RESEARCH ARTICLE

REVISED Rice husk and melaleuca biochar additions reduce soil CH_4 and N_2O emissions and increase soil physicochemical properties [version 2; peer review: 2 approved]

Previously titled: Rice husk and melaleuca biochar additions reduce soil CH_4 and N_2O emissions and increase soil organic matter and nutrient availability

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Abstract
Background: Biochar is a promising material in mitigating greenhouse gases (GHGs) emissions from paddy fields due to its remarkable structural properties. Rice husk biochar (RhB) and melaleuca biochar (MB) are amendment materials that could be used to potentially reduce emissions in the Vietnamese Mekong Delta (VMD). However, their effects on CH_4 and N_2O emissions and soil under local water management and conventional rice cultivation have not been thoroughly investigated.
Methods: We conducted a field experiment using biochar additions to the topsoil layer (0-20 cm). Five treatments comprising 0 t ha^{-1} (CT0); 5 t ha^{-1} (RhB5) and 10 t ha^{-1} (RhB10), and 5 t ha^{-1} (MB5) and 10 t ha^{-1} (MB10) were designed plot-by-plot (20 m^2) in triplicates.
Results: The results showed that biochar application from 5 to 10 t ha^{-1} significantly decreased cumulative CH_4 (24.2-28.0%, RhB; 22.0-14.1%, MB) and N_2O (25.6-41.0%, RhB; 38.4–56.4%, MB) fluxes without a reduction in grain yield. Increasing the biochar application rate further did not decrease significantly total CH_4 and N_2O fluxes but was seen to significantly reduce the global warming potential (GWP) and yield-scale GWP in the RhB treatments. Biochar application improved soil Eh but had no effects on soil pH. Whereas CH_4 flux correlated negatively with soil Eh ($P < 0.001$; $r^2 = 0.552$, RhB; $P < 0.001$; $r^2 = 0.502$, MB). Ameliorating soil aeration and functions by adding RhB and MB resulted in improving soil physicochemical properties, especially significant SOM and AN boosting, which indicate better soil health, structure, and fertility.
Conclusions: Biochar supplementation significantly reduced CH_4 and N_2O fluxes and improved soil mineralization and physicochemical

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properties toward beneficial for rice plants. The results suggest that the optimal combination of biochar-application rates and effective water-irrigation techniques for soil types in the MD should be further studied in future works.

Keywords
Biochar amendment, conventional rice farming, greenhouse gas emissions, melaleuca biochar, rice-husk biochar, soil fertility

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REVISED **Amendments from Version 1**

We have incorporated the reviewers' suggestions and recommendations in the new version. Accordingly, the revisions have been made as follows:

1) The term soil physiochemical properties has been used in the title.
2) A short explanation related to soil properties has been supplemented in the abstract section.
3) An additional explanation has been expanded to statistical analysis.
4) The use of abbreviations has been unified in all parts.

Any further responses from the reviewers can be found at the end of the article

Introduction

In Vietnam, the agricultural sector contributes approximately 30% of national greenhouse gases (GHGs) emissions (MONRE, 2017). For rice cultivation, paddy fields are the primary source of GHGs emissions (Nan *et al.*, 2020; Shinoda *et al.*, 2019), accounting for 50% of the sub-sectors in agricultural production and roughly 14.6% of national GHG emissions in Vietnam (MONRE, 2017). According to NDC (2020), Vietnam is committed to reducing 8% of total national GHGs emissions from domestic resources by 2030. Management and technological strategies will play a vital role in reducing the total carbon footprint. Biochar is a carbonized biomass product produced from thermochemical conversion of organic materials under oxygen-limited conditions (Lohri *et al.*, 2016; Wu *et al.*, 2012; Waqas *et al.*, 2018). Biochar applications have been noted as one of the most promising approaches for reducing GHGs emissions from rice production (Koyama *et al.*, 2015; Wu *et al.*, 2019a; *Nan et al.*, 2021), and IPCC recently recommended the method (Ji *et al.*, 2020). Previous studies have demonstrated that biochar incorporated into soil paddy fields positively rehabilitated soil properties such as pH neutralization, cation exchange capacity (CEC), and buffering capability, soil organic materials (SOM), and nitrogen storage (Qin *et al.*, 2016; Luo *et al.*, 2020); improved plant available water, microporosity, and soil aggregate stability, and decreased bulk density (Burrell *et al.*, 2016); effected on soil functions and fertility (Giagnoni *et al.*, 2019; Siedt *et al.*, 2021); and ameliorated nutrient availability of carbon (C), nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg) and Calcium (Ca) (Li *et al.*, 2019). Furthermore, biochar forms a great habitat for different microorganisms via providing macro-, meso- and micropores (Palansooriya *et al.*, 2019; Wu *et al.*, 2019a), supports microbial communities by providing labile C substrates for degradation (Smith *et al.*, 2010), stimulating biodiversity and abundance of methanotrophic microbes (Qin *et al.*, 2016). Moreover, the addition of biochar to the soil reduces GHGs emissions (Spokas and Reicosky, 2009; Koyama *et al.*, 2015; Nan *et al.*, 2020; Huang *et al.*, 2019) and increases rice yield under different favorable conditions (Yang *et al.*, 2019; Paiman and Effendy, 2020).

In the Vietnamese Mekong Delta (VMD), melaleuca is an abundantly available hard firewood resource, accounting for 176,295 ha (GIZ, 2009); the wood reserve of melaleuca is estimated at 13 million m^3. In addition, rice husk is known as a by-product of rice production, accounting for 20% of rice yields (Chungsangunsit *et al.*, 2009). It is estimated that VMD annually produces around 1.9 million tons of rice husk (Son *et al.*, 2017). Biomass (hardwood and crop residues) are often used as typical feedstock for making biochar pyrolysis owing to their multiple-porous structure (Nguyen *et al.*, 2018; Nan *et al.*, 2020), which facilitates the multifunctional purposes of soil amendment and pollutant remediation. Therefore, both melaleuca and rice husk could be used to produce biochar, which is then applied to rice paddy fields as a GHGs emission reduction strategy. Although previous studies have demonstrated the effectiveness of biochar incorporation on reducing GHGs emissions, little attention has been paid to the quantitative variation of rice husk biochar (RhB) and melaleuca biochar (MB) on GHGs emissions and soil improvement in VMD lowland conditions. Moreover, the majority of previous studies exclusively emphasize CH_4 and N_2O emissions on water practices by controlled irrigation, and alternative wetting and drying, and midseason drainage (Yang *et al.* 2019, Sriphirom *et al.* 2020, Uno *et al.* 2021), while atypical water irrigation regime has not been thoroughly elucidated.

Thus, we aimed (i) to elucidate the CH_4 and N_2O emissions and global warming potential (GWP) from the incorporation of RhB and MB into the paddy field soils under locally typical water management regimes in the VMD, and (ii) to determine the effects of RhB and MB amendments on soil physicochemical properties. We, therefore, conducted a field experiment with a variety of RhB and MB amendment amounts under conventional farming practices. Our field experimentation confirmed that RhB and MB application to rice paddy fields was feasible in reducing GHGs emissions. Simultaneously, biochar application improved soil availability of SOM and anaerobically mineralized N.

