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Residual Effects of Rice Husk Biochar and Organic Manure Application after 1 Year on Soil Chemical Properties, Rice Yield, and Greenhouse Gas Emissions from Paddy Soils

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Abstract: Biochar is stable in soil and can have long-term effects on its physicochemical properties. Hence, a pot experiment was conducted with medium-fertility (MF) and low-fertility (LF) soils after 1 year of rice husk biochar and organic fertilizer application to determine biochar's residual effects on soil chemical properties, grain yield, and greenhouse gas emissions. In previous years, biochar alone (at application rates of 5 and 10 t ha⁻¹) and biochar combined with chicken manure (CHM) or cow manure (at application rate of 5 t ha⁻¹) were applied to the soil. In the present year, the soils were fertilized with only chemical fertilizers. Results indicated that application of 10 t ha⁻¹ biochar combined with 5 t ha⁻¹ CHM (B10:CHM) produced the highest grain yield and total global warming potential (GWP_{total}) in both soils. Regarding grain yield, non-significant results were detected for B10:CHM, B5:CHM, and B10. This study revealed that biochar retains nutrients without annual reapplication and has long-term effects. Although biochar application can suppress N₂O emissions effectively, the combined application of biochar 10 t ha⁻¹ and organic manure significantly increased CH₄ emissions. Overall, B5:CHM can be recommended for rice cultivation since it improves grain yield without increasing GWP_{total}.

Keywords: rice husk biochar; organic manure; soil chemical properties; greenhouse gas emissions; soil fertility

1. Introduction

Over the past few decades, greenhouse gas (GHG) emissions have received increasing attention [1]. Increasing GHG emissions have been predicted to lead to an increase in the Earth's temperature of $4.5 \,^{\circ}$ C by 2100 [2]. Furthermore, a 50% increase in total GHG emissions is predicted to occur between 2000 and 2030, and this increase in GHG emissions will have a significant impact on climate change [3]. Consequently, climate change threatens the production of rice, wheat, and maize, which are staple foods worldwide. Cereal food supplies for 9.8 billion people will need to increase by 70–100% by 2050 [4].

Additional organic and inorganic fertilizers are being applied in agriculture to supply food demand for growing population. As a consequence, an estimated 80% of anthropogenic N₂O emissions and 70% of anthropogenic NH₃ emissions originate from agriculture, primarily from the use of fertilizers [5]. Currently, most rice-producing countries are facing the challenge of maintaining yield while simultaneously reducing GHG emissions from paddy fields [6]. Thus, a number of mitigation techniques, such as improved fertilizer management and better agricultural practices, must be considered to enhance agricultural production without increasing GHG emissions [7,8].

Biochar is a carbonaceous substance produced by pyrolysis of various feedstocks under oxygen-controlled conditions. In agriculture, biochar is considered an alternative



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Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). soil amendment because of its ability to store carbon, mitigate climate change, improve soil properties, and increase crop yields [9]. Biochar has a large porous structure and can attract positively charged ions (cations) such as potassium, calcium, magnesium, and ammonium. Thus, soils amended with biochar have an increased CEC, which in turn allows the soil to hold more nutrients and reduces nutrient leaching [10]. Moreover, biochar amendment can modify soil aeration, adsorption, soil water-holding capacity, pH, and the activity of microbial and enzymatic organisms, which can affect CH₄ transport and oxidation [11–13]. Following the application of biochar, CH₄-oxidizing bacteria (methanotrophs) are abundant, leading to the dominance of CH₄ oxidation over CH₄ production by methanogens, which reduces CH₄ emissions [14]. Zhang et al. [15] observed that the application of wheat straw biochar reduced overall N₂O emissions by 40–51% and 21–28% when compared with control treatments (without N fertilizer) and with N-fertilizer. It is possible that biochar increases N immobilization, interacts with carbon and nitrogen in the soil, modifies enzyme activity, and causes toxicity to nitrifiers and denitrifiers [16].

Another interesting and advantageous effect of biochar is its stability in soils owing to its crystalline nature [17]. Since the discovery of Terra Preta de Indio soils in the Amazon, biochar has received increasing attention as a potential soil amendment. Terra Preta soils have aged over time and have been proven to be highly productive and capable of retaining nutrients. When fresh biochar is added to the soil, it undergoes aging processes similar to those of Terra Preta soils [18]. Therefore, biochar is expected to have long-term effects on crop growth and soil physicochemical properties, as evidenced by its high char content in the Terra Preta soil [19]. Although several studies have been conducted regarding the effects of biochar on physical, biological, and chemical soil properties, as well as on plant growth [20], there is still a lack of information about the residual effects of biochar on rice growth and the mitigation of GHG emissions. In addition, Mon et al. [21] reported that further research is necessary to investigate the effect of biochar application alone or in combination with organic manures on N2O emissions from paddy soils. To address these issues, we investigated the residual effects of biochar fertilization 1 year after its application in rice production. The objectives of this study were (i) to examine the possible effects of biochar after 1 year of soil amendment on changes in soil chemical properties, (ii) to examine GHG emissions from two different soils, and (iii) to investigate whether biochar inputs should be reapplied annually to maintain grain yield. These objectives will explore the impact of biochar on soil properties, greenhouse gas dynamics, and crop productivity and provide insights into its potential role in sustainable agricultural practices.

