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Research article

Co-application of poultry-litter biochar with Azolla has synergistic effects on CH_4 and N_2O emissions from rice paddy soils

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ABSTRACT

Poultry-litter biochar and Azolla as green manure amendments are reported to enhance paddy soil fertility and rice yields. However, whether their co-application in lowland rice paddies has synergistic effects and whether those benefits are accompanied by greenhouse gas (GHG) emissions remains unknown. The objective of this study was to determine the effects of poultry-litter biochar (hereafter: biochar) and its co-application with Azolla as green manure (hereafter: Azolla), on the simultaneous methane (CH_4) and nitrous oxide (N_2O) emissions from a lowland paddy soil planted with rice during a single rice growing season in Tsuruoka, Yamagata, Japan. Biochar and Azolla amendments were applied once before rice was transplanted at a density of 20 t ha^{-1} and 133.9 kg N ha^{-1}, respectively. Compared with NPK, NPK + biochar, and Azolla only treatments, Azolla and biochar co-application (i.e., Azolla + biochar) significantly increased CH_4 emissions by 33%–197.6% in the early stages of rice growth (before 63 days after transplanting, DAT), but did not significantly influence CH_4 emissions at both late rice growth stages (after 63 DAT,) and whole rice growth period (112 DAT). Conversely, Azolla + biochar significantly reduced N_2O emissions by 83.0%–97.1% before 63 DAT, and by 76.4%–95.9% during the whole rice growth period at 112 DAT, with a significantly high interaction between biochar and fertilizer amendments. There were no significant N_2O emission differences among all treatments after 63 DAT. Additionally, Azolla + biochar significantly increased rice grain yield by 27.3%–75.0%, and consequently, decreased both yield-equivalent CH_4 emissions by 24.7%–25.0% and N_2O emissions by 81.8%–97.7%. Our findings suggest that the co-application of poultry-litter biochar and Azolla as green manure offers a novel approach to increase rice yield while reducing the emissions of non-carbon dioxide greenhouse gases.

1. Introduction

Flooded rice fields are a significant anthropogenic source of greenhouse gases (GHG), with an estimated global methane (CH_4) emission rate of 25–60 Tg yr^{-1} (Reay et al., 2010) and an annual global nitrous oxide (N_2O) contribution of 13%–24% (Saikawa et al., 2014). The global warming potential (GWP) by mass of CH_4 is 34 times while that of N_2O is 298 times that of carbon dioxide (CO_2) over 100 years (IPCC, 2013). According to Scialabba and Müller-Lindenlauf (2010) and Snyder et al. (2009) the exogenous application of inorganic and/or organic fertilizers to rice paddies exacerbates CH_4 and N_2O emissions. Accordingly, with the projected increase in rice demand by over 20% in the next 10–20 years, an increase in CH_4 emissions and a comparable increase in N_2O emissions resulting from increased fertilizer use is almost inevitable (Van Nguyen and Ferrero, 2006; Zou et al., 2009). Thus, it is important to find cultivation practices suitable to mitigate GHG emissions from constantly flooded rice paddies.

Despite the high overall contribution of chemical fertilizers to the carbon footprint of rice agriculture, their use is unavoidable to maintain rice growth and yield (Xu et al., 2013). However, given the current energy crisis, higher prices of inorganic fertilizers, and concerns about the detriments of climate changes, research interest in green manure use,

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especially in lowland rice, has been renewed (Brenzinger et al., 2018; Scialabba and Müller-Lindenlauf, 2010). Azolla, an aquatic fern often found in flooded rice fields, has long been used successfully as green manure to improve the N balance in lowland paddies in Vietnam and southern China, due to its symbiotic relationship with nitrogen (N)-fixing cyanobacteria *Anabaena azollae* (Cheng et al., 2015; Lu and Li, 2006). Nonetheless, the effects of green manure application on GHG emissions from lowland paddy fields remain contradictory. Bharati et al. (2000) demonstrated that incorporation of Azolla plus dual cropping significantly decreased CH_4 emissions by increasing the soil redox potential due to higher levels of dissolved oxygen (DO) concentration in the standing water effected by the floating Azolla cover. In contrast, Linquist et al. (2012) reported a significant increase in CH_4 emissions by 192% with the addition of green manure *Sesbania* compared to inorganic N fertilizers, mainly attributed to the amount of substrate available for methanogens. Meanwhile, Chen et al. (1997) reported substantial CH_4 and N_2O emissions from a rice field grown with Azolla as a cover, likely due to the exudation of Azolla root and decomposition of dead Azolla. Conversely, Kimani et al. (2018) reported that Azolla as a cover significantly decreased CH_4 emission by 34%, likely due to increased levels of DO concentrations and redox potential (Xu et al., 2017), and no significant influences on N_2O from a paddy soil planted with rice, attributed to no interferences by the Azolla cover (Cheng et al., 2006). The discrepancies in these results suggest, therefore, that the interactions between soil native and/or newly added N availability, management practices and other site-specific factors influence on CH_4 and N_2O emissions from lowland rice ecosystems (Linquist et al., 2012). Additionally, due to the accelerated decomposition rates of organic materials, a number of these benefits are short-lived and multiple applications per cropping seasons are required (Partey et al., 2014).

Biochar is the carbon-rich material obtained through the pyrolysis of biomass. Its application to agricultural soils leads to an increase in carbon sequestration and a corresponding decrease in GHG emissions subject to its high structural composition stability (chemically and biologically), characteristics that are of particular importance to the mitigation of climate change (Lehmann et al., 2006). Globally, biochar is readily produced from various sources of biomass under different pyrolysis conditions, resulting in products of varying properties, and consequently different soil amendment values. Accordingly, the use of biochar particularly in rice paddy ecosystems to decrease GHG emissions, though a promising option, remains contradictory (Kammann et al., 2017). For example, Singh et al. (2010) reported cumulatively higher N_2O emissions from poultry manure biochar amended soils by 32% compared to the control as result of higher labile native N contents of the biochar. In contrast, van Zwieten et al. (2010) reported reduced N_2O emissions to 4.0% of the applied and available N by poultry litter biochar compared to control soil, mainly due to an increase in NO_3^- adsorption. Similarly, contradictory observations on CH_4 emissions have been reported (Jeffery et al., 2016). Liu et al. (2011) found that bamboo chips and rice straw derived biochars amendments decreased methanogenic activities in the paddy soil, thereby significantly decreasing CH_4 emissions by 51.1% and 91.2%, respectively. Conversely, Zhang et al. (2012) revealed that amendment with wheat straw biochar at 40 t ha^{-1} significantly increased soil CH_4–C emissions by 34–41% probably due to increased substrate supply and the development of a conducive environment for methanogens, particularly in the early stages of rice growth (Jeffery et al., 2016). Meanwhile, some studies have reported no significant influences on CH_4 emissions, and a varied degree of N_2O emissions as depending on the feedstock source (Clough et al., 2013; Xie et al., 2013). These contrasting results may be due to differing soil conditions, biochar feedstock, pyrolysis methods, biochar application rate and intervals, as well as experimental duration and management practices (Saarnio, 2015; Song et al., 2016).

Given the shortcomings of either inorganic and/or organic fertilizers, and biochar use in lowland rice paddies as highlighted above, co-applications of inorganic and/or organic fertilizers and biochar amendments, though with differing effects, has been proposed as a suitable practice to achieve sustainable soil health, yield production, and GHG emissions mitigation (Rahman et al., 2020). For example, Abagandura et al. (2019) revealed a reduction in cumulative N_2O fluxes from a sandy loam soil amended with plant-based biochar plus dairy manure, attributed to improved aeration and a subsequent reduction in denitrification, and no significant effects on the cumulative. CH_4 fluxes, partly due similar soil water contents among treatments. Similarly, Wu et al. (2019) also reported a significant decrease in in cumulative N_2O emissions from a paddy soil co-treated with vermicompost and wheat straw derived biochar, attributed to suppression of carbon and nitrogen mobilization. In contrast, Lin et al. (2017) found a significant increase in N_2O emissions by 256% with wheat-straw biochar co-applied with N fertilizer, mainly due to increased soil pH and its influence on the ammonia-oxidizing bacteria abundance. Additionally, Zhang et al. (2010) found a significant increase in total CH_4 emissions by 41% with wheat straw biochar application in N fertilized soils, partly due to increased substrate for methanogens in the early stages of rice development. As highlighted here, there are multiple studies on the effects of plant biomass derived biochar co-applied with inorganic and/or organic fertilizers on agricultural GHG emissions. However, there are still few reports on the effects of animal manure derived biochar, and particularly poultry-litter biochar as a viable option to mitigate GHG emissions. With perhaps, the exception of Subedi et al. (2016) who observed significant increases in N_2O emissions by 0.65–3.41% from a soil amended with poultry-litter derived biochar in a laboratory study, attributed to a greater availability of volatile compounds which may have acted as potential substrate for the denitrifiers as well as increased availability of mineral N from the biochar itself (Cayuela et al., 2014). Furthermore, there are no studies on the combined effects of poultry-litter biochar and Azolla as green manure (herein Azolla) on both CH_4 and N_2O emissions in paddy soils.