Methods

Site description

A field experiment was carried out on a typical smallholding farmer's paddy field in Thoi An Dong Village, Can Tho city, Vietnam (10°3′44″N, 105°41′55″E). The study area was located in the center of the Mekong Delta, Vietnam, which is a tropical area influenced by the monsoon climate zone, with measured mean annual rainfall (2,088.4 mm), air temperature (27.5 °C), humidity (78.0 – 86.0%), sunshine (2,467.4 – 2,695.4 hours) in the period from 2015-2019 (DONRE, 2020). The precipitation and temperature during the experiment were recorded by a weather station placed at the farmer's house (~150 m from the field experiment). The soil was classified as Thionic Glycesol (International Union of Soil Sciences (IUSS) working group World Reference Base (WRB), 2015) (Dong *et al.*, 2012, Minamikawa *et al.*, 2021). The elementary properties were (0-20 cm depth) as follows: pH (H_2O), 5.41; EC, 0.9 mS cm^{-1}; bulk density, 0.92 $g.cm^{-3}$, silty clay texture (59.3% clay, 39.5% silt, 1.2% sand); organic matter, 87 g kg^{-1}; total N, 4.21 g kg^{-1}; cation exchange capacity (CEC), 37.4 meq 100 g^{-1}; exchangeable K, 0.54 meq 100 g^{-1}, exchangeable Mg, 5.47 meq 100 g^{-1}; exchangeable Ca, 10.5 meq 100 g^{-1}; and total C, 40.76 g kg^{-1}.

Biochar preparation

RhB was made on-site using a simple semi-industrial pyrolysis batch method (Oikawa *et al.*, 2016). Here a short iron bar was to set onto the ground. A stainless chimney pipe 1.5m long was vertically erected to the bar using wire. The pipe was kept at a 10-cm distance from the ground to release smoke generated during the pyrolysis process. Embers were placed adjacent to the bar to kick off the carbonization process. Then, rice husk was poured around the bar according to a coniform shape with 1.5 m height and 1.5 m diameter. RhB was generated from the bottom to the summit. After finishing the pyrolysis process (two days), RhB was watered to achieve ambient temperature.

MB was produced by a poor-oxygen pyrolysis process under a traditional bell-shaped charcoal production kiln for a 30-day batch. The kiln was made from baked bricks, clay, and sand mortar. The kiln's structure comprises a bell-shaped heating firewood chamber, a door used for firewood loading, and biochar unloading. A combustion chamber provided hot air for the carbonization process, while four chimneys were installed around the heating chamber discharging smoke during the carbonization process. Firewood was fully loaded according to each layer inside the heating chamber; the lowest layer was kept 10 cm away from the ground to ensure air convection. Before starting, the door was closed to begin the carbonization process. Air heating from the combustion chamber was slowly provided to the inner heating chamber to form carbonization. After 30 days of pyrolysis, the heating was switched off, and the combustion chamber was blocked off for an additional 15 days to cool to ambient temperature. The images of RhB and MB and their properties are shown in Figure 1 and Table 1, respectively.

Experimental design

The size of each experimental plot was 20 m^2 (4 m × 5 m) which were arranged in a randomized complete block design with three replications. Each plot was separated by soil banks and covered with mulch film. Five treatments with RhB and MB incorporated into the soil paddy field comprised 0 t ha^{-1} (conventional rice cultivation without biochar supplementation), 5 t ha^{-1}, and 10 t ha^{-1} (based dried weight) named CT0, RhB5, RhB10, MB5, and MB10, respectively. Biochar was manually spread on the soil surface of each pot and evenly incorporated into the plow layer of soil (approximately 20 cm) by shovels and rakes before sowing. Biochar additions were applied one time solely at the beginning of the experiment.

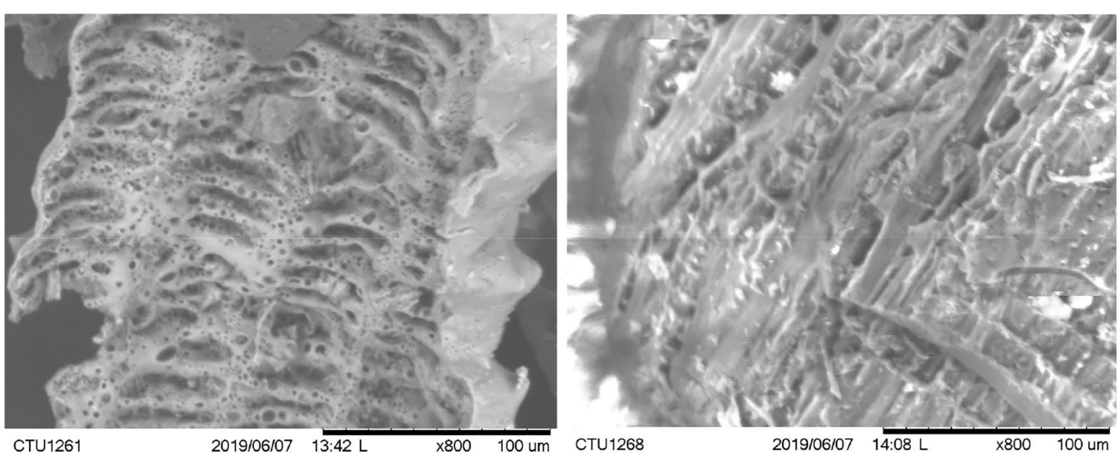

Figure 1. Scanning electron microscope (SEM) images of biochar produced from rice husk (a) and melaleuca (b) at X800 magnification.

Table 1. **Main properties of biochar derived from rice husk and melaleuca used in the field experiment.**

Items	Rice husk	Melaleuca
pH (H_2O)	9.56	7.54
EC (mS cm^{-1})	0.78	0.28
CEC (cmol (+) kg^{-1})	13.2	9.55
Total C (g kg^{-1})	253.5	291.8
Total N (g kg^{-1})	3.26	2.50
Total P (g kg^{-1})	0.13	0.33
Specific surface area (m^2 g^{-1})	51.93	2.04
Total pore volume (cm^3 g^{-1})	0.026	0.001

Rice cultivation and water management

According to the local crop calendar, the experiment time corresponded with the Spring-Summer (SS) season (the second crop) (Table 2). This is a transitional season between the dry and wet seasons. Rice straw and rice stubble from the previous rice crop cycle (Winter-Spring) were plowed by a hand tractor and underwent a 7-day fallow period before sowing. A short-duration variety of rice (IR50404 cultivar) typically grown in VMD was used in this field experiment (85-90 days of maturity). Pre-germinated seeds were sown on the wet-leveled soil using drum seeders at a rate equivalent to 120 kg ha^{-1}. The irrigation followed regionally typical water management based on the farmer's practical experience.

Fertilizer application

Inorganic fertilizers with the total amount of 80 kg N ha^{-1}, 40 kg P_2O_5 ha^{-1} and 40 kg KCl ha^{-1} were applied. The fertilization was divided into intervals at 9, 23, and 38 days after sowing (DAS) by broadcasting. Nitrogen (N) was applied as urea at a rate of 16-32-32 kg N ha^{-1} (broadcasted three times). Phosphorus (P) was applied as superphosphate at a rate of 8-16-16 kg P_2O_5 ha^{-1} tolerant (broadcasted three times). Whereas potassium (K) was applied as potassium chloride at a 20-0-20 kg KCl ha^{-1} rate (broadcasted twice). The rice cropping calendar and fertilizer application are shown in Table 2.

Measurements

Scanning electron microscope (SEM) images of RhB and MB were captured by microscope (TM-1000, Hitachi, Japan). Specific surface area and total pore volume were determined using BET Surface Area Analyzer (Quatachrome Nova 1000e, USA).

