2</sub> production from CM was shown



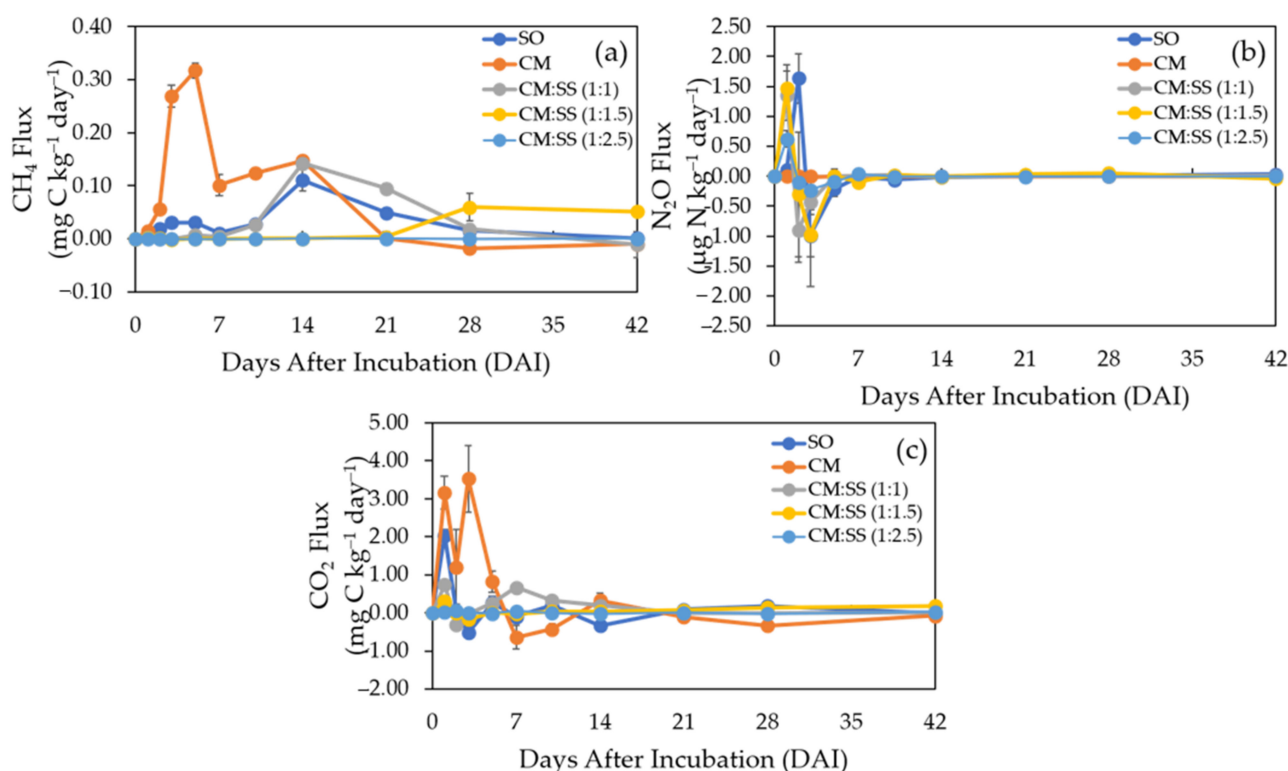
in CM:SS (1:1.5) ( $3.44 \text{ mgC kg}^{-1} \text{ period}^{-1}$ ), but this was not statistically significant relative to other treatments. However, the lowest  $\text{CO}_2$  production from CM was shown in CM:SS (1:2.5) ( $0.03 \text{ mgC kg}^{-1} \text{ period}^{-1}$ ) but not statistically significant relative to other treatments.

**Table 2.** GHG production from chicken manure in each treatment during the incubation experiment (Mean  $\pm$  Standard Error).

Treatments	GHG Production from Chicken Manure		
	$\text{CH}_4$ ( $\text{mgC kg}^{-1} \text{ Period}^{-1}$ )	$\text{N}_2\text{O}$ ( $\mu\text{gN kg}^{-1} \text{ Period}^{-1}$ )	$\text{CO}_2$ ( $\text{mgC kg}^{-1} \text{ Period}^{-1}$ )
CM	$0.97 \pm 0.08 \text{ b}$	$-0.03 \pm 0.25 \text{ a}$	$1.62 \pm 1.50 \text{ a}$
CM:SS (1:1)	$1.26 \pm 0.04 \text{ b}$	$-0.91 \pm 0.51 \text{ a}$	$1.77 \pm 0.67 \text{ a}$
CM:SS (1:1.5)	$1.00 \pm 0.25 \text{ b}$	$0.21 \pm 0.77 \text{ a}$	$3.44 \pm 0.42 \text{ a}$
CM:SS (1:2.5)	$-0.06 \pm 0.02 \text{ a}$	$-0.11 \pm 0.05 \text{ a}$	$0.03 \pm 0.13 \text{ a}$