2. Materials and Methods

2.1. Study Area and Treatments

A pot experiment was conducted from 4 June 2023 to 9 September 2023 under greenhouse conditions at Ehime University (33.83° N, 132.79° E), Ehime Prefecture, Japan. Three seedlings of the rice plant cultivar Koshihikari were transplanted into 0.02 m² Wagner pots with residual soils from the first season of rice cultivation [21]. Rice husk biochar (5 and 10 t ha⁻¹) and organic manure (5 t ha⁻¹) were thoroughly mixed into soils 1 year ago on 6 June 2022, and, subsequently, rice was cultivated in 2022. After rice cultivation, all pots were kept in a greenhouse at Ehime University.

For the 2023 experiment, we no longer applied biochar, chicken manure, or cow manure; we used the same pots from the first season to determine the residual effect of biochar fertilization over a one-year period. One week before transplanting, all pots were irrigated and basal fertilizer was applied. Supplemental nitrogen, phosphorus, and potassium (NPK; 15%-15%-15%) were applied at basal, 14 and 30 days after transplanting (DAT). All treatments received 1.33 g of pot⁻¹ NPK (15-15-15) fertilizer. Urea fertilizer, as the top dressing, was applied at 40, 47, and 54 DAT with an application rate of 0.13 g N pot⁻¹ (30 kg N ha⁻¹ on the weight basis) during each application. All pots were irrigated on the day of basal NPK fertilizer application. Daily irrigation was provided until 7 days before harvesting.

The two different soils were as follows: (1) medium-fertility (MF) soil, obtained by mixing rice nursery soil and sand at a ratio of 1:1, and (2) soil collected from Toon City, Ehime Prefecture, Japan, which had low fertility (LF). The initial chemical properties of the MF soil had pH 6.17, electrical conductivity (EC) 274 μ S cm⁻¹, exchangeable K content 0.15 cmol_(c) kg⁻¹, exchangeable Mg content 1.08 cmol_(c) kg⁻¹, exchangeable Ca content 10.3 cmol_(c) kg⁻¹, total N 0.18%, and total C 0.53%. LF soil had pH 7.86, EC 21 μ S cm⁻¹, exchangeable K content 0.06 cmol_(c) kg⁻¹, exchangeable Mg content 0.88 cmol_(c) kg⁻¹, exchangeable Ca content 9.84 cmol_(c) kg⁻¹, total N 0.02%, and total C 0.03%. The pot experiment was conducted in triplicate using a completely randomized design. Treatments included (i) no biochar application (C), (ii) application of rice husk biochar 5 t ha⁻¹ (B5), (iii) application of rice husk biochar 10 t ha⁻¹ (B10), (iv) 5 t ha⁻¹ of rice husk biochar combined with 5 t ha⁻¹ of chicken manure (B5:COM), (vi) 10 t ha⁻¹ of rice husk biochar along with 5 t ha⁻¹ of chicken manure (B10:CHM), and (vii) rice husk biochar 10 t ha⁻¹ along with 5 t ha⁻¹ of cow manure (B10:COM).

In this experiment, we used commercially available biochar that had been pyrolyzed at temperatures between 900 °C and 1000 °C. The following are the characteristics of rice husk biochar, pH 6.45, EC 856.3 μ S cm⁻¹, exchangeable K content 14,959 mg kg⁻¹, 44.9% of ash content, total N content 0.8%, total C content 33.7%, and cation exchange capacity (CEC) 25.4 cmol_(c) kg⁻¹. The laboratory analytical methods for determining NH₄⁺, NO₃⁻, available P, exchangeable cations, total N, and total C have been described in a previous study [21]. The chemical properties of the organic manure used in this study are listed in Table 1.

Table 1. Chemical properties of organic manures.

Measurements	Unit	Chicken Manure	Cow Manure
Total N	%	4.1	1.9
Total C	%	25.0	34.1
C/N		6.1	17.9
Available P content	${ m mg}~{ m kg}^{-1}$	1334	2548

2.2. Measurement of Grain Yield and GHG Measurements

The rice plants were harvested on 9 September 2023. To determine dry biomass production, we divided the harvested plants into the main stems and leaves as aboveground parts and roots as belowground parts. The samples were washed with water, air-dried, and oven-dried at 70 °C for 2 days. Grain yield was calculated by measuring the total grain weight per pot.

The gas samples were collected using the closed-chamber method. Once the chamber was installed, gas samples were collected at 0, 10, and 20 min. Gas samples were collected before and on the day of basal NPK fertilizer application, on the day of transplantation, and at 1, 3, 7, 14, 21, 28, 35, 42, 49, 70, 84, and 98 DAT. A short acrylic chamber with a volume of 0.0032 m³ was used during the early stages of rice cultivation (until 28 DAT), whereas a tall acrylic chamber with a volume of 0.017 m³ was used from 35 to 98 DAT. Both acrylic chambers have the same diameter of 16 cm, and the heights of the short and tall chambers are 16 and 85 cm, respectively. CH₄ and N₂O concentrations were analyzed using a gas chromatograph equipped with a flame ionization detector and an electron capture detector (GC- 14A, Shimadzu, Kyoto, Japan). The calculations of the CH₄ and N₂O fluxes and the cumulative emissions GWP_{CH4}, and GWP_{N2O} are described in [21]. The total GWP period⁻¹ (GWP_{total}) was estimated as the sum of GWP_{CH4} and GWP_{N2O} values.