A weather station (WS-GP1, Delta-T Devices, Cambridge, UK) was installed on-site to record hourly temperature and rainfall at the experimental site. Redox potential (Eh) at plow-layer soil (20 cm) was measured by using platinum-tipped

Table 2. **Rice cropping calendar in the field experiment.**

Cultivated schedule	Date of experiment[1)]	Days after sowing
Plowing	14/03/2019	−7
Biochar incorporation	21/03/2019	0
Sowing	21/03/2021	0
Starting irrigation	29/03/2021	8
Fertilization		
1st topdressing (16-8-20)[2)]	30/03/2019	9
2nd topdressing (32-16-0)[2)]	13/04/2019	23
3rd topdressing (32-16-20)[2)]	27/04/2019	38
Drainage	30/05/2019	70
Harvest	14/06/2019	85

[1)] dd/mm/yyyy.
[2)] The numbers in parenthesis indicate the amount (kg ha^{-1}) of fertilizers applied in terms of N, P and K, respectively.

electrodes pined into the ground at a depth of 5, 10, and 20 cm; a portable Eh meter (HM31P; TOA-DKK, Japan) was connected to the electrodes to record soil Eh values at corresponding times to gas sampling. Surface water levels were also recorded simultaneously with gas sampling, using a ruler to read values directly in a plastic-perforated tube pre-installed in each plot.

Topsoil samples (0-20 cm) in each plot were collected before adding biochar and harvest by an auger 3 cm diameter. Visible remaining biomass was eliminated before air drying and sieved at 2.0 mm. Initial soil samples (n = 15) were mixed into a collective sample for analysis. Harvest soil samples were collected for each plot separately. Physical soil properties were measured as follows: soil texture - Pipette Robinson method (Carter and Gregorich, 2008), bulk density - Core method, and the particle density of soil (Blake and Hartge, 1986). Biochar and soil chemical properties were detected as follows: pH (H_2O) – a portable pH meter (HANA, Germany), soil organic matter (SOM) and total organic C (TOC) – Walkley and Black (1934), total P - Bowman (1988), available P (AP) - Olsen and Sommers (1982), total N – semi-micro Kjeldahl method (Bremner, 1996), anaerobically mineralized N (AN)– a 7-day anaerobic incubation at 40 °C (Keeney and Bremner, 1996), and CEC and exchangeable cations – Thomas (1982).

Rice yield was determined by harvesting from a 2.5 m × 2.0 m area in each plot at physiological maturity and removed unfilled grains by water before sun drying. A grain moisture tester (Riceter f2, Kett Electric Laboratory, Tokyo, Japan) was used to measure moisture content. The presented rice yield was adjusted to a 14% moisture content.

The closed chamber method was used to collect gas samples. A chamber was made from transparent polyvinyl chloride (PVC) panels with a 1.5 mm thickness. The cross-sectional area was 0.25 m^2 (0.5 m × 0.5 m). The height of the chamber was 70 cm from the bottom to the top layer. The chamber inside was equipped with a circulating fan, a temperature meter, and a pressure control plastic bag as described in detail by Minamikawa (2015). The chamber was placed on a plastic pre-installed base (a groove 4.5 cm depth) in each plot and sealed off by water before sampling. After chamber closure, a syringe (50 mL) was utilized to take inside gas at 1, 11, 21, and 31 minutes. Then, gas samples were injected into a 20-mL evacuated vial. The gas sampling was carried out from 10 DAS to 73 DAS at 7-day intervals. The concentrations of CH_4 and N_2O were analyzed with a gas chromatograph (8610C, SRI Instruments, CA, USA) equipped with a flame ionization detector (FID) and an electron capture detector (ECD) for the analysis of CH_4 and N_2O, respectively. The columns for the analysis of CH_4 and N_2O were packed with Porapak Q (50–80 mesh); dinitrogen (N_2) was used as the carrier gas for both FID and ECD.

Porosity was calculated by dividing volume pores (based on the subtraction between bulk density and particle density of soils) by volume total (Flint and Flint, 2002). CH_4 and N_2O fluxes were calculated by a linear progression of gas concentration change over time, and total fluxes of CH_4 and N_2O were calculated using a trapezoidal integration method described by Minamikawa (2015). Global warming potential (GWP) was calculated based on CO_2 equivalence (1 CH_4 = 34 CO_2-eq; 1 N_2O = 298 CO_2-eq) at a 100-year scale of climate-carbon feedbacks (Myhre *et al.*, 2013). Yield-scale GWP was calculated by dividing the GWP by grain yield (Minamikawa *et al.*, 2021).

Statistical analysis

One-Way analysis of variance (ANOVA) was used to assess the effects of each biochar on grain yield, gas fluxes, GWP, yield-scale GWP, and soil improvement. The difference of treatments was carried out using Duncan's method for all pairwise multiple comparison procedures. Linear regression analyses were performed to assess the relationship between Eh change and methane emission. We also analyzed the relationship between biochar application rate and gas emissions. In the statistical analysis, we did not compare the difference between RhB and MB. All analyses were carried out using R stats Version 4.2.0 (R Project for Statistical Computing, RRID:SCR_001905). The results are presented in tabular form with the values including mean ± standard deviation (SD) and the different symbols with a confidence level of 95%. Significant different comparison among treatments was considered at Duncan's multiple range test (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$ and †$P > 0.05$) after passing homogeneity of variance.

Results

Weather and water management

The mean air temperature and the total rainfall during the experiment were 28.9 °C and 429 mm, respectively (Figure 2). High rainfall was observed between 40-60 and 65-80 DAS. Figure 3 shows that the flooding water regime was predominantly observed during the experimental regression. The trend of water levels variation was similar over treatments. Water was irrigated from 7 DAS, reflooded 3-5 cm from soil surface for fertilizing (9, 23, and 38 DAS), and respective multiple drainage practices (−10 to 5 cm) (Uno *et al.*, 2021) was carried out for the remaining periods. Fifteen days before harvesting (70 DAS), the soil was drained and kept saturated to minimize rice lodging and easy-to-harvest grain. Rice plants flowered and headed during 45-60 DAS.

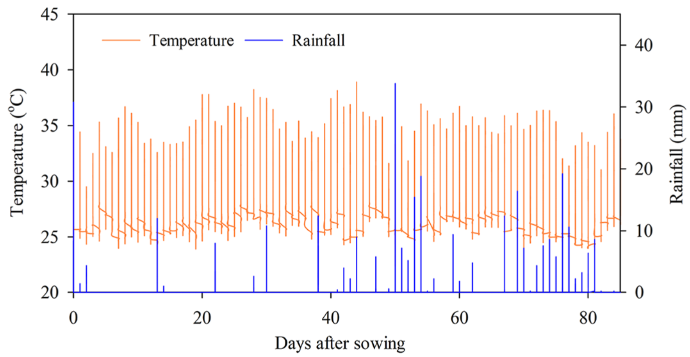

Figure 2. Temperature and rainfall during the field experiment.