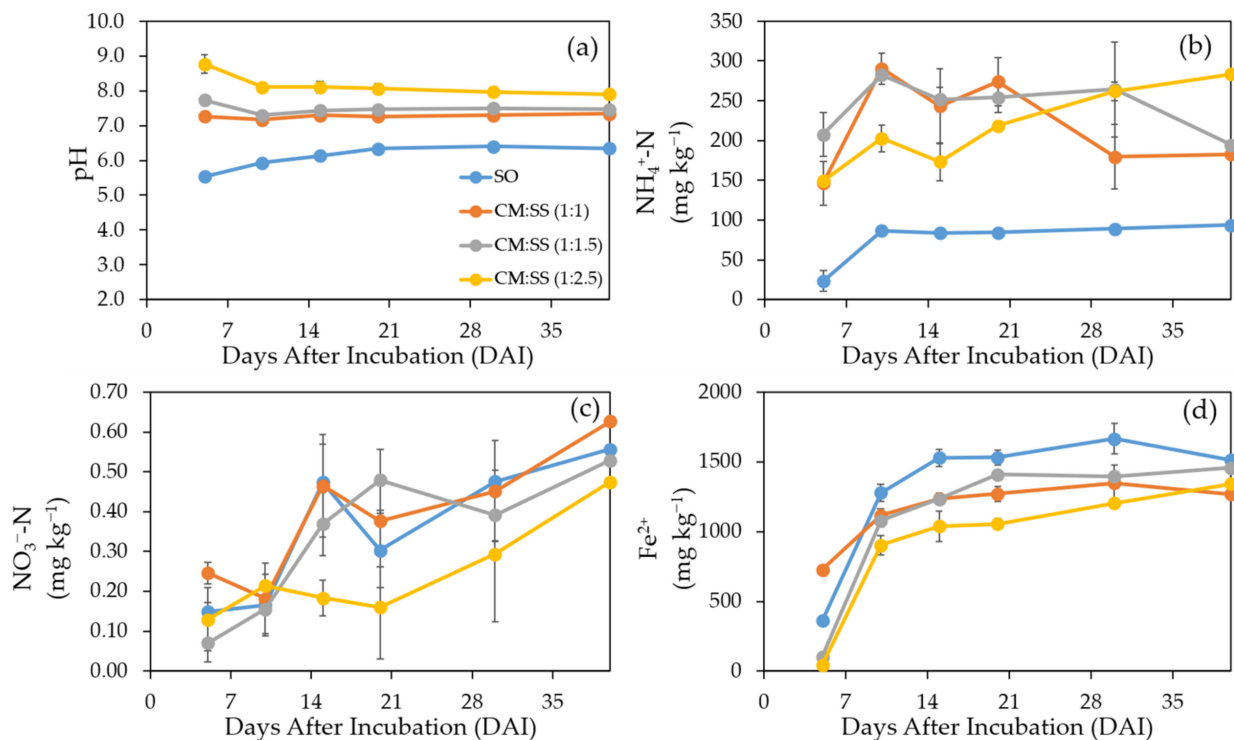
GHG: Greenhouse Gas, CM: chicken manure, SS: steel slag. CM:SS means the weight ratio given between chicken manure and steel slag. Different letters within the same column among the treatments indicate a significant difference ( $p < 0.05$ ).

The variations in  $\text{CH}_4$ ,  $\text{N}_2\text{O}$ , and  $\text{CO}_2$  fluxes during the incubation experiment are shown in Figure 1. The fluxes of  $\text{CH}_4$  were very low in the first seven days of incubation in all treatments, except in CM, but peaked at 14 DAI (Figure 1a). The highest  $\text{CH}_4$  flux was shown in CM ( $0.32 \text{ mgC kg}^{-1} \text{ day}^{-1}$ ) at 5 DAI, and the lowest flux was also in CM ( $-0.02 \text{ mgC kg}^{-1} \text{ day}^{-1}$ ) at 28 DAI. The  $\text{N}_2\text{O}$  fluxes fluctuated only in the first week of the incubation experiment in all treatments (Figure 1b). The highest  $\text{N}_2\text{O}$  flux was observed in SO ( $1.63 \mu\text{gN kg}^{-1} \text{ day}^{-1}$ ) at 2 DAI, and the lowest was observed in CM:SS (1:1.5) ( $0.97 \mu\text{gN kg}^{-1} \text{ day}^{-1}$ ). The  $\text{CO}_2$  fluxes increased sharply at 1 DAI in all treatments and then decreased at 2 DAI (Figure 1c). CM treatment increased again at 3 DAI and then declined sharply at 5 DAI. After 5 DAI, all treatments showed the same trend until the end of the incubation experiment.



**Figure 1.**  $\text{CH}_4$  (a),  $\text{N}_2\text{O}$  (b), and  $\text{CO}_2$  (c) flux during the incubation experiment. SO: soil only, CM: chicken manure, SS: steel slag. CM:SS is the weight given ratio between chicken manure and steel slag. Error bars represent standard error.