Based on the previous findings (as highlighted above), we hypothesized that while biochar, inorganic fertilizers and organic amendments show contrasting effects when applied independently, their co-applications may have synergistic effects, resulting in simultaneous positive effects on CH_4 and N_2O emissions. Previously, in the same batch of experiment, we reported a significant increase in seasonal CH_4 emission by 31.5% and a 3.4 fold N_2O emission decrease in Azolla amended paddy soil compared to NPK only treatment, mainly attributed to the increased substrates availability favoring methanogens as well as accelerating denitrification (Kimani et al., 2020). Therefore, in the current study, we investigated the effects of poultry-litter biochar amendment and its co-application with NPK and Azolla (i.e., NPK + biochar and Azolla + biochar) on the simultaneous CH_4 and N_2O emissions. The main objective of this study was to determine the effects of poultry-litter biochar amendment and its co-application with Azolla (incorporated as green manure and its successive growth as a cover crop), on the simultaneous CH_4 and N_2O emissions from a flooded rice paddy soil planted with rice in a single rice growth season.

2. Materials and methods

2.1. Experiment site, design, and management

The pot experiment was carried out on the ground at the Experimental Farm of Yamagata University (38°44′N, 139°50′E, 16 m a.s.l.) in 2017. Average daily air temperature in the rice growth season (7th June to 20th September 2017) was 0.1 °C above the historic average for 1981–2010, coupled with a daily average air temperature of 22.7 °C and 6.4 h sunshine time (Figure 1). The historical weather data was retrieved from the Japan Meteorological Agency database (http://www.data.jma.go.jp/obd/stats/etrn/index.php). Four treatments, each replicated four times, were employed: chemical fertilizer (NPK) and Azolla (as green manure) without and with 20 t ha^{-1} biochar (Table 1). The experimental soil was collected from a plough layer (0–30 cm) from a rice field at the

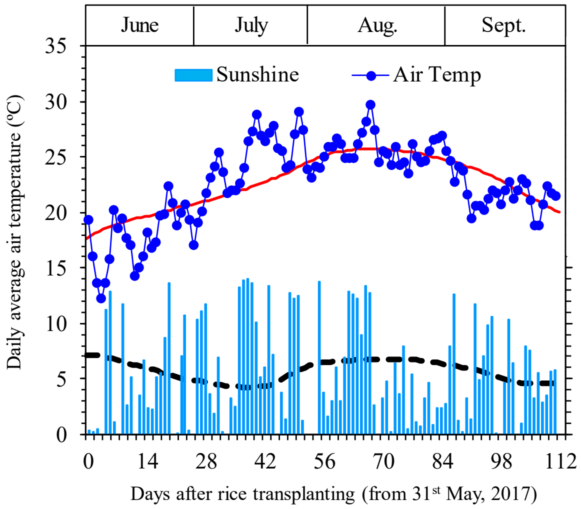

Figure 1. Daily sunshine time (■) and average air temperature (●) during the experimental period from 31st May to 20th September 2017 in Tsuruoka, Japan. The red bold and black dashed lines crossing air temperature and sunshine time are the daily average values for 1981–2010, respectively. Data were derived from the Japan Meteorological Agency.

university farm and classified as an Alluvial according to the United States Department of Agriculture (USDA) soil taxonomy. Before the start of the experiment, the soil was air-dried, mixed, and sifted using a 5-mm sieve. Basic soil properties (Table 2) were determined using the air-dried soil sample procedures. Soil pH (1:5 soil-in–water ratio mixture) and electrical conductivity (EC) were determined with a handheld pH meter (D-51, Horiba, Kyoto, Japan) and an EC meter (DS-51 conductivity meter, Horiba), respectively. Soil organic carbon (SOC) and total nitrogen (TN) were analyzed by dry combustion using a Sumigraph NC 220F Analyzer (Sumika Chemical Analysis Service, Ltd., Osaka, Japan). The NH_4^+-N and NO_3^--N concentrations were determined by the nitroprusside and hydrazine reduction methods, respectively (JSSSPN, 1986), and measured using Hitachi U-2900 Spectrophotometer (Hitachi High-Tech Science Corporation, Tokyo, Japan).

The poultry-litter biochar (a composition of poultry excreta and bedding materials sourced from commercial poultry farms in Kanazawa) used in this study was produced using commercial pyrolysis equipment under oxygen-limited conditions at 450 °C–500 °C (Meiwa Co., Ltd., Kanazawa, Ishikawa, Japan). The Azolla (*A. filiculoides* Lam.) species IRRI code FI 1001 (Cheng et al., 2015; Kimani et al., 2018, 2020) was used in this study. The primary properties of biochar and Azolla, determined as describe above, are as shown in Table 2. We also used Haenuki, a popular rice cultivar widely grown in Yamagata Prefecture, Japan.

One day before transplanting, 7 kg of soil (4.9 kg oven-dried soil equivalent, 30% water content per total weight of soil per pot) were mixed with: 66 g pot^{-1} biochar (equivalent to 20 t ha^{-1}, an amount within a range of rates shown to have significant effects on plant growth (Biederman and Harpole, 2013)) for the with biochar treatments only (NPK + biochar and Azolla + biochar), fresh green manure at 12.2 g Azolla dry weight pot^{-1} (equivalent to 0.40 g N pot^{-1}) in the Azolla and Azolla + biochar treatments only, and 0.87 g of KH_2PO_4 and 0.87 g $CO(NH_2)_2$ for the NPK without and with biochar treatments only. Germinated rice seeds were grown in a seedling tray (three seeds per cell), then transplanted (three seedlings per pot) five weeks after sowing into 16 plastic pots (19.5-cm diameter, 27-cm height, and 0.2-cm thickness). Next, 49 days after transplanting (DAT), all treatments were top-dressed with 0.43 g of KH_2PO_4 and 0.43 g $CO(NH_2)_2$ (Table 1). The total amount of N application were the same at 200.9 kg ha^{-1} between NPK and Azolla treatments. The flooding water depth was maintained at

Table 1. Summary of the experimental treatments with chemical fertilizers, Azolla and poultry-litter biochar application at the Experimental farm, Tsuruoka, Japan.

Treatment code	Amendments				Total N application
(in details)	Basal fertilizer application	Azolla incorporation	Biochar incorporation	Additional fertilizer application	(g pot^{-1})
NPK (Chemical fertilizer)	0.40 g N, 0.20 g P, and 0.25 g K per pot were applied by KH_2PO_4 and $CO(NH_2)_2$ as basal fertilizer before transplanting.	-	-	Top dressing was applied at 49 DAT by KH_2PO_4 and $CO(NH_2)_2$ at 0.20 g N, 0.10 g P, and 0.13 g K per pot.	0.60
NPK + biochar	Basal chemical fertilizer applied as above.	-	66 g per pot dry wt. biochar (20 tons ha^{-1} eqv.) mixed with soil at before transplanting.	Top dressing fertilizer applied as above.	0.60
Azolla (As green manure)	-	243 g fresh Azolla (95% water content, 12.2 g dry weight) incorporated as green manure at transplanting to provide 0.40 g N pot^{-1} eqv. [Azolla cover grew following Azolla incorporation].	-	Top dressing fertilizer applied as above.	0.60
Azolla + biochar	-	Fresh Azolla applied as above.	66 g per pot dry wt. biochar (20 tons ha^{-1} eqv.) mixed with soil at before transplanting.	Top dressing fertilizer applied as above.	0.60

Table 2. Characteristics of the experimental paddy soil, poultry-litter biochar, and Azolla (*A. filiculoides* Lam.).