2.3. Statistical Analysis

All data were analyzed using a two-way factorial analysis of variance with Real Statistics Resource Pack statistical software (Released 8.4). We performed multiple comparisons among the treatments using Tukey's honest significant difference test at a sig-

nificance level of p < 0.05. Multiple regression analysis was performed using grain yield, GWP_{CH4}, GWP_{N2O}, and GWP_{total} as explanatory variables to obtain standardized regression coefficients.

3. Results

3.1. Effects of Biochar Fertilization after 1 Year on Soil Nutrients

Changes in soil chemical properties under different treatments after rice cultivation are shown in Figure 1. One year after biochar fertilization, the soil pH significantly increased with the presence of biochar in the MF soil (Figure 1a). Compared with C, the application of B5, B10, B5:CHM, B5:COM, B10:CHM, and B10:COM increased the pH of the MF soil by 3.0%, 0.9%, 5.8%, 3.0%, 5.9%, and 1.2%, respectively. The pH values of LF soil after the various treatments were not significantly different from those of C (Figure 1a). There was an increase in the soil pH following the application of B5:CHM and B10:CHM in LF soil by 2.6% and 5.9%, respectively, compared to that in C. However, the application of B5, B10, B5:COM, and B10:COM decreased the soil pH by 2.2%, 1.5%, 0.5%, and 2.3%, respectively, compared with C.



Figure 1. Cont.







Figure 1. Soil pH (**a**), electrical conductivity (EC) (**b**), NH₄⁺-N (**c**), NO₃⁻-N (**d**), available phosphorus (**e**), exchangeable potassium (**f**), exchangeable magnesium (**g**), and exchangeable calcium (**h**). All values are expressed as mean \pm standard error. Values with different letters in the same column indicate significant differences at the 5% level. Medium fertility (MF), low fertility (LF). No biochar (C), 5 t ha⁻¹ of rice husk biochar application (B5), 10 t ha⁻¹ of rice husk biochar application (B10), 5 t ha⁻¹ of rice husk biochar + 5 t ha⁻¹ of chicken manure (B5:CHM), 5 t ha⁻¹ of rice husk biochar + 5 t ha⁻¹ of chicken manure (B10:CHM), 10 t ha⁻¹ of rice husk biochar + 5 t ha⁻¹ of cow manure (B10:COM).

Regarding soil EC, 10 t ha⁻¹ of biochar application alone or in combination with organic manure significantly increased the soil EC compared to C in both soils (Figure 1b). The same magnitude of soil EC was found in both soils in the following order: B10:CHM > B5:CHM > B10:COM > B10 > B5:COM > B5 > C. In both soils, all biochar treatments did not significantly differ in soil NH₄⁺-N; however, they significantly increased soil NH₄⁺-N compared to the control treatment (Figure 1c). No significant difference was detected among the treatments in soil NO₃⁻-N in either MF or LF soil (Figure 1d). The B10:CHM treatment resulted in the highest values of both NH₄⁺-N and NO₃⁻-N regardless of soil fertility.

In the MF soil, the highest available P content was achieved in the B10:COM treatment, followed by the B10:CHM, B5:COM, and B5:CHM treatments. In the LF soil, the highest available P was detected in the B10:COM treatment, which was not significantly different from those detected in the B10:CHM, B5:COM, and B10 treatments (Figure 1e). Notably, the application of B10, B5:CHM, B10:CHM, and B10:COM significantly increased the soil exchangeable K compared with C in both soils (Figure 1f). The order of soil exchangeable K for both soils, arranged from highest to lowest, was B10:CHM > B10:COM > B5:CHM > B10 > B5 > B5:COM > C. Significant differences were not detected in soil exchangeable Mg and Ca among the different treatments. Soils under treatment C showed the lowest available soil nutrients for all soil chemical property parameters.

3.2. Grain Yield under Different Biochar Amendments

The highest aboveground dry biomass and root dry biomass were achieved with the B5:CHM treatment in the MF soil; however, this increase was not significant compared to that of the B5, B10, and B10:CHM treatments (Table 2). In the LF soil, the B10:CHM treatment produced the maximum aboveground and root biomass. No significant increase in the aboveground dry biomass was observed in soils treated with biochar. The B10:CHM treatment showed the highest grain yield, followed by the B5:CHM and B10 treatments, in both soils. No significant differences in grain yield were noted among the B10:CHM, B5:CHM, and B10 treatments for either soil type. The order of grain yield in both MF and LF soils was B10:CHM > B5:CHM > B10 > B10:COM > B5 > B5:COM > C. There were no interaction effects between soil type and treatment.