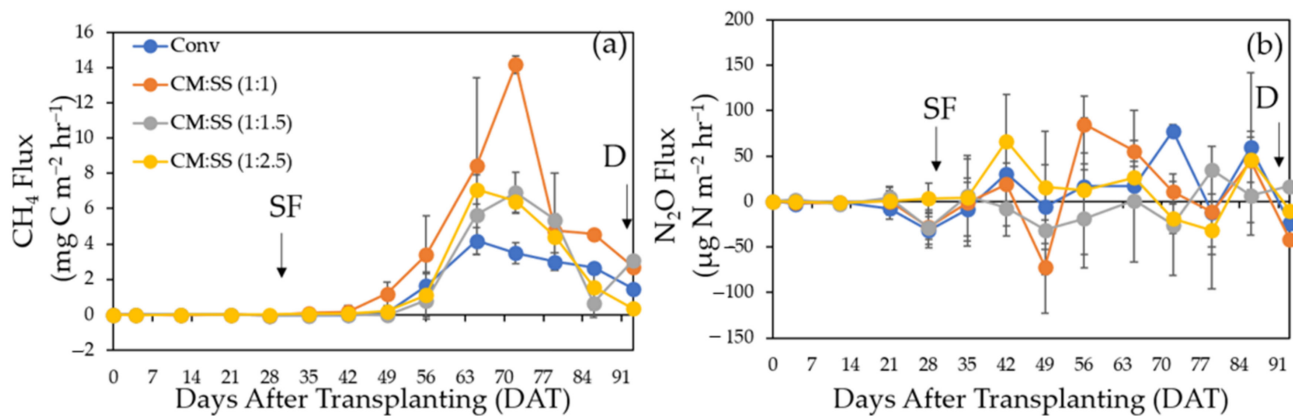
Variations in soil pH and concentrations of  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , and  $\text{Fe}^{2+}$  are shown in Figure 2. Soil pH was higher in the CM:SS treatments than in the SO treatment throughout the incubation period (Figure 2a). The highest pH value was observed in CM:SS (1:2.5) (8.77) at 5 DAI. However, the lowest pH value was observed in SO (5.53) at 5 DAI. SO had the lowest  $\text{NH}_4^+\text{-N}$  concentration during the 40 DAI (Figure 2b). The highest  $\text{NH}_4^+\text{-N}$  concentration was observed in CM:SS (1:1) (290.4  $\text{mg kg}^{-1}$ ). The concentration of  $\text{NH}_4^+\text{-N}$  in the CM:SS treatments tended to decrease at the end of the incubation experiment, except in CM:SS (1:2.5). CM:SS (1:2.5) had the lowest  $\text{NO}_3^-\text{-N}$  concentration from 15 to 40 DAI (Figure 2c). The highest  $\text{NO}_3^-\text{-N}$  concentration was observed in CM:SS (1:1) (0.63  $\text{mg kg}^{-1}$ ) at 40 DAI. The highest  $\text{Fe}^{2+}$  concentration was observed in SO at 30 DAI, whereas the lowest was observed in CM:SS (1:2.5) at 5 DAI (Figure 2d). In the SS amendment treatments,  $\text{Fe}^{2+}$  concentration was lower than that of SO at 5, 10, 15, 20, and 30 DAI.



**Figure 2.** Soil pH (a),  $\text{NH}_4^+\text{-N}$  (b),  $\text{NO}_3^-\text{-N}$  (c), and  $\text{Fe}^{2+}$  (d) concentrations of soil in each treatment during the pot experiment. All values are expressed as mean. SO: soil only, CM: chicken manure, SS: steel slag. Error bars represent standard error.