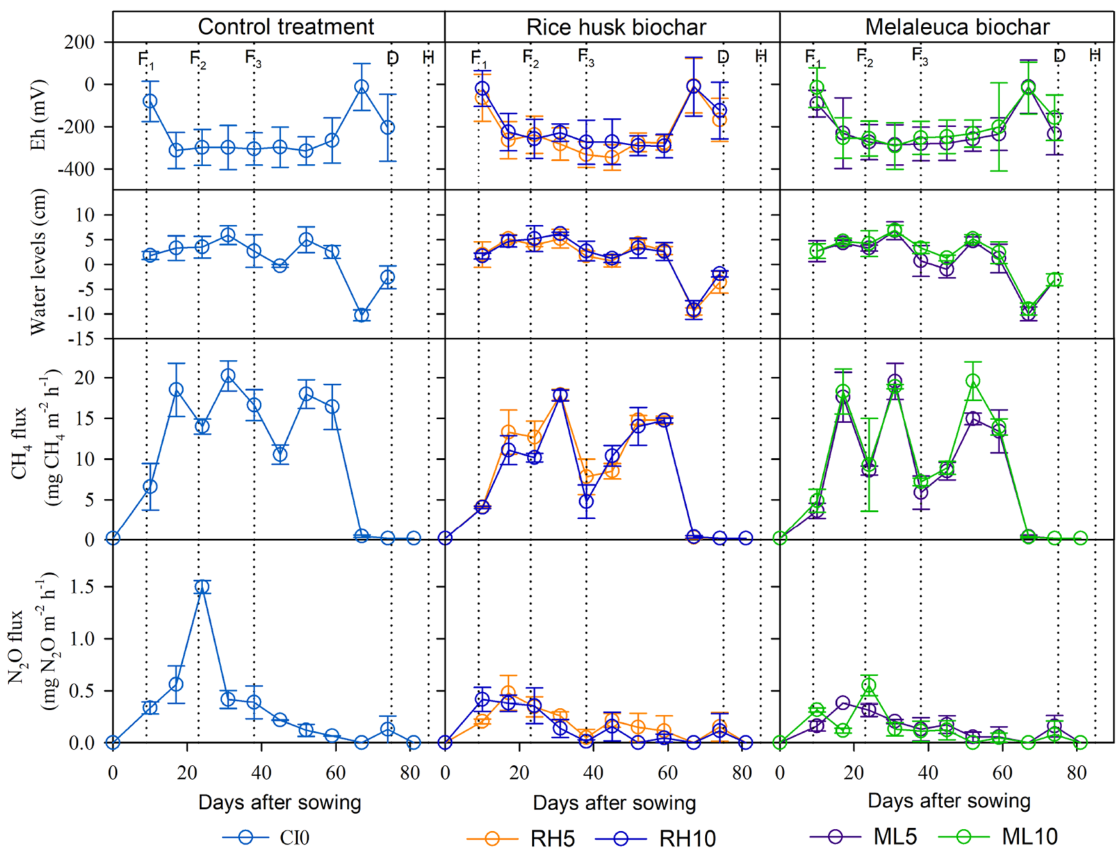

Figure 3. Time course changes in soil redox potential (Eh), water level, hourly CH_4 and N_2O fluxes in the paddy field applied without (left) or with RhB (center) or MB (right) during the field experiment. Error bars indicate the standard error ($n = 3$). Vertical dotted lines illustrate agronomic management of the first, the second and the third topdressing fertilizer (F_1, F_2 and F_3, respectively), drainage (D) and harvest (H).

CH_4 and N_2O emissions

CH_4 emissions gradually increased in the early rice growth stage (0-17 DAS) and almost stopped after drainage (70 DAS) (Figure 3). It should be noted that CH_4 flux was predominant in the period from 17 – 59 DAS and several CH_4 flux peaks were observed between treatments (i.e., three peaks were observed in MB5 and MB10). Maximum CH_4 flux peaks were reached simultaneously in all treatments after 31 DAS. Highest peaks between treatments are represented in a descending way as follows: CT0 > MB5 > MB10 > RhB5 > RhB10. Compared to the CT0 treatment, biochar application reduced total CH_4 emissions significantly (Table 3). Particularly, RhB5 and RhB10 mitigated total CH_4 flux from 24.2 to 28.0%, respectively, while MB5 and MB10 alleviated between 22.0 and 14.1%, respectively. Irrespective of RhB and MB, the CH_4 flux was insignificant with an increasing biochar addition rate from 5 to 10 t ha^{-1} ($P < 0.01$). There was a negative linear regression relationship between biochar application rate and total CH_4 emission ($P < 0.001$, $r^2 = 0.825$) (Figure 4).

Table 3. Grain, total CH_4 and N_2O fluxes, global warming potential (GWP) and yield-scaled GWP at 100 years scale in the paddy field applied without or with biochar[1)].

Treatment[2)]	Grain (g m^{-2})	CH_4 (g CH_4 m^{-2})	N_2O (g N_2O m^{-2})	GWP (g CO_2-eq m^{-2})	Yield-scaled GWP (g CO_2-eq m^{-2})
CT0	498 ± 47.6	18.6 ± 0.80^{aA}	0.39 ± 0.07^{aA}	749 ± 13.5^{aA}	1.51 ± 0.13^{aA}
RhB5	513 ± 56.2	14.1 ± 0.23^{b}	0.29 ± 0.07^{ab}	566 ± 25.5^{b}	1.12 ± 0.18^{b}
RhB10	510 ± 33.0	13.4 ± 0.30^{b}	0.23 ± 0.04^{b}	524 ± 2.76^{c}	1.03 ± 0.06^{c}
MB5	519 ± 9.86	14.5 ± 1.00^{B}	0.24 ± 0.01^{B}	563 ± 31.6^{B}	1.09 ± 0.08^{B}
MB10	517 ± 10.9	15.9 ± 0.90^{B}	0.17 ± 0.07^{B}	591 ± 10.8^{B}	1.14 ± 0.44^{B}
P value[3)]					
CT × RhB	†	***	*	***	**
CT × MB	†	**	**	***	**

[1)]Data represent as means ± SD (n = 3).
[2)]CT0, control treatment; RhB5 and RhB10, 5 and 10 t ha^{-1} rice-husk biochar amendment, respectively; MB5 and MB10, 5 and 10 t ha^{-1} melaleuca biochar amendment, respectively.
[3)]Statistical analysis did not compare between RhB and MB. The letters indicate significant difference according to Duncan's multiple range test (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$ and †$P > 0.05$). Normal and capital lowercases indicate a significant difference between CT0 vs. RhB and CT0 vs. MB, respectively.

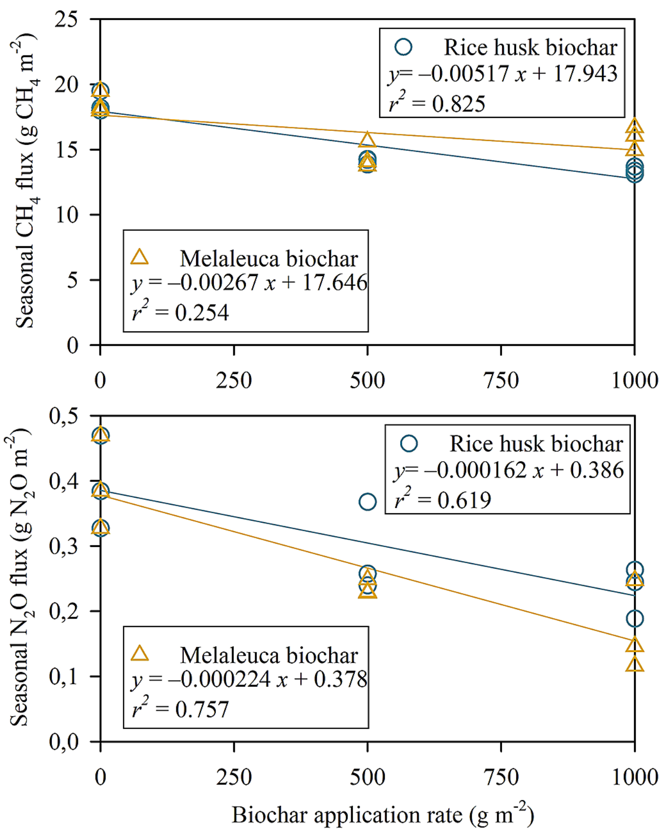

Figure 4. Relationship between biochar application rate and total CH_4 (above) and N_2O (below) fluxes during the field experiment. Each symbol represents one replication in each treatment.