	Soil	Biochar	Azolla
Organic C (g kg^{-1} DW)	14.50	284.50	339.90
Total N (g kg^{-1} DW)	1.40	26.70	33.80
C:N	10.36	10.66	10.06
pH (H_2O)	5.24	10.0	-
EC (μS cm^{-1})	170.0	2790.0	-
Available P (mg P_2O_5 kg^{-1})	70.0	2470.0	-
NH_4^+ (mg N kg^{-1} DW)	24.8	18.4	-
NO_3^- (mg N kg^{-1} DW)	101.6	550.8	-

about 5 cm above the surface of soil throughout the experiment period by continuously topping up with tap water. The surface cover of growing Azolla in the Azolla treatments without and with biochar was maintained throughout the rice growth period.

2.2. Quantification of CH_4 and N_2O fluxes, and night respiration (CO_2 flux)

Emissions of CH_4 and N_2O, as well as nighttime respiration (CO_2 flux) rates from rice pots placed in outdoor water tanks (two pots per tank) (65-cm length, 46-cm width, and 32-cm depth) filled with water, were measured using a static closed-top chamber (height, 100 cm; inside diameter, 20.5 cm; thickness, 0.3 cm) as described previously (Kimani et al., 2018). After closure, a small fan was used to mix the gas in the chamber, and a 30-mL gas sample from the chamber headspace of each experimental pot was collected at 0, 15, and 30 min with a syringe and transferred into a 19-mL pre-evacuated vial. As detailed previously (Kimani et al., 2018), gas sampling was conducted between 20:00–23:00 once a week in the first 84 DAT. After this date, sampling was done every two weeks until 122 DAT, a day before rice harvesting (113 DAT). All gas samples were analyzed at the Institute for Agro-Environmental Sciences, NARO using an automated analysis system for three gases of CO_2, CH_4, and N_2O (Kimani et al., 2018; Sudo, 2006). The GHG fluxes were calculated from the linear increase in gas concentrations inside the chamber per square metre per hour along with atmospheric pressure and temperature (Cheng et al., 2008; Kimani et al., 2018, 2020; Sudo, 2006).

2.3. Quantification of dissolved CO_2, CH_4, and nitrate in soil solution

For understanding the CH_4 and N_2O emissions with the C and N dynamics in the soils, the concentrations of dissolved CO_2 and CH_4 and nitrate (NO_3-N) in soil solutions were sampled using a 10 cm long microporous polymer tube (outside diameter, 2.5 mm; inside diameter, 1.5 mm) fitted to a PVC tube (length, 50 cm; outside diameter, 2.7 mm; inside diameter, 1.0 mm) and inserted vertically into the soil between the rice plant and pot edge at a depth of 10–15 cm one day after rice transplanting, as previously described (Kimani et al., 2018). The 9.5-mL soil solution sample was aspirated into a 19-mL semi-vacuum bottle fitted with a rubber stopper and a screw cap and filled with pure N_2 gas at 0.5 atm (Kimani et al., 2018). The concentrations of CO_2 and CH_4 in the headspace volume were measured in the laboratory using a gas chromatograph (GC-7A, Shimadzu, Kyoto, Japan), fitted with a thermal conductivity detector (TCD), and a flame ionization detector (FID), respectively. The CO_2 and CH_4 concentrations were calculated with Henry's law according to their respective concentrations in the headspace (Cheng et al., 2005, 2006). The NO_3-N concentration in soil solution was analyzed using colorimetric techniques at 450 nm by a spectrophotometer (UV–1200V, Shimadzu, Japan). The soil solution samples were collected on the same day after gas measurements.

2.4. Effects of poultry-litter biochar and Azolla co-application on net GHG emissions

Global warming potential (GWP), soil C sequestration, and the net GHG balance in g CO_2-equivalent (CO_2-eq) per square meter were calculated for all treatments. GWP was derived by combining cumulative CH_4 and N_2O emission fluxes. In these calculations, the GWP values for CH_4 and N_2O were considered to be 34 and 298, respectively, (IPCC, 2013). The GWP and soil C sequestration calculations were as below (Toma et al., 2019):

$$\mathrm{GWP_{CH_4}\ to\ CO_{2eq}} = \mathrm{CH_4\text{–}C\ emission} \times 16/12 \times 34 \tag{1}$$

$$\mathrm{GWP_{N_{2O}}\ to\ CO_{2eq}} = \mathrm{N_2O\text{–}N\ emission} \times 44/28 \times 298 \tag{2}$$

$$\mathrm{Soil\ C\ sequestration}\left(\mathrm{gCO_{2eqv}m^{-2}}\right) = \left(\mathrm{C_{tre}} - \mathrm{C_{bef}}\right) \times \left(\frac{\mathrm{S_{dw}}}{\mathrm{P_{area}}}\right) \times 44 \Big/ 12 \tag{3}$$

where C_{tre} is the soil C content in each treatment after rice cultivation (g kg^{-1} dry soil), C_{bef} is the soil C content before the experiment (14.50 g kg^{-1} dry soil), S_{dw} is the amount of soil in the pot at the start of the experiment (4.9 kg dry weight), P_{area} is the pot area (m^{-2}), multiplied by a ratio of molecular weight of CO_2 to C (44/12) to calculate C sequestration in CO_2 equivalent. The ratios of 16/12 and 44/28 were used to convert CH_4–C to CH_4 and N_2O–N to N_2O, respectively. Changes in the net GHG balance following the co-application of poultry-litter biochar and Azolla were calculated relative to the other treatments.

2.5. Investigation of plants growth, grain yield, and soil analysis

The rice height and tiller number data per treatment were collected once a week beginning on 8 DAT. At that time, top rice leaf greenness (SPAD value) was measured using a SPAD-502 Plus chlorophyll meter (Konica Minolta Inc., Tokyo, Japan). Data from four hills per treatment were averaged during the rice growth period. At maturity (113 DAT), rice was harvested and separated into grains and straw, then air-dried for one month and weighed to determine total yield (Cheng et al., 2009). After harvest, soil in the pots was divided into two equal parts from the center. One part was used for roots sampling (Kimani et al., 2018) and the other part was air-dried for soil characteristics measurements, such as soil pH, EC, C, and N contents (JSSSPN, 1986).

2.6. Statistical analysis

A two-way analysis of variance (ANOVA) to examine the direct and interaction effects of poultry-litter biochar and Azolla on soil properties, rice yield, cumulative CH_4 and N_2O emissions, night respiration (CO_2 flux), and the concentrations of soil solution dissolved CO_2, CH_4 and NO_3-N. Significant differences among means were compared using Tukey's HSD test at $P < 0.05$ (unless stated otherwise). All data were analyzed using SPSS 20 software (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Changes in CH_4 and N_2O fluxes and their cumulative emissions

The pattern and intensity of CH_4 and N_2O fluxes and their cumulative emissions during the rice growth period are shown in Figure 2a and b and Table 3, respectively. The interaction effect of poultry-litter biochar and fertilizer amendments on the total cumulative CH_4 emissions for the whole rice growth period was not significant ($P = 0.199$; Table 3). During the early rice growth stages (i.e., before heading; before 63 DAT), the co-application of biochar and Azolla significantly increased cumulative CH_4 emissions by 197.6, 95.3, and 33.0% compared to the NPK, NPK + biochar, and Azolla treatments, respectively ($P < 0.01$; Table 3). The bulk of the CH_4 was emitted from 35 DAT after the soils changed to reduced condition. Furthermore, Azolla + biochar (10.86–22.60 mg C m^{-2} h^{-1}) had significantly higher CH_4 fluxes compared with NPK (0.54–8.20 mg C m^{-2} h^{-1}), NPK + biochar (1.91–14.54 mg C m^{-2} h^{-1}) and Azolla (7.22–16.39 mg C m^{-2} h^{-1}) between 42–56 DAT, (Figure 2a). Amendment with biochar did not influence cumulative CH_4 emission during the late rice growth stages (i.e., heading to maturity; after 63 DAT) ($P = 0.682$; Table 3).