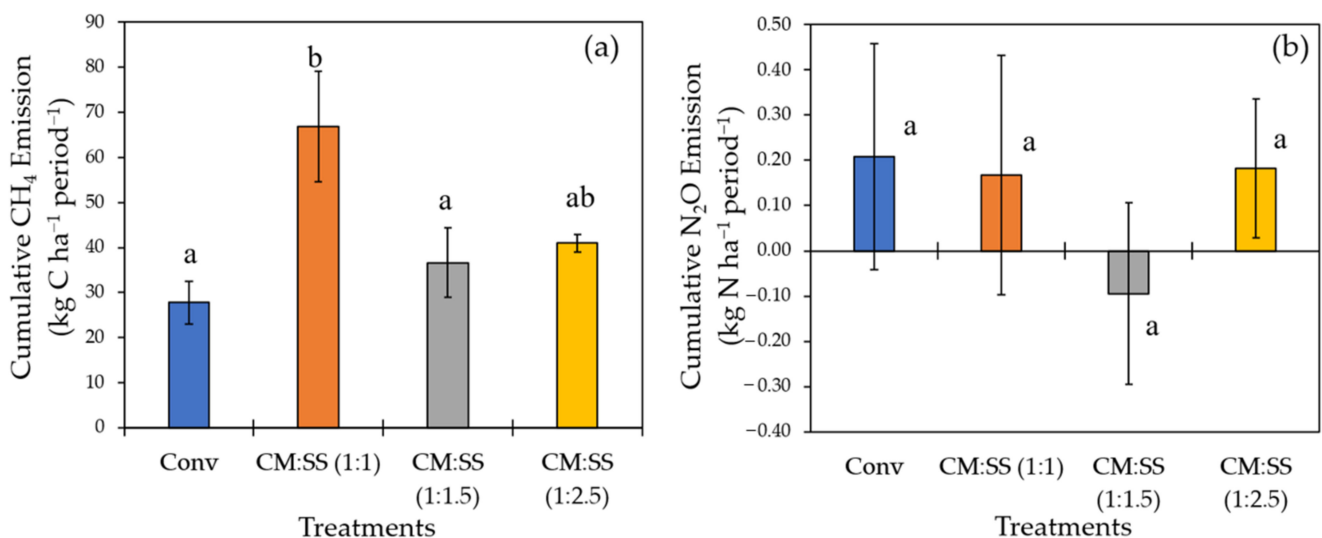
### 3.2. Pot Experiment

The  $\text{CH}_4$  and  $\text{N}_2\text{O}$  fluxes for all treatments are shown in Figure 3. The  $\text{CH}_4$  fluxes were low during the initial growth of rice plants but increased significantly at 49 DAT (Figure 3a). CM:SS (1:1) had the highest flux (6918.57  $\text{mgC m}^{-2} \text{h}^{-1}$ ) at 74 DAT.  $\text{N}_2\text{O}$  fluctuated during the rice plant growth period (Figure 3b). The timing of the  $\text{N}_2\text{O}$  flux peak also varied widely among the different treatments. Maximum  $\text{N}_2\text{O}$  fluxes were detected on 67 DAT in conventional (77.59  $\mu\text{gN m}^{-2} \text{h}^{-1}$ ), 53 DAT in CM:SS (1:1) (84.97  $\mu\text{gN m}^{-2} \text{h}^{-1}$ ), 74 DAT in CM:SS (1:1.5) (34.68  $\mu\text{gN m}^{-2} \text{h}^{-1}$ ), and 39 DAT in CM:SS (1:2.5) (66.35  $\mu\text{gN m}^{-2} \text{h}^{-1}$ ).



**Figure 3.** CH<sub>4</sub> flux (a) and N<sub>2</sub>O flux (b) in each treatment during the pot experiment. All values are expressed as means. Conv: conventional, CM: chicken manure, SS: steel slag, SF: supplementary fertilization, D: time when the pot was dried. Error bars represent standard error.

The cumulative CH<sub>4</sub> and N<sub>2</sub>O emissions are shown in Figure 4. Significant differences were found in the cumulative CH<sub>4</sub> emissions among the treatments (Figure 4a). The lowest cumulative CH<sub>4</sub> emission was observed in the Conv (27.8 kgC ha<sup>-1</sup>); however, there was no statistical significance relative to CM:SS (1:1.5) and CM:SS (1:2.5). The highest cumulative CH<sub>4</sub> emission was observed in CM:SS (1:1) (66.8 kgC ha<sup>-1</sup>), but not statistically significant relative to CM:SS (1:2.5). There was a decreasing tendency for cumulative CH<sub>4</sub> emissions in CM:SS (1:1.5) and CM:SS (1:2.5), with a decrease of 45.2% and 38.74% compared to the CM:SS (1:1). We observed no significant differences in cumulative N<sub>2</sub>O emissions between the Conv and CMSS treatments (Figure 4b). CM:SS (1:1.5) (−0.09 kgN ha<sup>-1</sup>) had the lowest cumulative N<sub>2</sub>O emissions among all treatments.



**Figure 4.** Cumulative CH<sub>4</sub> (a) and N<sub>2</sub>O (b) emissions in each treatment during the pot experiment. All values are expressed as mean. Conv: conventional, CM: chicken manure, SS: steel slag. Error bars represent standard error. Different letters among the treatments indicate a significant difference ( $p < 0.05$ ).

The GWP for CH<sub>4</sub> and N<sub>2</sub>O emissions varied considerably with treatment (Table 3). The GWP<sub>CH<sub>4</sub></sub> was higher than that of GWP<sub>N<sub>2</sub>O</sub> in all treatments. When both CH<sub>4</sub> and N<sub>2</sub>O emissions were combined, the overall GWP showed a decreasing trend in CM:SS (1:1.5) and CM:SS (1:2.5) compared to CM:SS (1:1).