In contrast, the linear regression of melaleuca biochar was poorly explained with increasing biochar amendment rate and total CH_4 flux (P = 0.095, r^2 = 0.254).

N_2O was released mainly in the early stage of rice growth in all treatments (Figure 3). The highest N_2O flux peaks were observed in the CT0 (24 DAS). All measured values were below 1.5 mg N_2O m^{-2} h^{-1}. As observed, N_2O flux flushed mainly during the fertilizing period from 9 to 38 DAS, even though experimental pots were predominantly flooded, especially in the CT0 accounted for 56.8% in total, while RhB and MB varied by 50.6-53.1% and 52.3-47.6%, respectively. Total N_2O emission was reduced in RhB or MB applied soil compared to CT0 (Table 3). Specifically, RhB10 significantly reduced by approximately 41.0%, whereas MB5 and MB10 by 38.5 and 56.4%, respectively. However, the reduction of total N_2O flux was insignificant in MB5. As a result, there were different negative linear relationships of biochar application rate and total N_2O flux (RhB, $P = 0.012$, $r^2 = 0.619$; MB, $P = 0.002$, $r^2 = 0.757$) (Figure 4).

Rice yield, GWP, and yield-scaled GWP

Biochar addition to the soil slightly increased rice yield compared to the CT0, but the statistical analysis was insignificant (Table 3). A similar pattern about emissions was seen among GWP, yield-scaled GWP, and total CH_4 flux due to CH_4 flux was greatest contribute to GWP, yield-scaled GWP. The RhB additions significantly decreased the GWP and yield-scaled GWP by 24.4 – 30.0% and 25.8 – 31.8% for RhB5 and RhB10, respectively. Although MB significantly diminished the GWP and yield-scaled GWP by 24.8 – 21.09% and 27.8 – 24.5%, respectively, there was no significant difference between MB5 and MB10.

Soil characteristics

A similar performance pattern of soil Eh condition was seen among treatments (Figure 3). Eh reduced after initial irrigation and was seen to reach a stable level (below -250 mV) during the rice growth period from 17 to 66 DAS. Whereas the final drainage rapidly increased the soil Eh condition (73 DAS) in all treatments. The supplementation of RhB and MB obviously improved soil Eh condition compared to the CT0 by 7.44 – 14.5% and 10.7 – 19.0%, respectively (Table 4). There was a negative linear relationship between hourly CH_4 flux and the Eh values in RhB ($P < 0.001$; $r^2 = 0.552$) and MB ($P < 0.001$; $r^2 = 0.502$) (Figure 5).

Table 4 represents the soil characteristic differences between treatments at the time of harvest. Overall, although biochar amendment was seen to increase soil pH slightly, statistical analysis implied no significant difference between treatments. Yet, biochar amendment significantly reduced the soil bulk density (RhB5, 19%; RhB10, 23%; MB5, 22.7% and MB10 26.8%) and ameliorated the soil porosity (RhB5, 8.2%; RhB10, 11.8%; MB5, 2.2%, and MB10 9.6%). However, increasing RhB and MB biochar application rate from 5 to 10 t ha^{-1} did not significantly change soil bulk density and porosity. Moreover, intensifying biochar incorporation significantly increased SOM by 38.6 – 52.7% for RhB and 25.4 – 45.9% for MB. Notably, AN in biochar-applied treatments was higher than that of the CT0 by 44.8 – 38.3% and 35.5 – 55.1% for RhB and MB, respectively. AP significantly increased in the MB treatments by 32.1 – 51.58% but did not in RhB. Although additional biochar increased the available and mineralized nutrients, statistical analysis results showed no significant difference between biochar application rates of 5 to 10 t ha^{-1} (Table 4) (Tran Sy *et al*., 2021).

Discussion

Effects of biochar incorporation on CH_4 and N_2O fluxes

Conventional practices without biochar application released 18.6 g CH_4 m^{-2} and 0.39 g N_2O m^{-2} (Table 3). These values are in accordance with previous findings conducted in the VMD (Vo *et al.* 2020; Minamikawa *et al.* 2021; Uno *et al.* 2021). Notably, RhB and MB amendments under typically local water management, and conventional practices significantly reduced CH_4 flux by 24.2 % in RhB5, 28.0 % in RhB10, 22.0 % in MB5, 14.1 % in MB10 and N_2O flux by 38.5 % in RhB5, 56.4 % in RhB10, 25.6 % in MB5, 41.0 % in MB10, and slightly improved rice yield (2.41-4.21%) (Table 3). Similarly, Yang *et al.* (2019) demonstrated that biochar additions (20 - 40 t ha^{-1}) under controlled irrigation in the Taihu Lake region, China mitigated both CH_4 and N_2O emissions by 35.7% and 21.5%, respectively, and simultaneously enhanced rice yield by 16.7-24.3%. Moreover, Wu *et al.* (2019b) reported that biochar additions (20 - 40 t ha^{-1}) significantly decreased CH_4 and N_2O fluxes by 11.2-17.5% and 19.5-26.3%, respectively, and increased grain yield by 7.9-9.2%. In line with our findings, a long-term biochar application (5 – 10 t ha^{-1}) in China's typical double rice plantation region also significantly decreased CH_4 flux by 26.18% (Qin *et al.* 2016). Nevertheless, Wang *et al.* (2011) reported that the biochar incorporation (50 ton ha^{-1}) into the soil significantly decreased N_2O flux by 41.4-93.5% in lab-scale experiments. In parallel, a meta-analysis based on 30 studies with 261 experimental treatments (lab-scale and pilot-scale) from 2007 to 2013 demonstrated that the addition of biochar reduced N_2O emissions by 54% (Cayuela *et al.* 2014). However, Koyama *et al.* (2015) reported that biochar application (10-40 t ha^{-1}) reduced CH_4 flux but did not N_2O. In the case of Liu *et al.* (2014), biochar supplementation (24-48 t ha^{-1}) significantly reduced CH_4 flux by 33.9-40.2%, while N_2O flux significantly increased by 150 to 190%. Overall, biochar amendment could reduce CH_4 flux from a rice paddy field, but in some cases, the effect on N_2O flux remains uncertain. Our study demonstrated that rice husk and melaleuca biochar applications with a range of 5-10 t ha^{-1} significantly reduced both CH_4 and N_2O fluxes within a Thionic Glycesol soil in the VMD. Albeit, the biochar application rate between 5 and 10 t ha^{-1} hardly obtained the disparity of CH_4 and N_2O emissions. Thus, a wide range of biochar application amounts should be evaluated to provide more tailored recommendations.

Table 4. Physiochemical properties[1] of soil applied without or with biochar.

