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Research Paper

Effect of biochar and its combined application with manure and fertilizer on nitrogen leaching, greenhouse gas (GHG) emissions, and grain yield under alternate wetting and drying (AWD) system

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Abstract. The application of biochar to the adoption of alternate wetting and drying (AWD) system is crucial to mitigate greenhouse gas (GHG) emissions, soil nutrient retention, and maximize crop productivities in rice cultivation. In this study, the lysimeter experiment was carried out to determine the ability of biochar and its co-application with manure or fertilizer on nitrogen (N) leaching, GHG emissions, and grain yield under the AWD system. The results showed that biochar application alone could not reduce the total NH_4^+ –N and NO_3^- –N losses as compared to control. N loss varied depending on the N input and peaked mainly in the combined treatment soil especially after fertilization and re-watering of the AWD system. However, the total CH_4 emissions were reduced by 20.92 to 25.75% in soils amended with biochar alone while N_2O emissions were sharply reduced by 6.84 to 13.46%, respectively, compared to control. Both CH_4 and N_2O peaked in the following day after fertilizer, while CH_4 reduced during AWD and N_2O increased. Biochar co-application was increased by N leaching loss with higher emissions due to high N input to the system. The results show that biochar application alone significantly ($p < 0.05$) increased rice yields by 9.49 to 14.10% compared to treatment without it. In addition, the co-application with both manure and fertilizer were increased grain yield but nevertheless high GHG emission. When compared biochar combination between manure and fertilizer, we found it was beneficial in terms of yield production with lower emissions in biochar with manure than with fertilizer.

Keywords: Biochar application, alternate wetting and drying, leaching, climate change, productivity.

INTRODUCTION

Rice is the most rapidly growing food source of the world which is consumed and cultivated in many countries especially in Asia. Due to the rapid population growth and economic development increased every year, however, it has been posing a growing pressure for an increase in food production, especially in rice production (Ma and Yuan 2015). Nitrogen (N) fertilizer is commonly used in rice cultivation and needs to improve productivity to meet the requirements. However, the excessive application of fertilizer in rice paddy field is the major anthropogenic source of atmospheric methane (CH_4) production and emission (de Miranda *et al*., 2015). Nitrous oxide (N_2O) is another GHGs contribution from the rice field associated with paddy water and N status (Skinner *et al*., 2014). The future for rice cultivation may hold many challenges, including the continued efforts to optimize resource used, reduce N leaching loss and mitigate GHG emissions. This needs to be set against the urgent and growing need to improve productivity to meet the sustainable rice cultivation approach.

An alternative method to increase N retention and reduce nitrogen leaching loss from paddy soil is the application of biochar into the agricultural soil. Biochar is a solid carbon-rich organic material generated by pyrolysis or gasification of biomass residues in the absence of oxygen at a relatively low pyrolusis temperature. Biochar application to agricultural soils has the potential to slow carbon and N release (Pereira *et al*., 2015) with the high content stable organic carbon in the biochar to change the soil properties. Recent studies have emphasized the application of biochar to the soil as a potential technique to increase nutrient bioavailability and decrease N leaching lose (Xu *et al.*, 2016). The recent result also indicated that biochar amendment plays a significant role in increasing crop yield. Moreover, biochar application to soils is currently being considered as a means of mitigating climate change by sequestering C into the soil, while concurrently improving soil properties and functions (Cayuela *et al*., 2014). Some mechanisms have been proposed to explain the apparent retention of N in biochar-amended soils and the reduction of N leaching. Biochar amendment effects on chemical and physical properties of soil by positive effects on pH leading to the direct absorption of ammonium-nitrogen (NH_4^+ –N) and nitrate-nitrogen (NO_3^- –N) (Novak *et al*., 2009). Additionally, biochar properties increase the capacity of cation exchange capacity (CEC) and enhance water holding capacity (WHC) of the soil (Ding *et al*., 2010; Zheng *et al*., 2013).