Throughout the rice growth period, Azolla + biochar significantly decreased total cumulative N_2O emission by 95.9, 76.4, and 86.1% compared to the NPK, NPK + biochar, and Azolla treatments, respectively, with a significantly high interaction between the biochar and fertilizer amendments ($P < 0.01$; Table 3). Additionally, Azolla + biochar treatment significantly reduced N_2O emission before 63 DAT by 97.1, 83.0, and 89.9% compared to the NPK, NPK + biochar and Azolla

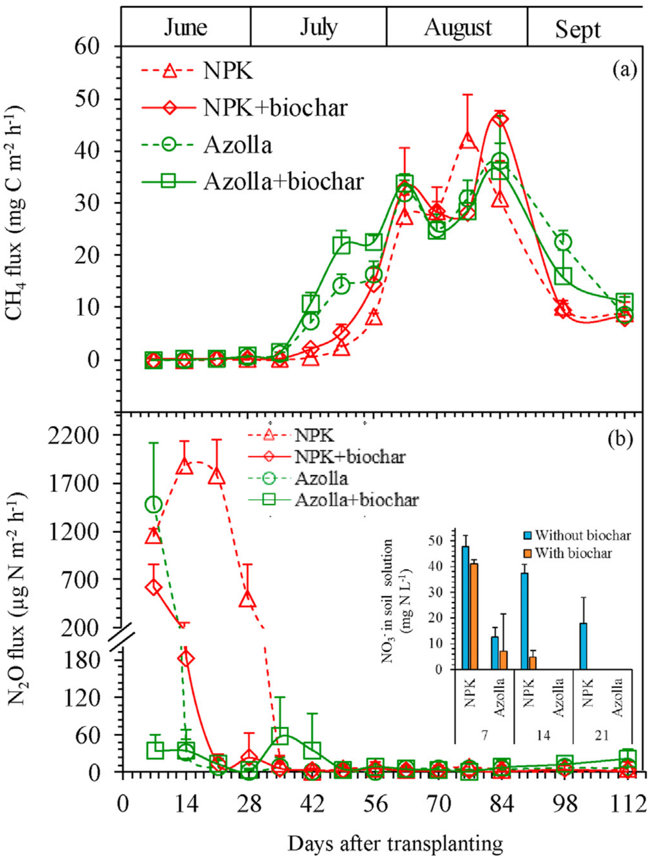

Figure 2. Changes in CH_4 (a) and N_2O (b) fluxes from pots treated with NPK and Azolla (as green manure) with and without biochar throughout the experimental period. Bars indicate the standard deviation (n = 4). The inset in (b) shows the concentration of NO_3^--N dissolved in the soil solution during the first three weeks on the day of gas sampling. The data for the no biochar amendment treatments were obtained from Kimani et al. (2020).

treatments, respectively, with a significantly higher interaction between biochar and fertilizer amendments ($P < 0.01$; Table 3). Furthermore, the co-application of biochar and Azolla significantly decreased N_2O fluxes within the first 28 DAT (Azolla + biochar: 33.6–2.87 µg N m$^{-2\ h-1}$; NPK: 1151.8–496.3 µg N m$^{-2\ h-1}$; NPK + biochar: 626.8–24.4 µg N m$^{-2\ h-1}$; Azolla: 1473.4–1.2 µg N m$^{-2\ h-1}$; Figure 2b). The addition of biochar did not influence the cumulative N_2O emission during the late rice growth stages (after 63 DAT) ($P = 0.505$; Table 3).

The amendment of poultry-litter biochar significantly influenced the total CH_4 and N_2O emissions per grain yield equivalent (at $P < 0.05$; Table 3). Azolla + biochar significantly decreased CH_4 emissions per grain yield equivalent compared with NPK (24.9%) and Azolla (24.7%) treatments, but not NPK + biochar treatment, and total N_2O emissions per grain yield equivalent by 97.7% (NPK), 81.9% (NPK + biochar), and 89.4% (Azolla), with a significantly high fertilizer × biochar interaction ($P < 0.01$; Table 3).

3.2. Changes in CO_2 night respiration

Nighttime CO_2 respiration fluxes, composed mainly of CO_2 emitted from rice plants in the NPK and NPK + biochar treatments, and both rice plants and floating Azolla masses in the Azolla and Azolla + biochar treatments, are shown in Figure 3a. Transient significant variations were observed between 8 and 35 DAT between the Azolla + biochar, NPK, and NPK + biochar treatments. However, between 42 and 112 DAT the Azolla + biochar treatment significantly increased nighttime CO_2 emissions compared to the NPK and NPK + biochar treatments, with no significant differences compared to Azolla throughout the rice growth period (Figure 3a). The highest CO_2 respiration peak was observed at 42 DAT with significantly high emissions in the Azolla + biochar treatment (559.7 mg CO_2–C m^{-2} h^{-1}) compared to NPK (381.3 mg CO_2–C m^{-2} h^{-1}) and NPK + biochar (464.5 mg CO_2–C m^{-2} h^{-1}), but not Azolla (484.0 mg CO_2–C m^{-2} h^{-1}). Consecutive smaller peaks at 84 DAT observed in all treatments were attributed to the high daytime and night temperature at sampling (Figure 3a, b). The average CO_2 respiration rates throughout the rice growth period were 220.8, 247.5, 316.7, and 369.9 mg CO_2–C m^{-2} h^{-1} for the NPK, NPK + biochar, Azolla, and Azolla + biochar treatments, respectively.

3.3. Changes in dissolved CO_2, CH_4, and NO_3^--N concentrations in soil solution

The concentration of CO_2 dissolved in the soil solution increased significantly in the biochar and/or Azolla amended treatments (Figure 4a). In the presence of Azolla, amendment with biochar (i.e., Azolla + biochar) significantly increased dissolved soil CO_2 concentration compared to NPK, NPK + biochar, and Azolla treatments between 7 and 42 DAT ($P < 0.01$; Figure 4a). Between 7and 42 DAT, the dissolved

Table 3. Cumulative CH_4 and N_2O emissions during the early (before 63 DAT) and late (after 63 DAT) rice growth stages, and total CH_4 and N_2O emissions per grain yield equivalent between four treatments.

Treatment		Cumulative CH_4 emission			Cumulative N_2O emission			CH_4 emission per grain yield	N_2O emission per grain yield
		Early	Late	Total	Early	Late	Total		
Fertilizer	Biochar	(g C m^{-2})			(mg N m^{-2})			(g C kg^{-1})	(mg N kg^{-1})
NPK	Without biochar	4.2 ± 0.5d	26.3 ± 2.4a	30.5 ± 2.9b	907.4 ± 137.6a	4.0 ± 3.6a	911.5 ± 140.3a	38.2 ± 4.5a	1156.2 ± 264.2a
(Chemical fertilizer)									
	With biochar	6.4 ± 1.3c	27.9 ± 2.8a	34.4 ± 4.1ab	152.6 ± 39.4b	4.0 ± 3.3a	156.5 ± 41.3b	32.0 ± 5.3ab	143.9 ± 29.6b
	% change by plus biochar	52.4	-	-	-83.2	-	-82.8	-	-87.6
Azolla	Without biochar	9.4 ± 1.0b	30.7 ± 3.0a	40.1 ± 3.6a	257.5 ± 110.8b	8.0 ± 5.8a	265.5 ± 113.8b	38.1 ± 7.2a	246.6 ± 94.8b
(As green manure)									
	With biochar	12.5 ± 0.6a	27.9 ± 1.5a	40.5 ± 1.4a	26.0 ± 10.1c	11.0 ± 4.2a	37.0 ± 13.9c	28.7 ± 2.4b	26.1 ± 10.1c
	% change by plus biochar	33.0	-	-	-89.9		-86.1	-24.7	-89.4
ANOVA results									
Fertilizer		**	ns	**	**	*	**	ns	**
Biochar		**	ns	ns	**	ns	**	*	**
Fertilizer x Biochar		ns	ns	ns	**	ns	**	ns	**

Values are means ± standard deviation (n = 4). Different letters following values within the same column indicate significant differences among treatments (Tukey's HSD test [ns; not significant; *; $P < 0.05$; **; $P < 0.01$]). The data for the no biochar amendment treatments were obtained from Kimani et al. (2020).