Treatment[2]	pH	Eh[3] (mV)	Bulk density ($g\ cm^{-3}$)	Porosity (%)	SOM ($g\ kg^{-1}$)	AP ($mg\ kg^{-1}$)	AN ($mg\ kg^{-1}$)
CT0	4.69 ± 0.10	-242 ± 12.3^{cB}	0.97 ± 0.10^{aA}	53.3 ± 0.75^{bB}	31.8 ± 0.36^{cC}	19.0 ± 3.95^{B}	10.7 ± 1.03^{bB}
RhB5	4.81 ± 0.21	-224 ± 6.12^{b}	0.78 ± 0.04^{b}	61.5 ± 7.20^{ab}	43.2 ± 2.08^{b}	21.8 ± 3.48	15.5 ± 0.52^{a}
RhB10	5.26 ± 0.64	-207 ± 2.13^{a}	0.74 ± 0.13^{b}	65.1 ± 2.07^{a}	47.6 ± 1.13^{a}	25.2 ± 4.04	14.8 ± 0.60^{a}
MB5	4.82 ± 0.13	-216 ± 16.3^{A}	0.75 ± 0.06^{B}	55.5 ± 1.92^{A}	39.1 ± 3.03^{B}	25.1 ± 2.81^{AB}	14.5 ± 1.10^{AB}
MB10	4.68 ± 0.17	-196 ± 5.78^{A}	0.71 ± 0.05^{B}	62.9 ± 4.19^{A}	45.0 ± 1.30^{A}	28.8 ± 2.79^{A}	16.6 ± 2.97^{A}
P value[4]							
CT × RhB	†	*	*	*	***	†	***
CT × MB	†	*	***	*	***	*	*

[1]Data represent as means ± SD (n = 3).
[2]Abbreviations are the same as Table 3.
[3]Mean value is based on the whole values measured during the experimentation in each plot at 3 soil levels depth comprising 5, 10 and 20 cm between 10 and 64 DAS.
[4]Statistical analysis was carried out as the same as Table 3.

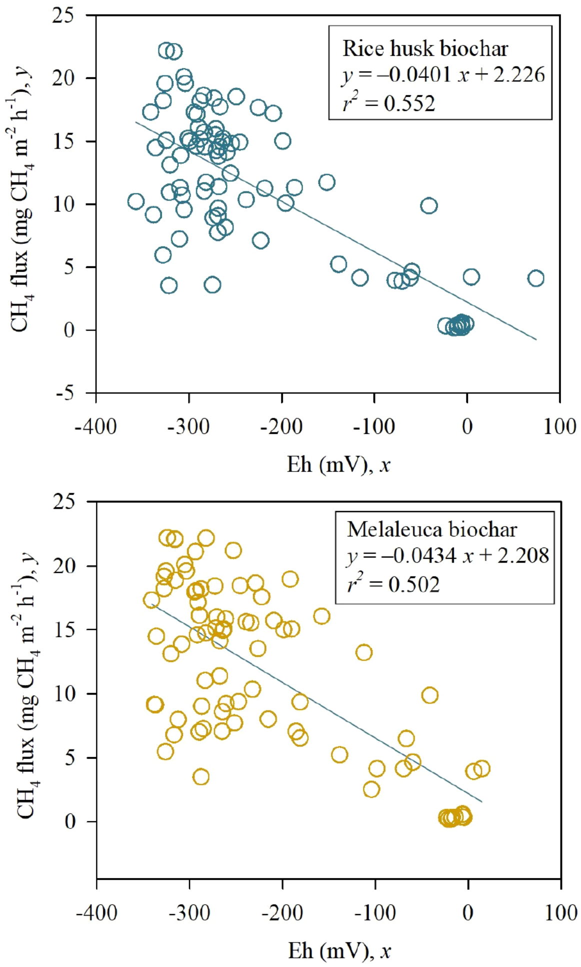

Figure 5. Relationship between the hourly CH_4 flux and Eh in the field applied with RhB (above) or MB (below).

The CH_4 mitigation by biochar application consistently pertains to the increasing soil oxidation rate and methanotrophs community. Although we did not determine the number of methanogens and methanotrophs, Nan *et al.* (2021) demonstrated that biochar application stimulates the abundance in either methanogens or methanotrophs, with a high amount of methanotrophs detected in most cases resulted in decreasing of CH_4 flux. Moreover, Wu *et al.* (2019a) reported that biochar applications to fertilized paddy field soils increased the total type I *pmo*A (preferred the CH_4 environment) and type II *pmo*A (more dynamic in low CH_4 conditions) methanotrophs compared to non-amended biochar, indicating that CH_4 flux mitigation by promoting potential CH_4 oxidation. Thus, we adopted a hypothesis that the balance of activities between methanogens and methanotrophs in a site-specific environment results in either an increasing or decreasing CH_4 flux. Feng *et al.* (2012) revealed the main mechanisms of CH_4 flux reduction in a biochar-supplemented field were by (1) increased methanotrophic proteobacterial abundance significantly and (2) decreased the methanogenic to methanotrophic proportion substantially. Thus, an increase of methanotrophs dynamic in paddy field soil by biochar addition can be expected to play a vital role in mitigating CH_4 fluxes. Our study demonstrated that rice husk and melaleuca biochar could promote low-GHG emissions in the rice production system in the VMD.

We achieved N_2O flux reduction by incorporating biochar into the topsoil layer when compared to the non-amended biochar field. However, several hypotheses supposed that soil applied with biochar could not decrease the N_2O flux (Koyama *et al.*, 2015; van Zwieten *et al.*, 2010). Similar to our field study, several findings achieved a total N_2O flux reduction (Shaukat *et al.*, 2019; Zhang *et al.*, 2010). The mitigation of N_2O flux in biochar-treated soils could be attributed to soil moisture contents and nitrification processes (Ameloot *et al.*, 2016). In agreement with the hypothesis, Shaukat *et al.* (2019) demonstrated that fields with biochar added retained 9-14% higher moisture contents than fields without biochar amended and resulted in a significant reduction of the N_2O flux. Supporting the idea, Wang *et al.* (2013) revealed the relationship between the denitrifying community and N_2O flux change, where biochar supplementation significantly shifted the abundance of NO_3-utilizing bacteria (carrying the *nir*K and *nir*S genes), leading to less N_2O generation and more N_2O-consuming bacteria (carrying the *nos*Z gene). Moreover, Cayuela *et al.* (2013) used ^{15}N gas-flux to observe the reduction of $N_2O/(N_2+N_2O)$ and demonstrated that biochar facilitated the last step of denitrification. The key mechanisms of N_2O flux reduction under biochar amendment were by (i) stimulated nitrification generation via electron donation, a decrease in total denitrification by serving as an alternative electron acceptor by acting as electron shuttle to soil NO_3^- consuming microorganisms (Cayuela *et al.*, 2013), and (ii) based on the entrapment of N_2O in water-saturated soil pores and co-occurrent stimulation of microbial N_2O reduction deriving in an overall decrease of the $N_2O/(N_2O + N_2)$ ratio (Harter *et al.*, 2016). Therefore, biochar could be attributed as a decisive factor to inhibit N_2O production and simultaneously stimulate N_2O utilization. As such, these findings and the above-discussed mechanisms strongly support our findings in suggesting N2O flux reduction from biochar amendment in the rice paddy field.

Our study showed that N_2O emission was mainly concentrated during fertilization, which indicates fertilization provides more available N driving for soil N_2O emission. Xie *et al.* (2013) observed ^{15}N abundance significantly intensified by the application of ^{15}N-enriched urea. Our study did not measure NH_4^+ or NO_3^- concentration during fertilizing, so the mechanism remains uncertain. N_2O emission via the nitrification process directly pertains to soil physical, chemical, and biological properties (Huang *et al.*, 2019). Thus, we speculate that N fertilizing increased the nitrification activities and stimulated the strong metabolism of potential N_2O-producing bacteria. Minamikawa *et al.* (2021) reported that higher N availability levels in soil than rice plant uptake demands resulted in increasing N_2O emissions. Although N-fertilizing obviously promoted N_2O emissions for the majority of the time, N_2O emission peaks of biochar-amended soil were lower than that of biochar-unamended soil. This would indicate that biochar potentially changed the functionality and diversity of denitrifiers within the soils and inhibited the conversion of NO_2^- and NO_3^- to N_2O (Zhang *et al.*, 2010).