Treatments	Aboveground Dry Weight (g pot ⁻¹)		Root Dry We	ight (g pot $^{-1}$)	Grain Yield (g pot ⁻¹)		
	MF	LF	MF	LF	MF	LF	
С	$34.3\pm0.5~\mathrm{d}$	$21.5\pm0.6b$	$4.2\pm0.1\mathrm{b}$	2.80 ± 0.4 a	$34.8\pm1.7~\mathrm{e}$	$18.9\pm1.0~\mathrm{e}$	
B5	$44.5\pm0.7~\mathrm{a}$	33.2 ± 1.6 a	5.9 ± 0.4 a	3.50 ± 0.3 a	$47.1\pm1.5~\mathrm{c}$	$27.7\pm0.8~{\rm c}$	
B10	$44.9\pm0.5~\mathrm{a}$	29.9 ± 2.5 a	5.6 ± 0.6 a	3.60 ± 0.2 a	53.9 ± 1.0 a	$35.4\pm1.0~\mathrm{a}$	
B5:CHM	46.3 ± 1.1 a	$30.8\pm1.0~\mathrm{a}$	6.3 ± 0.3 a	$4.00\pm0.1~\mathrm{a}$	58.1 ± 1.6 a	39.3 ± 4.0 a	
B5:COM	$35.8\pm0.5~\mathrm{c}$	26.7 ± 1.6 a	5.8 ± 0.2 a	3.53 ± 0.1 a	$37.8 \pm 2.3 \text{ d}$	$23.8\pm1.7~\mathrm{d}$	
B10:CHM	45.8 ± 1.9 a	$32.4\pm0.7~\mathrm{a}$	5.9 ± 0.4 a	$4.07\pm0.5~\mathrm{a}$	58.5 ± 1.0 a	41.7 ± 1.5 a	
B10:COM	$40.1\pm0.6~\text{b}$	$29.6\pm0.8~a$	5.3 ± 0.3 a	$3.63\pm0.2~\mathrm{a}$	$50.0\pm1.4\mathrm{b}$	$28.1\pm3.0~b$	
Between two soils	<0.001		<0.001		<0.001		
Within treatments	< 0.001		<0	< 0.001		<0.001	
Soils \times treatments	0.1	135	0.6	798	0.4	979	

Table 2. Dry biomass weight and grain yield (mean \pm standard error).

Values with different letters in the same column indicate significant differences at the 5% significance level. Medium fertility (MF), low fertility (LF). No biochar (C), 5 t ha⁻¹ of rice husk biochar application (B5), 10 t ha⁻¹ of rice husk biochar application (B10), 5 t ha⁻¹ of rice husk biochar + 5 t ha⁻¹ of chicken manure (B5:CHM), 5 t ha⁻¹ of rice husk biochar + 5 t ha⁻¹ of chicken manure (B5:CHM), 10 t ha⁻¹ of chicken manure (B10:CHM), 10 t ha⁻¹ of rice husk biochar + 5 t ha⁻¹ of chicken manure (B10:CHM), 10 t ha⁻¹ of rice husk biochar + 5 t ha⁻¹ of cow manure (B10:CHM), 10 t ha⁻¹ of rice husk biochar + 5 t ha⁻¹ of cow manure (B10:CHM), 10 t ha⁻¹ of rice husk biochar + 5 t ha⁻¹ of cow manure (B10:CHM), 10 t ha⁻¹ of rice husk biochar + 5 t ha⁻¹ of cow manure (B10:CHM), 10 t ha⁻¹ of rice husk biochar + 5 t ha⁻¹ of cow manure (B10:CHM), 10 t ha⁻¹ of rice husk biochar + 5 t ha⁻¹ of cow manure (B10:CHM), 10 t ha⁻¹ of rice husk biochar + 5 t ha⁻¹ of cow manure (B10:CHM), 10 t ha⁻¹ of rice husk biochar + 5 t ha⁻¹ of cow manure (B10:CHM), 10 t ha⁻¹ of rice husk biochar + 5 t ha⁻¹ of cow manure (B10:COM).

3.3. CH₄ and N₂O Fluxes and GHG Emissions

Figure 2 depicts the CH₄ and N₂O fluxes in both soils. The CH₄ fluxes in both MF and LF soils fluctuated during the early growth stages of the paddy rice. The CH₄ fluxes under different treatments peaked during the late growth stages. At 84 DAT, B10:COM-treated MF and LF soils reached maximum peaks of 5302.9 and 4498.9 μ gC m⁻² h⁻¹, respectively (Figure 2a,b).

Regarding N₂O fluxes, the variation was wide throughout the growing season, not only in the MF soil but also in the LF soil. The maximum peak was observed in B10:COM (54.9 μ gN m⁻² h⁻¹) at the beginning of rice cultivation, 1 DAT, in the MF soil, whereas in B5:COM (24.2 μ gN m⁻² h⁻¹) was seen at 35 DAT in the LF soil. The B10:CHM and B5 treatments showed almost the same peak, with the maximum peak of B5:COM at 1 and 84 DAT in the LF soil (Figure 2c,d).

The cumulative emissions of CH₄ and N₂O in the pot experiments for both soils are listed in Table 3. The highest cumulative CH₄ emissions were recorded for the B10:CHM treatment. The cumulative CH₄ emissions from the MF soil were in the following order: B10:CHM > B10:COM > B10 > B5:CHM > B5:COM > B5 > C, whereas those from the LF soil were in the following order: B10, CHM > B10, COM > B5, COM > B5, C > B5, and CHM > B10. Interaction effects were observed between soil type and treatment on cumulative CH₄ emissions. With respect to cumulative N₂O emissions, the order of magnitude in the MF soil was C > B5 > B5:COM > B5:CHM > B10:COM > B10:CHM, whereas that in the LF soil was C > B5 > B5:COM > B10:COM > B10:COM > B10:CHM > B10.