CO_2 concentration in the Azolla + biochar treatment was between 202.4 and 368.7 μg C mL^{-1} compared with NPK (59.4–129.0 μg C mL^{-1}), NPK + biochar (184.5–206.5 μg C mL^{-1}), and Azolla (80.0–206.4 μg C mL^{-1}). The concentration of CO_2 dissolved in the soil solution for all treatments converged at 56 DAT and no significant differences occurred thereafter.

The concentration of CH_4 dissolved in the soil solution was significantly higher in the biochar and/or Azolla amended treatments compared with NPK treatment (Figure 4b), and the effect of biochar and Azolla co-application on dissolved CH_4 was significantly higher than in the NPK and NPK + biochar, but not in the Azolla treatment between 7–49 DAT ($P < 0.01$; Figure 4b). Between 7–49 DAT, the dissolved CH_4 concentration in the Azolla + biochar treatment was between 0.02 and 1.08 μg C mL^{-1}, while those of the NPK and NPK + biochar treatments were between 0.0–0.16 μg C mL^{-1} and 0.0–0.71 μg C mL^{-1} for the Azolla treatment. The concentration of CH_4 dissolved in the soil solution for all treatments increased uniformly with the highest levels recorded at 112 DAT (the last sampling day).

The concentrations of NO_3^--N in soil solution were significantly lower in the Azolla + biochar treatment compared to the NPK, NPK + biochar, and Azolla treatments ($P < 0.01$; insert Figure 2b). Over three weeks, the concentration of NO_3^--N in the soil solution was significantly lower in Azolla + biochar treatment (0.0–7.12 mg N L^{-1}) while those of the NPK, NPK + biochar, and Azolla treatments ranged between 0.0–47.78 mg N L^{-1}. Nitrate-N was not detectable in the soil solutions after the three weeks for all treatments.

3.4. Rice yield and biomass

The addition of poultry-litter biochar and/or Azolla significantly influenced rice plant shoot height, total biomass, grain yield, and harvest index at harvest ($P < 0.05$, Table 4), with no significant interactions between the biochar and fertilizer amendments for all rice plant growth parameters. Poultry-litter biochar and Azolla co-application (i.e., Azolla + biochar) significantly increased rice grain yield by 75.0% (NPK), and 27.3% compared with both NPK + biochar and Azolla only treatments. Azolla (30.3) and Azolla + biochar (36.3) treatments recorded significantly lower maximum tiller numbers compared with NPK (46.0) and NPK + biochar (47.3) treatments. However, there were no significant differences in the productive tillers among treatments (Table 4). The dry biomasses of floating Azolla cover in Azolla and Azolla + biochar treatments at harvest were 15.6 and 14.9 g pot^{-1}, respectively.

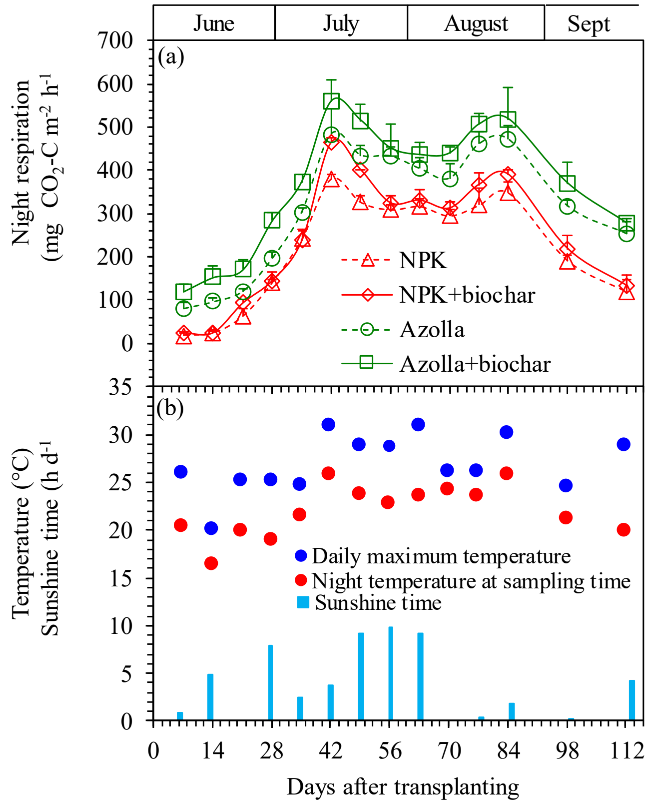

Figure 3. Changes in night respiration (CO_2 emissions) of rice plants from pots treated with NPK and Azolla without and with biochar throughout the experimental period (a). Bars indicate standard deviation (n = 4). The daily maximum temperature and temperature at sampling time (21:00) and sunshine time on the day of gas sampling are shown in (b). The data for the no biochar amendment treatments were obtained from Kimani et al. (2020).

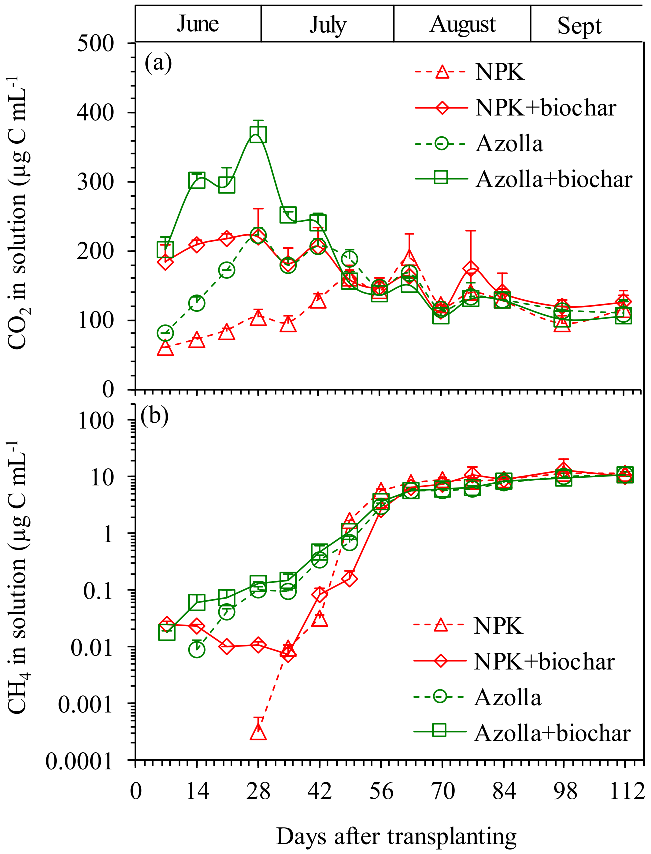

Figure 4. Changes in the concentration of CO_2 (a) and CH_4 (b) dissolved in the soil solution in pots treated with NPK and Azolla with and without biochar throughout the experimental period. Bars indicate the standard deviation (n = 4). The data for the no biochar amendment treatments were obtained from Kimani et al. (2020).

3.5. Changes in soil chemical properties at harvest

The chemical properties of soil at harvest are shown in Table 5. The co-application of biochar and Azolla significantly and positively influenced the soil pH and EC values with a significant synergistic interaction between biochar and fertilizer amendments ($P < 0.05$; Table 5). Additionally, biochar and Azolla amended treatments significantly increased the soil organic C and total N ($P < 0.01$). In the presence of Azolla, the poultry-litter biochar application significantly increased soil organic C by 44.1% (NPK), 25.9% (NPK + biochar), and 17.4% (Azolla), and total N by 33.3% (NPK), 17.6% (NPK + biochar), and 11.1% (Azolla). There were no significant differences in the C/N ratios among treatments (Table 5).

Table 4. Synergistic effects of poultry-litter biochar and Azolla on maximum and productive tiller number, shoot dry weight at harvest, total biomass, grain yield and harvest index.