Water management is a crucial factor in the strategy of GHGs reduction, although we achieved the GHGs reduction under typical water management when most of the time the soil was flooded. Multiple-flooded times in this study were due to the combination of high rainfall in the transition season (rainfall, Figure 2; water level, Figure 3) and the typical flooding water management practice of the farmers in the region. Uno *et al*. (2021) conducted a 2-year field experiment in An Giang province in the VMD and demonstrated that AWD (known as multiple drainages) significantly reduced CH_4 by 35%, while found no difference in N_2O emissions, but a 22% yield improved. Moreover, Minamikawa *et al.* (2021) registered that the intermittent irrigation technique is also a promising approach to mitigate CH_4 emissions by reductive soil conditions. Thus, integrating AWD and intermittent irrigation by incorporating biochar into the soil under the MD's edaphology, climate, and traditional practices could be feasible for further works.

Relationship between biochar amendment ratios and CH_4 and N_2O fluxes

There is a negative correlation between CH_4 flux and RhB application rate ($P < 0.001$, $r^2 = 0.825$) (Figure 4). It is indicated that CH_4 flux decreased with the increase of rice-husk biochar application (Xiao *et al.* 2018). On the other hand, although increasing MB application rate could mitigate the CH_4 emission, the relationship found a poor explanation ($P = 0.095$, $r^2 = 0.254$). This contrast could be partly attributed to biochar-carbonized properties. MB was low in the specific surface area and total pore volume compared to RhB (Table 1). Ji *et al.* (2020) revealed that biochar structure intimately related to anaerobic CH_4 oxidation and created a suitable environment for CH_4-consuming bacteria.

Similarly, we found a negative correlation between the N_2O flux reductions and the application rate of RhB ($P = 0.012$, $r^2 = 0.619$) and MB ($P = 0.002$, $r^2 = 0.757$). In agreement with our finding, a meta-analysis of Cayuela *et al.* (2014) showed a negative relationship between biochar application rates and reduced N_2O flux, where sufficient N_2O reduction was 1-2% biochar amendments, whereas, incorporating more than 10% of biochar into the soil was found to reach up to 80%. In line with our study, Huang *et al.* (2019) also showed a negative relationship between biochar application rates and N_2O flux. Overall, the increase of biochar application rates could potentially stimulate the CH_4 and N_2O reduction. However, for CH_4 and N_2O fluxes, the application of 5 and 10 t ha^{-1} remains unclear.

Effect of biochar incorporation on Soil Eh and CH_4 emission

Our study found that the negative linear relationship between soil Eh and hourly CH_4 flux with RhB ($P < 0.001$; $r^2 = 0.552$) and MB ($P < 0.001$; $r^2 = 0.502$) (Figure 5). Similar results were also observed by Wang *et al*. (2018). This indicates that an increase of soil redox potential decreased CH_4 emission, which is in line with the report by Towprayoon (2020). Moreover, soil Eh remained below −250 from 17 to 66 DAS in our study (Figure 4), implying a favorable condition for CH_4 emission (Wang *et al.* 1993). Final drainage rapidly increased soil Eh and reduced CH_4 flux (Figure 3), indicating the strong sensibility of soil Eh and CH_4 flux under water management.

Biochar application increased soil Eh compared to non-amended soils (Table 4). This indicates that biochar was the critical factor contributing to the positive effects of anaerobic CH_4 oxidation activities known as the electronic accepting capacities (EAC) of biochar (Nan *et al.*, 2021). The supplementation of biochar intensifies oxygen-containing functional groups (carboxyl, carbonyl, quinone phenolic hydroxyl group) and positively improves biochar redox potential (Klüpfel *et al.* 2014; Wu *et al.* 2016). The increase of Eh and the reduction of CH_4 emissions could also be explained by the porosity and absorbability characteristics of biochar, which enable robust CH_4-utilizing bacteria activities and intensify the diffusion and metabolism process. In a similar way, biochar incorporation into soils improves soil aeration, creating a favorable environment for methanotrophic bacteria resulting in soil Eh amelioration and better reduction of CH_4 oxidation (Feng *et al.*, 2012).

Effects of biochar incorporation on grain yield, GWP and Yield-scaled GWP

Although biochar amendments could improve yield (2.41-4.21%) (Table 3), multiple comparison analyses found no significant difference between amended and unamended soils. Several studies have found similar results (Qin *et al.* 2016; Nguyen *et al.* 2016). The undistinctive grain yield could be partly attributed to spatial and temporal variations, i.e., climatic conditions, field practices, soil substrates (Xie *et al.* 2013).

Biochar-amended soil significantly decreased GWP by 21.1-30.0% and yield-scaled GWP by 24.5% - 31.8% (Table 3). It was indicated that RhB and MB application potentially mitigates total CH_4 and N_2O emissions without scarifying grain yield. Yang *et al.* (2019) performed a double-season field experiment on biochar applications ranging from 20 to 40 t ha^{-1} and found that the average GWP and yield-scaled GWP reduced by 18.7% - 16.4%, and 80.3% - 41.6%, respectively. Similarly, Zhang *et al.* (2019) observed a six-year field experiment on biochar-applied soils at rates of between 20-40 t ha^{-1} and showed a GWP and yield-scaled GWP reduction by 12.1-18.4% and 35.9-56.4%, respectively. Here we observed that CH_4 flux was the key contributor in the GWP and yield-scaled GWP via the field experiment in the VMD's transition season, while N_2O flux was more neglectable. Thus, future works should emphasize on reducing the GWP, yield-scaled GWP, and concentrate on the CH_4 mitigation technology solutions rather than N_2O emissions.

Effects of biochar incorporation on soil improvement

Soil improvement under short-term and long-term biochar applications has been widely recognized. Our study showed that biochar amendment insignificantly increased soil pH (Table 4), which indicated no effect of biochar addition on soil pH perfection as suggested by previous studies (Yang *et al.*, 2019). However, biochar amendment significantly decreased the soil bulk density and improved soil porosity in comparison to non-amended soils. Furthermore, higher applied biochar rates showed lesser soil bulk density and higher porosity indicating that biochar directly upgraded soil physiology. Amelioration of soil surface area and porosity by biochar amendment intensifies soil aeration and functions of aeration, such as CH_4 oxidation, and provides habitat for methanotrophs (Nan *et al.*, 2021). Moreover, it stimulates NH_4^+ absorbance ability resulting in suppressing nitrification processes and N_2O flux reduction in the field (Wang *et al.*, 2020).

It is evident that increasing biochar application boosted SOM and AN, with a slightly increased available P through the season (Table 4). The increase of SOM and AN showed a high nutrient availability in the soil. Notably, the soil improvement did not increase soil CH_4 and N_2O emissions as above-mentioned and discussed. AN could be used as a soil health indicator (García *et al.*, 2020). The interdependence among AN, SOC, and particulate OC was demonstrated by a positive correlation (Domínguez *et al.*, 2016). In connection with our study, Yang *et al.* (2019) observed that biochar amendment slightly increased SOC, significantly increased NH_4^+ by 47.7%, and significantly decreased NO_3^- by 30.4%. Incorporating biochar into soils could inhibit nitrification and produce more NH_4^+ than NO_3^- consisting of an anoxic environment (water level and redox potential; Figure 2). Increasing NH_4^+ concentrations and declining NO_3^- concentrations would partly explain the enhanced CH_4-consuming figure and N_2O oxidation (Xiao *et al.*, 2018). Overall, biochar application offers benefits not only for nutrient availability but also for GHGs mitigation.