Figure 2. Cont.





Figure 2. Dynamics of CH₄ flux from MF soil (**a**), CH₄ flux from LF soil (**b**), N₂O flux from MF soil (**c**), and N₂O flux from LF soil (**d**) during pot experiments. Mean \pm standard error was used to express the data. Medium fertility (MF), low fertility (LF). No biochar (C), 5 t ha⁻¹ of rice husk biochar application (B5), 10 t ha⁻¹ of rice husk biochar application (B10), 5 t ha⁻¹ of rice husk biochar + 5 t ha⁻¹ of chicken manure (B5:CHM), 5 t ha⁻¹ of rice husk biochar + 5 t ha⁻¹ of cow manure (B5:COM), 10 t ha⁻¹ of rice husk biochar + 5 t ha⁻¹ of chicken manure (B10:CHM), 10 t ha⁻¹ of rice husk biochar + 5 t ha⁻¹ of cow manure (B10:COM).

Table 3. Cumulative CH₄ and N₂O emissions during the pot experiment.

Treatments	Cumulative ((mg C m ⁻²	CH ₄ Emission ² 96 Days ⁻¹)	Cumulative N ₂ O Emission (mg N m ⁻² 96 Days ⁻¹)		
-	MF	LF	MF	LF	
С	$1440\pm42.1~\mathrm{bc}$	$1431\pm173.7~\mathrm{c}$	$16.30\pm1.8~\mathrm{a}$	17.22 ± 0.5 a	
B5	$1400\pm58.4~{ m bc}$	$1434\pm43.9\mathrm{b}$	$12.50\pm5.7~\mathrm{a}$	16.69 ± 4.2 a	
B10	$1742\pm130.0\mathrm{b}$	$1180\pm228.3~\mathrm{bcd}$	0.69 ± 4.5 a	$-5.40\pm5.2~\mathrm{c}$	
B5:CHM	$1487\pm159.4~{\rm c}$	$1396 \pm 24.6 \text{ d}$	2.19 ± 1.0 a	-1.62 ± 0.9 a	
B5:COM	$1429\pm 6.3\mathrm{bc}$	2343 ± 218.3 a	6.56 ± 3.8 a	10.60 ± 2.8 a	
B10:CHM	$3059\pm25.3~\mathrm{a}$	2674 ± 34.2 a	$-4.44\pm4.1\mathrm{b}$	-3.74 ± 4.6 b	
B10:COM	$2810\pm32.3~\mathrm{a}$	$2486\pm76.6~\mathrm{a}$	-1.79 ± 0.9 a	$6.01\pm4.7~\mathrm{a}$	
Between two soils	0.3	3461	0.5	801	
Within treatments	<0.	.001	<0.	001	
Soils × treatments	<0.	.001	0.5	420	

Data are expressed as mean \pm standard error. Different letters within the same column denote significant differences between groups at the 5% significance level, as determined by the LSD test. MF: medium-fertility soil; LF: low-fertility soil. No biochar (C), 5 t ha⁻¹ of rice husk biochar application (B5), 10 t ha⁻¹ of rice husk biochar + 5 t ha⁻¹ of chicken manure (B5:CHM), 5 t ha⁻¹ of rice husk biochar + 5 t ha⁻¹ of chicken manure (B10:CHM), 10 t ha⁻¹ of chicken manure (B10:CHM), 10 t ha⁻¹ of cow manure (B10:COM).

3.4. GWP

The GWP_{total} was calculated from the sum of GWP_{CH4} and GWP_{N2O} and is presented in Figure 3. Concerning GWP_{total}, B10:CHM released the highest amount of GWP_{total} in both MF and LF soils. The lowest GWP_{total} values were recorded for B5:COM and B10 in MF and LF soils, respectively. The magnitude of GWP_{total} in the MF soil, ranked from highest to lowest, was B10:CHM > B10:COM > B10 > C > B5 > B5:CHM > B5:COM, whereas that of GWP_{total} in the LF soil, ranked from highest to lowest, was B10:CHM > B10:COM > B5:COM > C > B5 > B5:CHM > B10.



Figure 3. GWP_{total} under various treatments. Data are expressed as mean \pm standard error. The different letters above the bar indicate statistically significant differences at the 5% level according to the LSD test. MF: medium-fertility soil, LF: low-fertility soil. No biochar (C), 5 t ha⁻¹ of rice husk biochar application (B5), 10 t ha⁻¹ of rice husk biochar application (B10), 5 t ha⁻¹ of rice husk biochar + 5 t ha⁻¹ of chicken manure (B5:CHM), 5 t ha⁻¹ of rice husk biochar + 5 t ha⁻¹ of cow manure (B5:COM), 10 t ha⁻¹ of rice husk biochar + 5 t ha⁻¹ of chicken manure (B10:CHM), 10 t ha⁻¹ of rice husk biochar + 5 t ha⁻¹ of cow manure (B10:COM).