Treatment		Maximum tiller	Productive tiller	Shoot height at harvest	Total biomass	Grain yield	Harvest index
Fertilizer	Biochar	(No. hill^{-1})		(cm)	(kg m^{-2})		(%)
NPK	Without biochar	46.0 ± 2.7a	32.3 ± 2.9a	81.8 ± 3.7c	2.6 ± 0.3b	0.8 ± 0.1c	31.5 ± 3.6c
(Chemical fertilizer)							
	With biochar	47.3 ± 2.6a	32.5 ± 1.3a	86.3 ± 1.4bc	3.0 ± 0.2a	1.1 ± 0.1b	36.0 ± 1.0bc
	% change by plus biochar	-	-	-	15.4	37.5	-
Azolla	Without biochar	30.3 ± 1.3b	28.3 ± 2.9a	88.5 ± 2.8ab	2.7 ± 0.2b	1.1 ± 0.1b	40.3 ± 3.3ab
(As green manure)							
	With biochar	36.3 ± 1.3b	32.8 ± 2.1a	90.4 ± 0.6a	3.2 ± 0.2a	1.4 ± 0.1a	43.7 ± 1.8a
	% change by plus biochar	-	-	-	18.5	27.3	-
ANOVA results							
Fertilizer		**	ns	**	ns	**	**
Biochar		ns	ns	*	**	**	*
Fertilizer x Biochar		ns	ns	ns	ns	ns	ns

Values are means ± standard deviation (n = 4). Different letters following values within the same column indicate significant differences among treatments (Tukey's HSD test [ns; not significant; *; $P < 0.05$; **; $P < 0.01$]). The data for the no biochar amendment treatments were obtained from Kimani et al. (2020).

Table 5. Soil properties at harvest among four treatments after the co-application of poultry-litter biochar and Azolla.

Treatment		pH (H_2O)	EC	Total N	Soil Organic C	C/N
Fertilizer	Biochar	(1:2.5)	(μS cm^{-1})	(g kg^{-1} dry soil)		
NPK	Without biochar	5.1 ± 0.1b	80.1 ± 14.4d	1.5 ± 0.0c	14.5 ± 0.2d	10.0 ± 0.1a
(Chemical fertilizer)						
	With biochar	6.8 ± 0.1a	222.7 ± 15.3a	1.7 ± 0.1b	16.6 ± 0.6c	10.0 ± 0.2a
	% change by plus biochar	33.3	178.0	13.3	14.5	-
Azolla	Without biochar	5.2 ± 0.0b	118.7 ± 12.0c	1.8 ± 0.1b	17.8 ± 0.8b	10.0 ± 0.1a
(As green manure)						
	With biochar	6.7 ± 0.1a	184.6 ± 24.3b	2.0 ± 0.1a	20.9 ± 1.0a	10.2 ± 0.3a
	% change by plus biochar	28.8	55.5	11.1	17.4	-
ANOVA results						
Fertilizer		ns	ns	**	**	ns
Biochar		**	**	**	**	ns
Fertilizer x Biochar		*	*	ns	ns	ns

Values are means ± standard deviation (n = 4). Different letters following values within the same column indicate significant differences among treatments (Tukey's HSD test [ns; not significant; *; $P < 0.05$; **; $P < 0.01$]). The data for the no biochar amendment treatments were obtained from Kimani et al. (2020).

Table 6. Net CO_2-equivalent GHG emissions, soil C sequestration, and net GHG balance between the four treatments throughout the rice growth period.

Treatment		Total CH_4 emission	Total N_2O emission	Soil C sequestration	Net GHG balance
Fertilizer	Biochar	(g CO_2eq m^{-2})			
NPK	Without biochar	1381.2 ± 129.2b	426.8 ± 65.7a	-1.6 ± 128.2c	1806.3 ± 29.6c
(Chemical fertilizer)					
	With biochar	1557.4 ± 187.2ab	73.3 ± 19.3b	-1731.3 ± 444.4b	-100.7 ± 451.2b
Azolla	Without biochar	1815.6 ± 162.2a	124.3 ± 53.3b	-1821.2 ± 422.1b	118.7 ± 372.9b
(As green manure)					
	With biochar	1834.1 ± 65.2a	17.3 ± 6.5c	-3485.8 ± 541.4a	-1634.4 ± 511.2a
ANOVA results					
Fertilizer		**	**	**	**
Biochar		ns	**	**	**
Fertilizer x Biochar		ns	**	ns	ns

Values are means ± standard deviation (n = 4). Different letters following values within the same column indicate significant differences among treatments (Tukey's HSD test [ns; not significant; *; $P < 0.05$; **; $P < 0.01$]). The data for the no biochar amendment treatments were obtained from Kimani et al. (2020).

3.6. Global warming potential, soil C sequestration, and net greenhouse gas balance

The GWP of CH_4 and N_2O made up 93.6%–99.1% and 0.9%–6.4%, respectively, of the combined GWP (CH_4 plus N_2O) in the Azolla and Azolla + biochar treatments, as well as 76.4%–95.5% for CH_4 and 4.5%–23.6% for N_2O in the NPK and NPK + biochar treatments (Table 6). The application of Azolla significantly increased total CH_4 emissions g CO_2-eq m^{-2} (at $P < 0.01$) and combined GWP (at $P = 0.029$, Table 6). However, the co-application of poultry-litter biochar and Azolla had no significant influence on total CH_4 emissions g CO_2-eq m^{-2}, but significantly decreased total N_2O emissions g CO_2-eq m^{-2}, with a significantly high interaction between fertilizer and biochar amendments ($P < 0.01$, Table 6). Subsequently, in the presence of Azolla, the application of biochar did not significantly influence the combined GWP ($P = 0.086$) and no significant differences were observed in the combined GWP between treatments ($P = 0.056$). Application of biochar and/or Azolla significantly influenced soil C sequestration at harvest ($P < 0.01$) and the net GHG balance ($P < 0.01$) compared with NPK only treatment, with no significant interaction between biochar and fertilizer amendments (Table 6).

4. Discussion

4.1. Effect of poultry-litter biochar and Azolla on CH_4 emissions

Previously, Kimani et al. (2020) reported that incorporation of Azolla as green manure significantly increased CH_4 emissions during the early rice growth stages (i.e., before 63 DAT) by 123.3% and total cumulative CH_4 emissions by 31.5% compared to the NPK treatment (Table 3). This was largely attributed to the decomposition of the organic amendments by incorporated Azolla (Ying et al., 2000). Similarly, in this study, amendment with biochar in the presence of Azolla (i.e., Azolla + biochar), significantly increased CH_4 emissions both in the early rice growth stages before 63 DAT by 197.6% (NPK), 95.3% (NPK + biochar), and 33.0% (Azolla), and total cumulative CH_4 emissions (at 112 DAT) by 32.8% compared with NPK only treatment, with no significant emission differences relative to NPK + biochar or Azolla (Table 3). Furthermore, biochar amendment with chemical fertilizer (i.e., NPK + biochar) significantly increased cumulative CH_4 emissions before 63 DAT by 52.5% compared with NPK, but reduced the emissions by 46.7% relative to Azolla treatment. No significant cumulative CH_4 emissions were observed among treatments at the late rice growth stages (i.e., after 63 DAT) (Table 3).

In our observations, the significant increase in cumulative CH_4 emissions before 63 DAT following the addition of biochar (at $P < 0.01$, Table 3), are consistent with Knoblauch et al. (2011) and Zhang et al. (2012) who revealed a 26%–68% CH_4 emission increase in paddy soils after biochar applications. Similarly, Kim et al. (2013) reported increased CH_4 emissions of approximately 60% within 40 DAT from green manure amended plots relative to NPK only plots. During the early rice growth stages before 63 DAT, no significant differences on rice growth parameters among all treatments were observed (Figure 5a, b); however, biochar and Azolla amendments significantly increased the concentrations of CO_2 and CH_4 dissolved in the soil solutions compared to NPK (Figure 4a, b). This was likely as a result of increased microbial biomass and microbial activity after the application of biochar, which may have amplified the decomposition of both the newly-added (in this case Azolla) and native soil organic matter (SOM), as well as the decomposition of labile C pools derived from biochar (Steinbeiss et al., 2009). Therefore, the effect of biochar and Azolla applications on CH_4 emissions before 63 DAT is mostly attributed to increased availability of carbon substrates following application of Azolla as green manure and/or biochar and their co-application (Jeffery et al., 2016).