Conclusions

This study assessed the effects of rice husk biochar or melaleuca biochar amendment at 5 or 10 t ha^{-1} on CH_4 and N_2O emissions and the physiochemical soil properties after rice cultivation under typical water management and conventional practice regime in the VMD. Incorporating biochar into soils significantly mitigated CH_4 and N_2O emissions without reducing grain yield. Consequently, a lower GWP and yield-scaled GWP from biochar-amended soils were achieved. Although higher biochar applications decreased CH_4 and N_2O emissions, there was no significant difference between biochar-amended rates. Biochar significantly increased soil Eh conditions. There was a negative linear relationship between soil Eh and CH_4 emission rate for biochar-applied fields. N_2O emissions from biochar fields were relatively low and mostly concentrated during the fertilization period. Biochar amendments improved soil fertility via physical properties of soils by decreasing bulk density and intensifying porosity and the chemical characteristics of the soils by ameliorating SOM, AN and AP, but did not affect soil pH. Similar to GHG emissions, biochar application rates of between 5 and 10 t ha^{-1} could not obtain significant soil improvement. This study will help lower-GHG emissions from rice farming practices in the VMD. Further works should study the combination of biochar-application rates and effective water irrigation techniques on different soils in the VMD.

Data availability

Underlying data

Figshare: Biochar reduces GHGs from paddy fields. https://doi.org/10.6084/m9.figshare.16625137.v1 (Tran Sy *et al.*, 2021).

This project contains the following underlying data:

- Nam et al_Raw data biochar_F1000research.xlsx

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Current Peer Review Status: ✔ ✔

Version 2

Reviewer Report 25 February 2022

https://doi.org/10.5256/f1000research.121175.r123613

✔ **Azeem Tariq**
Department of Plant and Environmental Sciences, University of Copenhagen, Copenhagen, Denmark

No further comments.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Climate change mitigation, GHG emissions, sustainable crop rotations, ecosystem modeling

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 21 February 2022

https://doi.org/10.5256/f1000research.121175.r123614

✔ **Bui Truong Tho**
The Centre of Hi-tech Application in Agriculture, Chau Thanh district, Vietnam

The authors have revised the article and it is better than previous one. I totally agree with the new version.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Hi-tech Application in Agriculture, Climate change mitigation, GHG emissions, Sustainable crop rotations, Plant ecophysiology.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 08 February 2022

https://doi.org/10.5256/f1000research.77750.r120157

Bui Truong Tho
The Centre of Hi-tech Application in Agriculture, Chau Thanh district, Vietnam

This article, "Rice husk and Melaleuca biochar additions reduce soil CH_4 and N_2O emissions and increase soil organic matter and nutrient availability" aims to evaluate the impacts of two biochar amendments on soil CH_4 and N_2O emissions and its effect on soil physiochemical properties in Mekong Delta based on 80 days of field study.

The topic is interesting and poorly treated in literature. The data are correctly processed and the results are well discussed. However, there are some minor issues and my reviews are focused on improving a revision of the current article, and I hope the authors can accommodate my suggestions.

1. Site description: "air temperature (27.5 – 27.5 °C)" I think there is a mistake, authors should double-check the information.

2. Statistical analysis: Authors should confirm that all data were test homogeneity of variance for using One-Way analysis of variance (ANOVA).

3. Authors used "Vietnamese Mekong Delta (VMD)", some places used "Mekong Delta (MD)", it should unified in all the manuscript.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Hi-tech Application in Agriculture, Climate change mitigation, GHG emissions, Sustainable crop rotations, Plant ecophysiology.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 09 Feb 2022

Thao Huynh Van, College of Environment and Natural resources, 3/2 street, Can Tho University, Can Tho city, Vietnam

Thank you very much for your constructive comments. All your comments have been carefully read and revised by the authors. Our responses are enclosed as follows:

Comment 1#. Site description: “air temperature (27.5 – 27.5 °C)” I think there is a mistake, authors should double-check the information.
Reply 1: Thank you for finding this mistake. We re-referred to the original document and corrected the information in the manuscript. The air temperature should be 27.5^{o}C instead.

Comment 2#: Statistical analysis: Authors should confirm that all data were test homogeneity of variance for using One-Way analysis of variance (ANOVA).
Reply 2: We amended additional information related to the homogeneity of variance in the statistical analysis. The revised is as follows “Significant different comparison among treatments was considered at Duncan’s multiple range test (***P < 0.001, **P < 0.01, *P < 0.05 and †P > 0.05) after passing homogeneity of variance”.

Comment 3: Authors used “Vietnamese Mekong Delta (VMD)”, some places used “Mekong Delta (MD)”, it should be unified in all the manuscript.
Reply 3: We have unified the abbreviation in all parts of the manuscript which is “VMD”.

Best regards,
Authors.

Competing Interests: No competing interests were disclosed.

Reviewer Report 04 January 2022

https://doi.org/10.5256/f1000research.77750.r99426

Azeem Tariq
Department of Plant and Environmental Sciences, University of Copenhagen, Copenhagen, Denmark

The authors of "Rice husk and melaleuca biochar additions reduce soil CH_4 and N_2O emissions and increase soil organic matter and nutrient availability" present an effort in assessing the effects of two biochar amendments on soil CH_4 and N_2O emissions and effects on soil physiochemical properties based on 80 days of field study. The overall paper has been written in a good English and in a logical manner. However, there are some minor issues that authors need to deal before considering this paper for indexing.

Authors used the term “soil organic matter and nutrient availability” in title, but they have focused on different soil properties e.g. soil porosity, soil bulk density, soil redox potential, soil organic matter and nitrogen. Authors should use the term soil physiochemical properties instead.

Authors did not explain the results related to soil properties in the abstract section (e.g. there is no explanation about soil organic matter and nitrogen contents in abstract).

Authors stated that they used 95 % confidence level, but the result section predicted that authors have used different levels of probability for difference analysis. Author should explain the analysis carefully in the statistical analysis section in Material and Methods.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Climate change mitigation, GHG emissions, sustainable crop rotations, ecosystem modeling

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 09 Feb 2022

Thao Huynh Van, College of Environment and Natural resources, 3/2 street, Can Tho University, Can Tho city, Vietnam

Thank you very much for giving us constructive comments that helped us improve this paper immensely. All your comments have been carefully read and revised to elucidate underlying aspects. Our responses to your comments are as follows:

Comment 1: Authors used the term "soil organic matter and nutrient availability" in title, but they have focused on different soil properties e.g. soil porosity, soil bulk density, soil redox potential, soil organic matter and nitrogen. Authors should use the term soil physiochemical properties instead.
Reply 1#: Thank you very much for the recommendation for the title term of "soil physiochemical properties". The recommendation has been adopted. The revision is as follows "Rice husk and melaleuca biochar additions reduce soil CH_4 and N_2O emissions and increase soil physicochemical properties."

Comment 2: Authors did not explain the results related to soil properties in the abstract section (e.g. there is no explanation about soil organic matter and nitrogen contents in abstract).
Reply 2#: We revised the abstract with additional information related to the explanation and implication for improving soil physiochemical characteristics. The revision is as follows "The results showed…Ameliorating soil aeration and functions by adding RhB and MB resulted in improving soil physicochemical characteristics, especially significant SOM and AN boosting, which indicate better soil health, structure, and fertility".

Comment 3: Authors stated that they used 95% confidence level, but the result section predicted that authors have used different levels of probability for difference analysis. Author should explain the analysis carefully in the statistical analysis section in Material and Methods.
Reply 3#: We added more information to the statistical level to make it clearer to readers. The supplementations are as follows "The results are…Significant different comparison among treatments was considered at Duncan's multiple range test (***P < 0.001, **P < 0.01, *P < 0.05 and †P > 0.05)"

We look forward to hearing from you.
Best regards,
Authors.

Competing Interests: No competing interests were disclosed.