3.5. Standardized Regression Coefficients

Multiple regression analysis was performed using standardized data to obtain standardized regression coefficients. Standardized regression coefficients were used to determine which independent variables were more influential than the dependent variables. In Table 4, we compare and present the values of the standardized regression coefficients for the first growing season (2022) [21] and second growing season (2023) to understand the specific effects of biochar, organic manure, and soil type on grain yield, GHG emissions, and GWP_{total} for each year.

Response Variables	Explanatory Variables							
	Grain Yield		GWP _{CH4}		GWP _{N2O}		GWP _{total}	
Year	2022	2023	2022	2023	2022	2023	2022	2023
Biochar	0.24 *	0.42 ***	0.30 ***	0.41 *	0.62 ***	-0.65 ***	0.17	0.36 *
CHM	0.51 ***	0.33 ***	-0.07 *	0.38 *	-0.66 ***	-0.38 ***	-0.09	0.31
COM	0.04	0.33 ***	0.86 ***	0.46 *	-0.002	-0.01	0.16	0.45 *
Soil fertility	0.41 ***	0.73 ***	-0.07 *	0.05	0.39 ***	-0.06	0.04	0.05

Table 4. Standardized regression coefficients derived from the analysis of treatment factors.

CHM: chicken manure; COM: cow manure. GWP_{CH4}, global warming potential of CH₄; GWP_{N2O}, global warming potential of N₂O; GWP_{total}, sum of the global warming potentials of GWP_{CH4} and GWP_{N2O}. *: significant at p < 0.05, ***: significant at p < 0.001.

During the growing season (2023), the soil type had the greatest effect on grain yield, followed by biochar application. Biochar application has shown a tendency to improve grain yield by 2023. In 2022, chicken manure had a greater effect on grain yield; however, this effect decreased by 2023. Regarding GWP_{CH4}, cow manure had the most influential effects in 2023. Biochar application significantly suppressed GWP_{N2O} in 2023. Notably, biochar application did not affect the GWP_{N2O} in 2022; however, it showed the greatest effect in suppressing N₂O emissions in 2023. According to our results, cow manure produced the highest GWP_{total} in 2023.

4. Discussion

4.1. Soil Chemical Properties Changes over 1 Year under Different Treatments

After 1 year of different biochar fertilization treatments, the B10:CHM, B5:CHM, and B10 treatments showed residual effects on soil chemical properties, especially on soil EC, available P, and exchangeable K in both MF and LF soils. Over time, B10:CHM showed the highest soil EC, NH_4^+ , NO_3^- , and exchangeable K among the treatments regardless of soil fertility. Organic manures provide a range of essential nutrients, including macronutrients and micronutrients [22]. In our previous study [21], chicken manure can release more nutrients due to its low C/N compared to cow manure. Moreover, biochar can become more porous with time, and the surface area increases. Chemically, it may increase surface functional groups (e.g., hydroxyl, carboxyl, and phenolic groups) and nutrient availability (e.g., changes in nitrogen content or the presence of mineral ions) [23]. Consequently, the higher application rate of biochar may have a higher surface area and help to retain the nutrients released from chicken manure.

Treatments with biochar $(10 \text{ th} \text{a}^{-1})$ alone or biochar $(10 \text{ th} \text{a}^{-1})$ combined with organic manure significantly increased the soil EC in both MF and LF soils (Figure 1b). Generally, biochar is alkaline and contains ash, which may lead to increased soil base cations, thereby increasing the soil EC [24]. According to our findings, soil EC increased with higher biochar application rates, which is consistent with the results of a previous study [25], and soil EC values significantly increased with higher application rates of corn stover and switchgrass biochars. They reported that the alkalinity, the CaCO₃ content of biochar, and the release of weakly bound nutrients, such as cations and anions, into the soil solution contributed to the increased soil pH, EC, and CEC. In our study, the increased soil EC was a good result because our experimental soils had very low EC, especially the LF soil, which tended to easily lose nutrients.

The highest available P content in both soils was recorded for all combined applications of biochar and organic manure (Figure 1e). The cow manure used in this experiment contained more available P than the chicken manure; therefore, the highest soil availability of P was observed in the B10:COM treatment. Organic amendments significantly influence soil P transformation through complex physiological, chemical, and biological mechanisms [26]. The soil exchangeable K content was highest in the B10:CHM treatment; however, it was not significantly higher than that in the B10:COM, B5:CHM, and B10 treatments (Figure 1f). It is possible that the high exchangeable K content in rice husk biochar led to an increase in the soil exchangeable K content. According to Silber et al. [27], the majority of the potassium in biochar is soluble and released into the soil in a plant-available form. Although we applied both chicken and cow manure at an application rate of 5 t ha⁻¹, the nutrient content variation in the manure and biochar led to differences in K availability in each treatment. For instance, B5, B10, B5:CHM, B5:COM, B10:CHM, and B10:COM-treated soils received K_2O in the following amounts: 0.35, 0.5, 0.71, 0.45, 0.86, and 0.6 g K_2O pot⁻¹, respectively, from biochar, manures, and supplemental fertilizers. Therefore, B10, B5:CHM, B10:CHM, and B10:COM significantly increased the exchangeable K in both soils.