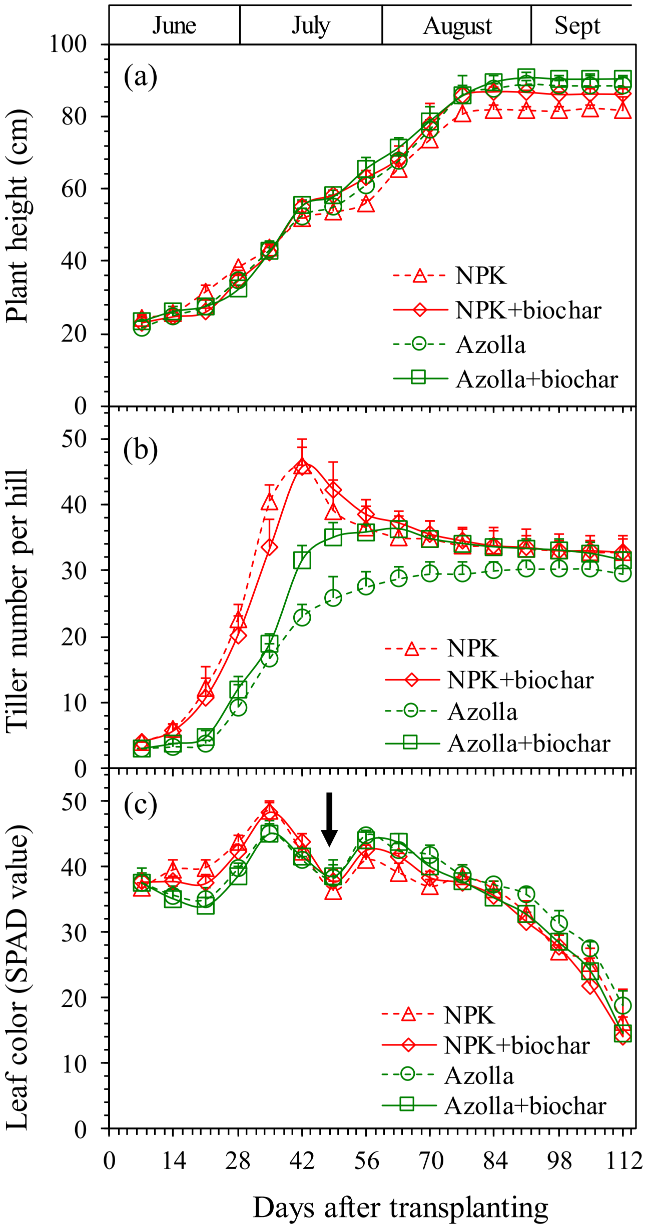

Figure 5. Plant height (a), tiller number (b), and leaf color measured in SPAD values (c) of rice plants from pots treated with NPK and Azolla without and with biochar throughout the experimental period. Bars indicate standard deviation (n = 4). The arrow indicates the day fertilizer was added to the pots. The data for the no biochar amendment treatments were obtained from Kimani et al. (2020).

Unlike in the early growth stages of rice, application of biochar did not significantly influence cumulative CH_4 emissions during the later stages (after 63 DAT; Figure 2a, Table 3). In the same batch of treatments, Kimani et al. (2020) found that the percentage of CH_4 emitted after 63 DAT relative to the seasonal CH_4 emission following incorporation of Azolla as green manure (76.5%), was lower compared with NPK (86.2%) (Table 3). CH_4 emissions in flooded paddy soils are particularly affected by C availability (Wang et al., 2017). However, the highly stable nature of biochar is said to cause no significant changes in C availability (Jones et al., 2011). Moreover, the positive priming of soil organic matter and other organic matter inputs by biochar has been observed to persist for a short-term, due to the relatively small amounts of easily-mineralizable fraction of biochar (Zimmerman et al., 2011). According to Partey et al. (2014) and Saarnio (2015), the labile C pools resulting from root exudates and root litters are thought to be significantly more compared to organic matter and/or biochar labile fractions. Considering this, our results could partly be ascribed to low soil C availability and supply after 63

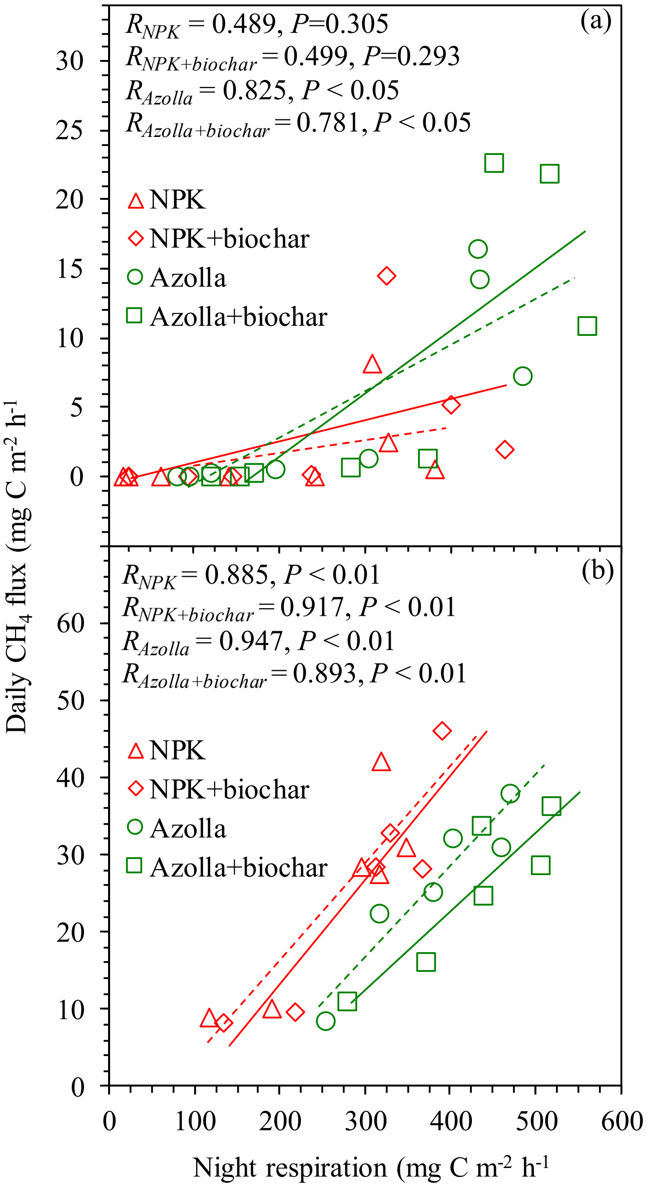

Figure 6. Relationship between daily CH_4 flux and night respiration (CO_2 emissions) between treatments during the early (before 63 DAT) (a) and late (after 63 DAT) (b) rice growth stages (n = 6). The data for the no biochar amendment treatments were obtained from Kimani et al. (2020).

DAT following the application of biochar and Azolla as suggested by the minor changes in dissolved CO_2 concentrations in the soil solution after 42 DAT vis a vis the initial (before 63 DAT) concentrations (Figure 4a). Additionally, different to the correlation observations in the early growth stages of rice between the daily CH_4 flux and night respiration (CO_2 emissions) where only the Azolla and Azolla + biochar amended treatments showed positive effects ($P < 0.05$, Figure 6a), the significantly high and positive correlations observed from all treatments during the late rice growth stages (at $P < 0.01$; Figure 6b), suggest likely changes in carbon sources for methanogens, from either the initial SOM, incorporated Azolla as green manure, or biochar addition, to the photosynthetic products of rice plants (Aulakh et al., 2001; Minoda et al., 1996; Sass and Cicerone, 2002).

According to Feng et al. (2013) decreasing yield equivalent agricultural CH_4 emissions remains a major global test. Cheng et al. (2018) reported a strong relationship between CH_4 emission and rice biomass. Similarly, (Sriphirom et al., 2020) found a significant reduction in yield-scaled CH_4 emissions by 15.2%–25.5%, and higher rice biomass from biochar amended treatments compared with control under conventional or water management practices. In this study, compared with NPK and Azolla treatments, Azolla + biochar treatment significantly increased rice yield (Table 4) and reduced yield-scaled CH_4 emissions (Table 3). The stimulatory effect of biochar on rice yield productivity is attributed to enhanced nutrient retention and addition, as well as improved nutrient turnover (Biederman and Harpole, 2013). As a result, the co-application of poultry-litter biochar and Azolla as green manure may be an alternative and feasible farming management practice for sustainable rice production.