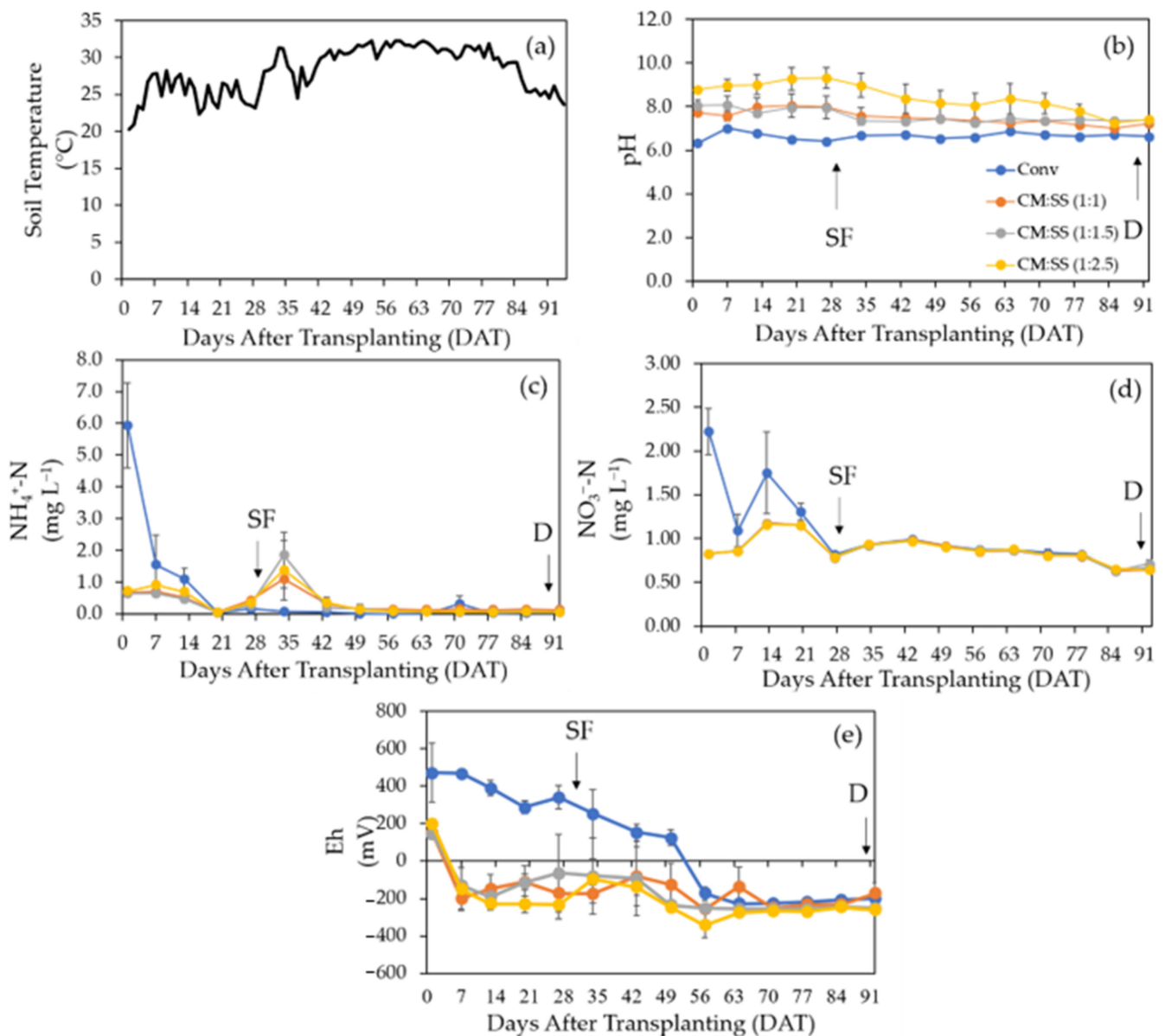
**Table 3.** Global warming potential of CH<sub>4</sub> (GWP<sub>CH4</sub>) and N<sub>2</sub>O (GWP<sub>N2O</sub>) (kg CO<sub>eq</sub> ha<sup>-1</sup>) in the pot experiment.

	Treatments			
	Conv	CM:SS (1:1)	CM:SS (1:1.5)	CM:SS (1:2.5)
GWP <sub>CH4</sub>	1260 a	3030 b	1660 a	1860 ab
GWP <sub>N2O</sub>	97.7 a	78.5 a	-44.2 a	85.5 a
Total	1360 a	3110 b	1620 ab	1940 ab

GWP<sub>CH4</sub> and GWP<sub>N2O</sub> represent carbon dioxide equivalent values of cumulative CH<sub>4</sub> emission and cumulative N<sub>2</sub>O emission, respectively. All values are expressed as mean. Conv: conventional, CM: chicken manure, SS: steel slag. Error bars represent standard error. Different letters within the same row among the treatments indicate a significant difference ( $p < 0.05$ ).