Before cultivation, the MF and LF soils had a pH of 6.17 and 7.86, respectively. Soil pH tended to increase in the B5, B10, B5:CHM, B5:COM, B10:CHM, and B10:COM treatments compared to C in MF soil (Figure 1a). Thus, biochar has the potential to increase the pH of MF soil. According to Gul et al. [28], negatively charged functional groups on the surface of biochar, such as hydroxyl, phenolic, and carboxylic groups, increase soil pH by binding excess H⁺ ions from the soil solution to the biochar surface. With respect to LF soil, no significant difference in pH was noted among the treatments; however, for some treatments, such as B5, B10, B5:COM, and B10:COM, the soil pH was reduced relative to that of C. Abeishamkesh et al. [29] revealed that the high calcium carbonate content in calcareous soils induces a high buffering effect on soil pH, resulting in no pH change when using rice husk biochar on alkaline soils. Therefore, biochar can either increase or decrease soil pH, depending on the original soil pH.

Non-significant differences were observed in soil NH₄⁺ among the various biochar treatments, indicating that biochar application alone could retain soil nitrogen even after the second rice-growing season. Incorporating biochar produced from agricultural residues provides an adequate amount of silicon (Si) and increases nutrient availability by directly importing nutrients, such as N, P, and K. Schmidt et al. [30] demonstrated that biochar is a slow-release N source and capable of providing plants with N over a long period, owing to its high N-retention capacity. Different biochar applications did not improve soil exchangeable Mg. Our experimental soils had highly exchangeable Ca in both MF and LF soils, with the values being 10.3 and 9.84 cmol_(c) kg⁻¹, respectively. Therefore, no significant differences among the treatments were observed in soil-exchangeable Ca relative to C (Figure 1h). Our findings are supported by a previous study [10] in which biochar altered the soil pH, CEC, and exchangeable Ca²⁺ and Mg²⁺ levels; however, the effectiveness and magnitude of the changes depended on the soil's original properties.

4.2. Yield Response to Various Rice Husk Biochar Fertilizations

Biochar has been reported to enhance soil properties, photosynthetic rates, and root morphological attributes, resulting in an increase in dry plant biomass [31]. Plants responded better when biochar was applied in combination with organic fertilizers because the organic fertilizer acted as a buffer to mitigate the physiological and biological processes of the soil medium, thus providing nutrient requirements for plant growth [32]. According to our results, biochar application combined with organic manure produced a higher dry biomass, with B5:CHM producing the highest aboveground and root dry biomass in the MF soil, whereas B10:CHM produced the highest in LF biomass.

As mentioned previously, B10, B5:CHM, B10:CHM, and B10:COM significantly improved the soil nutrients. This finding indicates that rice responded through improved plant growth depending on the available soil nutrients, showing the highest grain yield with B10:CHM, which had non-significant results with the application of B5:CHM and B10 in both soils (Table 2). The combined application of biochar and organic manure or chemical fertilizer results in better soil properties than biochar alone [33]. Biochar can improve crop productivity by increasing its capacity for water retention and immobilizing potentially harmful metals through expanded micropores and oxygen-containing functional groups [34]. Manure can also produce labile or transitory organic-binding molecules that combine with biochar to form stable aggregates, thereby increasing soil quality and soil nutrients [35]. A biochar-organic compost amendment, which is rich in organic nutrients, helps prevent the leaching of nutrients from the compost into deeper soil layers or groundwater, thereby increasing nutrient availability in the root zone. In addition, biochar has a high surface area and porous structure that can adsorb and retain nutrients from organic compost [36]. Thus, the combination of biochar and organic manure has a major effect on the morphology and physiology of roots by improving soil nutrients, leading to increased crop yield [37].

In the first growing season (2022), biochar application alone achieved yield improvement compared to that in C (no biochar) [21]. After 1 year of biochar amendment, biochar application alone continued to increase grain yield compared with that in C in both MF and LF soils. This sustained positive influence of biochar on soil quality and rice yield over the two crop-growing seasons is supported by other studies [38,39]. The increase in grain yield was mainly due to the higher K content, the presence of ash, and the high exchangeable capacity of the biochar. Furthermore, the characteristics of biochar, such as its high specific surface area, porosity, and the presence of a wide range of functional groups, make it an ideal matrix for fertilizers that release nutrients slowly (slow-release and controlled-release fertilizers) [40]. Biochar persists in the soil and improves soil water retention over time by altering the pore architecture of the soil and encouraging plant growth, making it useful in agricultural applications [20,41]. Consequently, biochar can show residual effects over time and does not need to be reapplied annually, making it cost-effective.