4.2. Effect of poultry-litter biochar and Azolla on N_2O emissions

The effects of biochar on N_2O emissions remain conflicting, ranging from stimulation (Lin et al., 2017), and reduction (Abagandura et al., 2019). In our study, Azolla + biochar significantly reduced the cumulative N_2O emission before 63 DAT compared with NPK (97.1%), NPK + biochar (83.0%), and Azolla (86.1%) (Figure 2a, Table 3). Similarly, NPK + biochar significantly reduced cumulative N_2O emissions before 63 DAT by 82.8% relative to NPK treatment, with a significantly high interaction between biochar and fertilizer (at $P < 0.01$; Table 3). There were no significant cumulative N_2O emissions observed after 63 DAT among the four treatments. During the entire rice growth period, Azolla + biochar significantly reduced the total cumulative N_2O emissions at 112 DAT by 95.9% (NPK), 76.4% (NPK + biochar), and 86.1% (Azolla), with a significant interaction between biochar and fertilizer (at $P < 0.01$; Table 3).

Nitrification and denitrification have been identified as the predominant pathways for N_2O production (Charles et al., 2017). According to Miller et al. (2008) availability of easily decomposable organic C and/or NO_3^- stimulates microbial metabolic activity, leading to increased oxygen consumption in the soil, and hence favoring denitrification. In our study, however, application of Azolla as green manure and/or biochar, and their co-application, did not result in additional N_2O production even though the microbial metabolisms, as seen by concentrations of CO_2 dissolved in the soil solutions in the early stages of rice growth, were significantly greater in NPK + biochar, Azolla, and Azolla + biochar treatments relative to NPK only treatment (Figure 4a). In the same set of experiment, Kimani et al. (2020) reported that on average, incorporation of Azolla as green manure (AGM) significantly decreased both early (before 63 DAT) and seasonal N_2O emissions by 71.3% (Table 3). Similarly, Song et al. (2016) reported significant reductions in N_2O emissions in the first 30 days or after 90 days, with no significant differences in the 30–90 day period after biochar amendment. The significantly higher effects of Azolla and/or biochar, and their co-application, on N_2O emissions before 63 DAT would be explained by the effective reduction of N_2O to N_2 during denitrification due to increased availability of easily decomposable carbon from both Azolla and/or biochar (Cayuela et al., 2014; Miller et al., 2008).

Biochar application to soils has been reported to mitigate N_2O emissions through nitrification by probably altering the soil physical, chemical, and biological properties (Kammann et al., 2017). Additionally, the inhibition of microbial pathways as a result of biochar toxicity, immobilization and adsorption of NH_4^+/NO_3^-, and aeration regulation, are believed to inhibit nitrification and subsequent N_2O emissions (Clough et al., 2013). Furthermore, amendment with biochar pyrolyzed at 400 °C and 600 °C has been reported to significantly decrease soil inorganic N by increasing the NH_4^+ adsorption by 62–81% (Zhang et al., 2015). In our study, the biochar was produced between 450 °C - 500 °C. Additionally, application of biochar significantly reduced the NO_3^--N in the soil solution (insert Figure 2b), in addition to possible suppression of carbon (C) and of nitrogen (N) mobilization from both the native and freshly-added organic matter sources. Application of biochar amendments has been reported to induce a negative priming effect, inhibiting the decomposition of native soil organic carbon (SOC) and the stimulation effect of inorganic N on SOC degradation (Saarnio, 2015; Zimmerman et al., 2011).

Soil pH is a key variable affecting both N_2O production and consumption, as well as the N_2O/N_2 ratio of emissions, with the effect of biochar on the denitrification of N_2O suggested to mostly depend on its pH and the C/N ratios (Cayuela et al., 2014, 2015; Clough et al., 2013). According to van Zwieten et al. (2010) an increase in soil pH under flooded soil conditions possibly enhances the final stage of denitrification (i.e., reduction of N_2O to N_2). In the current study, application of biochar significantly increased the soil pH by 33.3% (NPK + biochar, 6.8 pH unit) and 28.8% (Azolla + biochar, 6.7 pH unit) compared with NPK (5.1 pH unit) and Azolla (5.2 pH unit) treatments, respectively, (Table 5). Similarly, Clough et al. (2004) reported lower cumulative N_2O fluxes from soil at field capacity with pH values $\geq$ 5.9. On the other hand, the lack of significant influence on N_2O emissions after 63 DAT following biochar application in our observations (Table 3), might be a result of a decrease in the liming effect of biochar (Cayuela et al., 2014; van Zwieten et al., 2010).

In this study, co-application of poultry-litter biochar and Azolla as green manure simultaneously decreased N_2O emissions and increased grain yield, thereby decreasing yield-scaled N_2O emissions during the rice growth period. Thus, the co-application of biochar and Azolla is an optimal practice for mitigating N_2O emissions and increasing rice yield.

4.3. Effect of poultry-litter biochar and Azolla on GWP, soil C sequestration and net GHG balance

In our study, the combined GWP (CH_4 plus N_2O) ranged from 1630.7 to 1939.9 g CO_2eq m^{-2} in all treatments. Biochar and Azolla amendments had no significant effects on the combined GWP (Table 6). In the same batch of experiment, incorporation of Azolla as green manure plus its subsequent growth as a dual crop significantly increased the combined GWP by 7.3% compared with NPK treatment (Kimani et al., 2020), and this was attributed to the significantly higher seasonal CH_4 emissions. The contribution of CH_4 emissions to the combined GWP is considered higher than that of N_2O emissions (Tirol-Padre et al., 2018). In the current observations, however, compared to the NPK treatment, biochar and/or Azolla amendments did not significantly influence the net CH_4 emissions, but significantly reduced net N_2O emissions with significant interactions between biochar and fertilizer (Tables 3 and 6). Additionally, application of biochar and/or Azolla amendments significantly increased the amounts of carbon sequestered in the soil, and subsequently significantly decreased net GHG (Tables 3 and 6). According to Lehmann et al. (2006) application of biochar, independently or in combination with other amendments, is seen as a practical tool to mitigate GWP by enhancing soil C sequestration. Additionally, although the contribution of N_2O emissions to the combined GWP during rice cultivation is considered lower than that of CH_4 emissions (Tirol-Padre et al., 2018), our observations suggest that the co-application of biochar and Azolla in lowland rice fields could be a suitable management approach to reduce agricultural N_2O emissions without increasing CH_4 emissions and the subsequent GWP. However, the evaluation of appropriate years of long-term application is considered important in the future.

Nevertheless, the would-be role of biochar in climate change mitigation requires a comprehensive assessment of the energy consumption and carbon release from fossil fuels resulting from its production (Kammann et al., 2017), as well as the actual effect of biochar amendments on GWP. In other words, it is imperative to consider the balance of GHG gases from the production of biochar and the sinks its use may create (Mukherjee et al., 2014; Oomori et al., 2016). Considering this, future research should focus on evaluating the long-term effects of poultry-litter biochar and Azolla co-application to rice paddy fields and the resulting combined GWP and soil C sequestration. Moreover, future studies should also aim to provide a quantifiable basis for management recommendations to achieved maximum sustainable benefits and environmental safety.

5. Conclusion

The co-application of poultry-litter biochar and Azolla as green manure significantly increased CH_4 and decreased N_2O emissions during early rice growth stages but had no significant impact during later stages. Overall, the co-application of biochar and Azolla significantly decreased seasonal N_2O emissions, but did not significantly influence seasonal CH_4 emissions throughout the whole rice growth period. Subsequently, biochar and Azolla co-application significantly increased rice grain yield, and the soil organic C, total N, pH, and EC values. In the presence of Azolla, amendment with biochar significantly decreased both grain yield equivalent CH_4 and N_2O emissions. Although the co-application of biochar and Azolla did not influence the global warming potential, it significantly increased soil C sequestration and decreased net GHG balance. Consequently, the co-application of biochar and Azolla in conjunction with chemical fertilizers during the rice booting stages showed promising potential in increasing grain yield while reducing non-CO_2 GHG emissions. However, it should be noted that our results are based on a pot experiment, spanning a single rice crop season. Long-term field studies should be carried out in the future to confirm our results in field conditions.

Declarations

Author contribution statement

Samuel Munyaka Kimani: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Weiguo Cheng: Conceived and designed the experiments; Analyzed and interpreted the data.

Putu Oki Bimantara: Performed the experiments.

Xingkai Xu: Analyzed and interpreted the data.

Satoshi Hattori, Keitaro Tawaraya, Shigeto Sudo: Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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