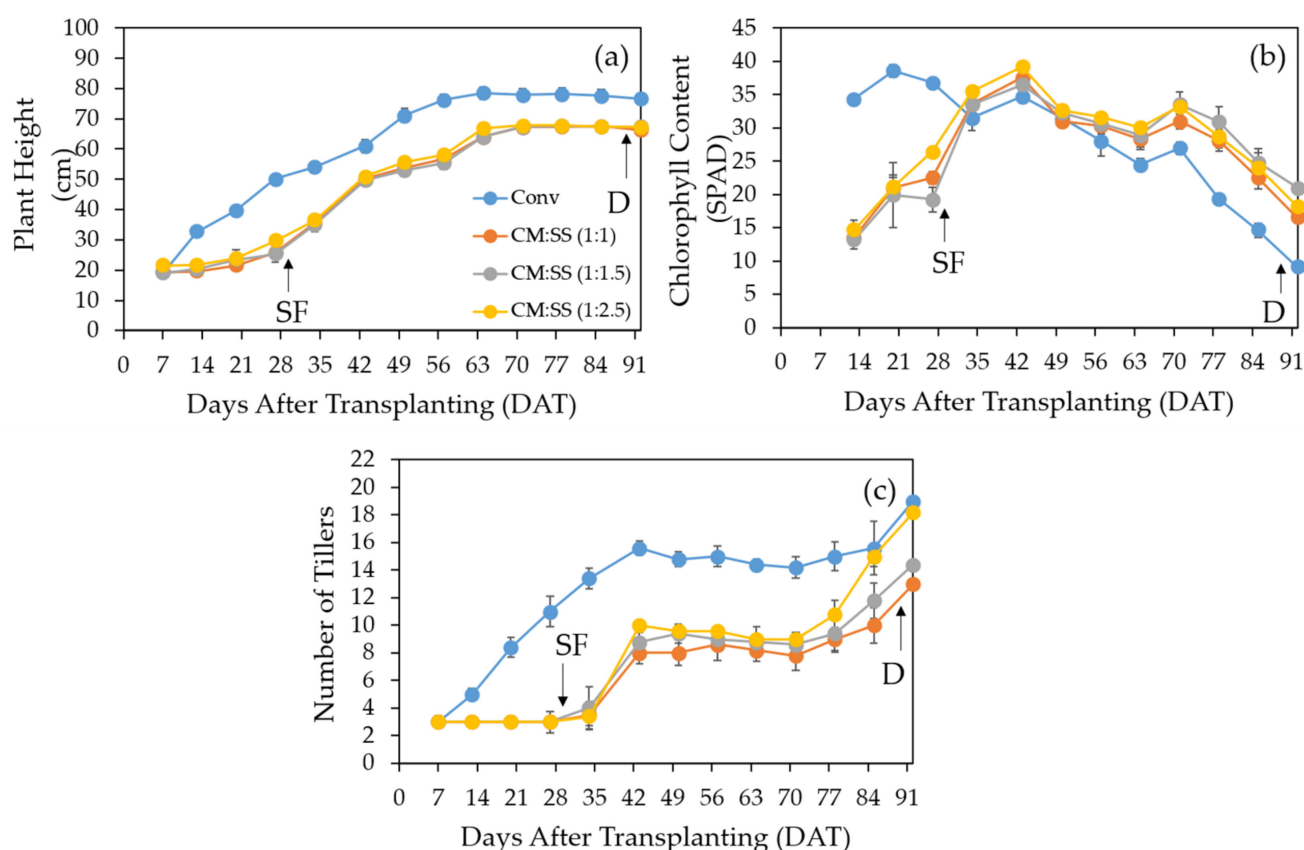
Variations in soil temperature, pH, NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N concentrations, and Eh during rice cultivation in the pot experiment are shown in Figure 5. The mean daily temperature during the pot experiment (June–September) was 28.1 °C (Figure 5a). The highest temperature was observed on 9 August (32.2 °C), while the lowest was observed on 18 June (20.2 °C). Soil water pH was higher in all CM:SS treatments than in Conv during the entire experimental period (Figure 5b). The highest pH value was observed in CM:SS (1:2.5) (9.3) at 27 DAT. The lowest pH value was observed for Conv (6.3) at 1 DAT. NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N concentrations in soil water were increased in Conv at 1 DAT but decreased sharply at 7 DAT (Figure 5c,d). Both NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N concentrations in the Conv treatment showed the highest values at 1 DAT. NH<sub>4</sub><sup>+</sup>-N concentration increased rapidly at 34 DAT in all CM:SS treatments after application of supplemental NPK fertilizer but decreased sharply at 43 DAT. However, NO<sub>3</sub><sup>-</sup>-N concentrations from all treatments were almost the same, except in Conv. The Eh decreased sharply in all treatments within three weeks after transplanting, except in the Conv treatment (Figure 5e). The Eh in all CM:SS treatments was lower than that in Conv during the experiment. The Eh decreased sharply again at 50 DAT in all CM:SS treatments but decreased gradually and sharply at 57 DAT in the Conv treatment.

Variations in plant height, chlorophyll content, and the number of tillers during rice cultivation in the pot experiment are shown in Figure 6. The plant height ranged from 19.08 to 21.5 cm at 7 DAT. Conv was the highest plant height starting 7 to 92 DAT. All the CM:SS treatments have almost the same plant height from 7 to 92 DAT. Chlorophyll content in Conv was the highest at 20 DAT, then gradually decrease until the end of the experiment. All chlorophyll content in CM:SS treatments started to increase gradually from 20 DAT then increase sharply after the supplementary fertilization at 30 DAT. Chlorophyll content in all CM:SS treatments reached their maximum number at 43 DAT, then gradually decrease until the end of the experiment. The number of tillers in Conv increased sharply from 7 to 43 DAT. All the CM:SS treatments started to increase the number of tillers at 34 DAT. At the end of the experiment, the number of tillers in CM:SS (1:2.5) showed almost the same number as Conv. Generally, throughout the experiment, all the CM:SS treatments had the same trend in plant height, chlorophyll content, and the number of tillers.



**Figure 5.** Soil temperature (a), Soil water pH (b),  $\text{NH}_4^+\text{-N}$  (c) and  $\text{NO}_3^-\text{-N}$  (d) concentrations, and Eh (e) in each treatment during the pot experiment. All values are expressed as mean. Conv: conventional, CM: chicken manure, SS: steel slag, SF: supplementary fertilization, D: the time when the pot was dried. Error bars represent standard error.

The dry biomass and grain yields are listed in Table 4. Conv had the highest biomass and grain weight, and was statistically significant relative to the other treatments. The CM:SS (1:1), CM:SS (1:1.5), and CM:SS (1:2.5) did not maintain the biomass yield in Conv. Although there was no significant difference, all components (dry biomass and grain yield) in the CM:SS treatments increased with the increasing ratio of SS application.