4.3. GHG Emissions during Pot Experiment

The B5 treatment suppressed cumulative CH₄ emissions in MF soil, whereas the B10 treatment suppressed cumulative CH_4 emissions in LF soil (Table 3). Notably, the cumulative emissions of CH₄ under the B5:CHM treatment were not statistically significant compared to those under the B5 and B10 treatments in MF and LF soils. Thus, B5:CHM is acceptable for suppressing CH_4 emissions associated with low-carbon substrates for methanogenesis [21]. In addition, a higher amount of biochar (10 t ha^{-1}) combined with organic manure, particularly in the B10:CHM and B10:COM treatments, contributed to the highest cumulative CH₄ emissions from both soils. Standardized regression coefficients revealed that biochar had a greater effect on cumulative CH₄ emissions, whereas chicken and cow manure had notable effects. Although we did not reapply biochar or organic manure during the growing season, the B10:COM-treated soils received the highest carbon input, followed by the B10:CHM-treated soils, in the first year of the growing season. Therefore, combined application of biochar (10 t ha^{-1}) and organic manure, which has a relative abundance of carbon substrates, could increase organic matter decomposition under flooded conditions. As a result, B10:CHM and B10:COM produced CH₄ fluxes and higher CH₄ emissions from both soils. Mer et al. [42] suggested that methane production, oxidation, and emissions from flooded paddy fields were significantly affected by the application of organic inputs. The labile components of biochar can be decomposed to create a favorable environment for methanogenic activity by providing sources of methanogenic substrates [43,44]. Some studies have shown that mid-season drainage, multiple drainages, and water-saving irrigation methods, such as alternate wetting and drying (AWD), are highly effective methods for reducing methane emissions [45,46]. In our study, we maintained flooded conditions without mid-season drainage or other drainage due to pot and soil conditions, as well as weather conditions. Prolonged flooding was one of the factors that promoted soil reduction and low redox potential, which enhanced the favorable conditions for the production of CH_4 . This reduction may improve the environment in which methanogens that create methane can proliferate and produce methane [47]. Thus, we assumed that biochar application might not significantly affect methane production compared with the control (no biochar) under flooded conditions. CH₄ fluxes under different treatments fluctuated sharply during the late growth stage (Figure 2a,b). The peak in the later season has been linked to the supply of plant-borne C through the decomposition of tissues and root exudates [48]. Additionally, 52% of the CH₄ emissions from paddy soils were from the release of labile carbon from root exudation, which is the source of methanogenesis [49]. Therefore, we may observe the effectiveness of biochar on CH₄ emissions when applied along with mitigation strategies such as drying paddy soils in the later phase of rice growth or adopting suitable water management practices.

Unlike CH₄ emissions, cumulative N₂O emissions were significantly reduced by the B10:CHM and B10 treatments (Table 3). This was probably because biochar enhanced the last step of the denitrification of N₂O to N₂ under reduced soil conditions. This result corresponds with that of [50], who reported that the aging of biochar suppresses soil N₂O emissions by promoting complete denitrification, thus suppressing N₂O emissions. Biochar supports the reduction of N₂O to N₂ by acting as an electron shuttle, coupled with its liming effects, to facilitate electron transfer to soil-denitrifying microorganisms [51].

We observed the highest N_2O peak immediately at 1 DAT in the MF soil, whereas N_2O fluctuations under various treatments began in the LF soil at 1 DAT. According to the standardized regression coefficients, biochar, organic manure, and soil type negatively affected the cumulative N_2O emissions (Table 4). Therefore, the application of supplemental fertilizers is likely associated with cumulative N_2O emissions. Moreover, the soil pH plays an important role in biological processes through N transformation. The most favorable soil pH for nitrifier populations and/or nitrification activity was neutral or higher [52]. In our experiment, the MF soil had a pH of 6.17 and the LF soil had a pH of 7.86, which may have led to favorable conditions for a wide variation in N_2O fluxes in both soils.

Global warming potential (GWP) is an indicator of global warming due to each GHG, enabling comparisons between the amount of energy absorbed by 1 ton of gas emissions over an average 100-year time horizon based on 1 ton of CO_2 emissions [45]. Our results revealed that GWP_{CH4} was more dominant than GWP_{N2O} under different treatments (Figure 3), indicating methane emission from rice cultivation contributed to the GWP in this study. Naser et al. [53] showed that the net GWP of rice paddies was dominated by CH_4 . The B5:COM treatment showed a decreasing trend for GWP_{total} in the MF soil; however, the B10 treatment showed a decreasing trend for GWP_{total} in the LF soil. Our results demonstrate that GWP_{total} under the B5:CHM treatment was not significantly different from that under the B5:COM treatment in the MF soil and the B10 treatment in the LF soil. The overall results indicate that B5:CHM treatment can be recommended for agricultural production to maintain crop yield while not increasing the GWP_{total}.

5. Conclusions

It is worth noting that biochar fertilization showed residual effects on grain yield and soil nutrients. In particular, B10:CHM improved soil chemical properties and obtained the highest grain yield; however, it was not significantly different from B5:CHM in both MF and LF soils. The higher grain yield was mainly due to the increased soil nutrients, particularly NH4⁺-N, available P, and exchangeable K content. Prolonged flooding with an abundance of carbon was one of the factors promoting favorable conditions for the production of CH_4 , and the combined application of biochar 10 t ha⁻¹ and organic manure significantly increased CH_4 emissions. N₂O emissions can be effectively suppressed by biochar application. Although B10:CHM produced the highest grain yield, it did not effectively suppress the cumulative CH₄ emissions or GWP_{total}. B5:CHM tended to reduce GWP_{total} compared with C in both soils. Overall, the balanced application rates of biochar (5 t ha^{-1}) and chicken manure (5 t ha^{-1}) were found to be the most effective for increasing rice grain yield without increasing GHG emissions for rice cultivation. This study provides valuable insights into the optimal application rates and methods for biochar and organic manure application to maximize rice yield while contributing to environmental conservation. As part of more intensive research, a combined application of biochar and organic manure along with water management practices should be conducted to examine their potential effects on GHG emissions.

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