**Figure 6.** Plant height (a), chlorophyll content (b), number of tillers (c) in each treatment during the pot experiment. All values are expressed as mean. Conv: conventional, CM: chicken manure, SS: steel slag, SF: supplementary fertilization, D: the time when the pot was dried. Error bars represent standard error.

**Table 4.** Dry biomass and grain yield after harvest in the pot experiment.

Treatment	Dry Biomass (g pot <sup>-1</sup> )		Grain (g pot <sup>-1</sup> )	
	Above Ground	Root	Fresh Matter	Dry Matter
Conv	29.0 b	4.87 b	7.18 b	7.04 b
CM:SS (1:1)	11.7 a	2.13 a	3.22 a	3.16 a
CM:SS (1:1.5)	14.6 a	2.81 a	3.88 a	3.81 a
CM:SS (1:2.5)	15.9 a	2.84 a	4.02 a	3.94 a

Biomass and grain yield of rice in each treatment in the pot experiment. All values are expressed as mean. Conv: conventional, CM: chicken manure, SS: steel slag. Error bars represent standard error. Different letters within the same column among the treatments indicate a significant difference ( $p < 0.05$ ).

## 4. Discussion

### 4.1. GHG Emissions in the Incubation and Pot Experiments

Cumulative CH<sub>4</sub> emissions in both the incubation and pot experiments showed that CH<sub>4</sub> emissions could be suppressed by a higher rate of SS. Slag-type fertilizers contain high amounts of iron, silica, and calcium. Further, active iron oxide can be used as an oxidizing agent. The iron content of the SS ameliorant has been shown to act as an electron acceptor that decreases methanogenic activity and mitigates CH<sub>4</sub> emissions from rice paddies [5]. CH<sub>4</sub> production was inhibited by electron acceptors, such as NO<sub>3</sub><sup>-</sup>, Fe<sup>3+</sup>, and SO<sub>4</sub><sup>2-</sup>, when added to paddy soils [12]. Beal et al. [13] reported that the presence of ferric ions could support the oxidation of CH<sub>4</sub> under anaerobic conditions. An increase in the ferric iron concentration could escalate CH<sub>4</sub> oxidation under anaerobic conditions, thereby reducing CH<sub>4</sub> flux. In this study, the Fe<sup>2+</sup> content in the incubation experiment showed that in the SS amendment treatments, Fe<sup>2+</sup> content was reduced compared to that in the SO treatment

(Figure 2d). Iron oxide in the SS may have acted as an oxidant and suppressed the reduction of flooded soil.

In the incubation experiment, CM:SS (1:2.5) showed the lowest CH<sub>4</sub> emission and was statistically significant relative to other CM:SS treatments and the SO treatment. However, in the pot experiment, CM:SS (1:1.5) showed the lowest CH<sub>4</sub> emission among the CM:SS treatments, but no statistical significance was found relative to CM:SS (1:2.5). In the pot experiment, CH<sub>4</sub> emissions from CM:SS (1:1.5) were lower than that from the other CM:SS treatments, possibly due to the lower organic C content (5.51 gC m<sup>-2</sup>) than CM:SS (1:1) (5.78 gC m<sup>-2</sup>) and CM:SS (1:2.5) (5.63 gC m<sup>-2</sup>). Organic C can provide C as an energy source for methanogenic bacteria to produce CH<sub>4</sub> [14]. Organic C is an important factor affecting CH<sub>4</sub> production capacity, and the readily decomposed organic matter in paddy fields increases CH<sub>4</sub> emissions under an anaerobic environment [15].

In the CM:SS (1:1) treatment in which SS application was lower than that of the other CM:SS treatments, cumulative CH<sub>4</sub> emission was the highest, possibly due to insufficient SS application to suppress CH<sub>4</sub> production. This finding is similar to that of Lee et al. [16], who found that iron slag silicate fertilizer failed to effectively suppress CH<sub>4</sub> production in soil, which might be due to its electron acceptor activity being insufficient to receive all electrons detached from the reduction process because of the high organic matter content. Conv treatment had the lowest CH<sub>4</sub> emissions as it did not contain additional organic matter (only chemical fertilizer). The application of organic matter increases CH<sub>4</sub> production in submerged soil conditions because methanogenic bacteria use labile organic C in organic matter as substrates to perform metabolism [17]. According to Wang et al. [6], the optimal pH for CH<sub>4</sub> production is approximately neutral. In CM:SS (1:1), the soil water pH ranged from 7.00–7.97, which was lower than that of the other CM:SS treatments.

There were no significant differences in N<sub>2</sub>O emissions in the incubation and pot experiments between treatments due to the higher variation in N<sub>2</sub>O flux. However, CM:SS treatments tended to decrease with CM and SS mixture ratio in the pot experiment. The SS amendment increased soil pH in the incubation experiment (Figure 2a) and soil water in the pot experiment (Figure 5b), which may be due to the release of base cations, such as Ca<sup>+2</sup>. The soil water pH from the CM:SS treatments was higher than seven from 1 to 42 DAI in the incubation experiment. Further, the soil water pH was also the highest from the early until end growth of paddy in the pot experiment indicated that the denitrification process might be suppressed. Noubactep [18] reported that reduced N<sub>2</sub>O emissions could be caused by an increase in the iron oxide concentration, suppressing microbial activities, including N<sub>2</sub>O production. In this study, N<sub>2</sub>O emissions in the incubation and pot experiments were not significantly different based on the application of CM and SS. However, in the pot experiment, CM:SS (1:1.5) treatment had the lowest cumulative emission among all treatments (−0.09 kg N ha<sup>-1</sup>), but was not statistically significant from the other treatments. Although there was no statistical difference in cumulative N<sub>2</sub>O emissions, CM:SS (1:1.5) reduced N<sub>2</sub>O emissions by 142% compared to Conv. As shown in our study, the effects of CM:SS fertilizer on N<sub>2</sub>O production in paddy soils deserves further investigation.

Our results demonstrate that amending the CM and SS ratio reduces CH<sub>4</sub> emissions from rice cultivation. Although the different ratios of CM and SS treatments could not mitigate CH<sub>4</sub> emission compared to conventional treatment, in which organic matter was not applied, treatment with higher rate SS, CM:SS (1:1.5), and CM:SS (1:2.5) had lower CH<sub>4</sub> emission than CM:SS (1:1). The application of organic matter is important for maintaining soil productivity. The use of the CM and SS mixture in this study is one of the solutions to utilize organic matter instead of chemical fertilizer in rice fields without impacting global warming.

#### 4.2. Plant Growth, Biomass, and Rice Yield

The use of organic and chemical fertilizers by farmers has been reported to increase yield, sustain soil productivity, and improve soil physicochemical properties. Some studies have shown that amending SS has a good impact on plant growth and yield components.

Ali et al. [19] reported that SS application increased the grain yield by 17% at a rate of 4 Mg ha<sup>-1</sup> compared to the control. Moreover, Susilawati et al. [20] reported that SS application could increase the grain yield by 4.8–5.6% at one of the study sites in Indonesia during the dry and rainy seasons. However, in this study, Conv produced the highest biomass and rice yield among the CM:SS treatments due to the higher rate of chemical fertilizer application (30 g pot<sup>-1</sup>). CM:SS treatments could not maintain the yield produced using Conv, which might be due to the higher volatilization rates under high soil pH at high temperatures. According to Jones et al. [21], high soil pH and high temperatures might cause higher volatilization rates due to the increasing soil concentrations of ammonia dissolved in soil water and the inability of warm soil water to hold as much ammonia gas. The soil water pH in the CM:SS treatments ranged from 7.0–9.3, indicating a high pH during the pot experiment. Shamsuddin et al. [22] reported that rice roots grow normally in soil when the pH value is approximately 6. The suppression of CM mineralization under alkaline conditions also contributed to the lower yield in the different CM and SS mixtures. In fact, in the incubation experiment, CO<sub>2</sub> emissions were low in the treatments with a higher ratio of SS (Table 1), indicating that SS could suppress microbial activity.

Although grain yield in the CM:SS treatments was not statistically significant, CM:SS (1:2.5) had the highest yield among the CM:SS treatments. Under the same N application rate, the yield was found to increase at a higher rate of SS application. SS is mainly composed of CaO and SiO<sub>2</sub>, which are essential nutrients for paddy fields. SS can increase SiO<sub>2</sub> availability and increase rice yield by promoting photosynthesis [23].

The chlorophyll content in CM:SS treatments had a better value than Conv at the end of the experiment. In CM:SS (1:2.5), the number of tillers showed almost the same number with Conv. These indicate that available nutrients were released slowly and steadily from manure decomposition. Moe et al. [24] reported that organic fertilizers release nutrients slowly, thus, rice plants might grow slowly at the early growth stage. Conv had the highest yield because the release of N from chemical fertilizer was faster than that from the CM and SS mixture, thereby allowing plants to uptake the available N faster in Conv. Therefore, it is better to place the CM and a SS mixture into the soil before transplanting.

## 5. Conclusions

Herein, CM:SS (1:2.5) was identified as the best CM and SS ameliorant for mitigating CH<sub>4</sub>, N<sub>2</sub>O, and CO<sub>2</sub> emissions in the incubation experiment. However, in the pot experiment, CM:SS (1:1.5) was the best ameliorant for mitigating CH<sub>4</sub> and N<sub>2</sub>O relative to CM:SS (1:1) and CM:SS (1:2.5). The total GWP in the pot experiment showed that CM:SS (1:1.5) had the lowest value among the treatments. Further, the CM:SS (1:1, 1:1.5, and 1:2.5) treatments did not significantly increase dry biomass and grain yield compared to conventional treatment.

**Author Contributions:** Conceptualization, B.N., S.A., H.U., and Y.T.; methodology, M.I.F. and Y.T.; formal analysis, M.I.F.; investigation, M.I.F.; resources, Y.T.; data curation, M.I.F.; writing—original draft preparation, M.I.F.; writing—review and editing, B.N., S.A., H.U., and Y.T.; visualization, M.I.F.; supervision, B.N., S.A., H.U., and Y.T.; project administration, Y.T.; funding acquisition, Y.T. All authors have read and agreed to the published version of the manuscript.

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