Many early studies have stated that effective water management practices reduce GHG emissions especially CH_4 and reduce water use in rice production is the alternate wetting and drying (AWD) system (Liang *et al*., 2016). Standard AWD required to follow strict standards during the rice growing period, whenever the water level naturally declines to 15 cm below the soil surface, the paddy fields need to water up to 5 cm (Humphreys *et al*., 2010). Sanchis *et al*. (2012) reported a positive effect of AWD on reducing CH_4 emission, however, there was an offset by the increase in N_2O emissions. The sporadic aerobic and anaerobic conditions of topsoil carry out by AWD can modify the pattern of N_2O lead to an increase in gas quantity (Hoang *et al*., 2019). Nitrogen in the soil can possibly cause increase especially NO_3^- -N in water leachate due to the AWD system (Tan *et al*., 2013).

Recent studies have indicated that combined applications of biochar with organic or inorganic fertilizers could lead to enhanced soil physical, chemical, and biological properties, as well as plant growth. Organic amendments, such as manure, therefore, are useful tools to sustainably maintain or increase soil organic matter, preserving and improving soil fertility and crop yield. Thus, biochar combined application with manure and with fertilizer is considered to be necessary to promote higher productivity. Therefore, the aim of this study is to investigate the effect of biochar and its combined application with manure and with fertilizer on N leaching, soil GHG emissions, and grain yield under the AWD system during two consecutive rice cultivation seasons. Rice growth characteristics, water use, and biomass were also evaluated.

MATERIALS AND METHODS

Site and amendment description

The experimental area was located at King Mongkut’s University of Technology Thonburi (KMUTT), Bangkunthien campus (13° 34' 33.87" N and 100° 26' 34.45" E) in southern Bangkok during the wet season (2018) and dry season (2019). Rice was cultivated under the control of greenhouse facilities. The soil tested a paddy field at KMUTT Ratchaburi Campus (13˚ 35' 10" N and 99˚ 30' 21" E) in Ratchaburi Province, with clay loam soil texture, was used in this study. Biochar applied in this study was acidic because due to the use of local plant with slow pyrolysis under low pyrolysis temperature. The physical and chemical properties of the initial soil, biochar, and manure used in this study are shown in Table 1. The local climate during the study was recorded throughout the experimental period (September 2018 to June 2019). The monthly air temperatures for both crops are shown in Figure 2a and b.

Treatment design and crop management

This study was focused on two continuous cropping seasons which are the first crop (September to December 2018) and the second crop (February to June 2019) seasons. The experiment was conducted in a draining lysimeter which was designed using a plastic tank. The size of the lysimeter tank was 100 cm length and weight with 50 cm of its height. The lysimeter was separately installed in each treatment following complete randomized design. Each lysimeter contained its PVC holes at the basal plate area to monitor the soil nutrient leaching. In the upper case of the filled soil lysimeter where the close chamber measurement take place, a stainless steel square base was permanently installed as the base of close flux chamber gas measurement. The schematic diagram of the lysimeter is described in Figure 1. This study was adopted the AWD system refer to Lampayan *et al*. (2015). The fields required to flood up to 5 cm water height over the soil surface by water pump whenever the water level was decreased to 15 cm below the soil surface which measured by the PVC water tube

Table 1. Basic soil, biochar, and manure properties used in this study.

Parameters	Initial soil	Biochar	Manure
% Sand	23	–	–
% Silt	29	–	–
% Clay	48	–	–
Texture	Clay Loam	–	–
pH	7.5	5.80	8.81
OM (%)	0.92	28.23	26.84
TN (%)	0.06	0.29	1.29
TC (%)	0.37	65.94	38.79
P (mg kg^{-1})	8.51	0.02	0.60
K (mg kg^{-1})	92.00	21.00	28.50
Ca (mg kg^{-1})	6146	13.00	8.01
Mg (mg kg^{-1})	277.00	13.1	0.21
EC (dS m^{-1})	0.85	1.79	*nd
CEC (cmol kg^{-1})	22.6	79.60	*nd
Bulk density (g cm^{-3})	1.65	*nd	*nd

*nd referred to no data available.

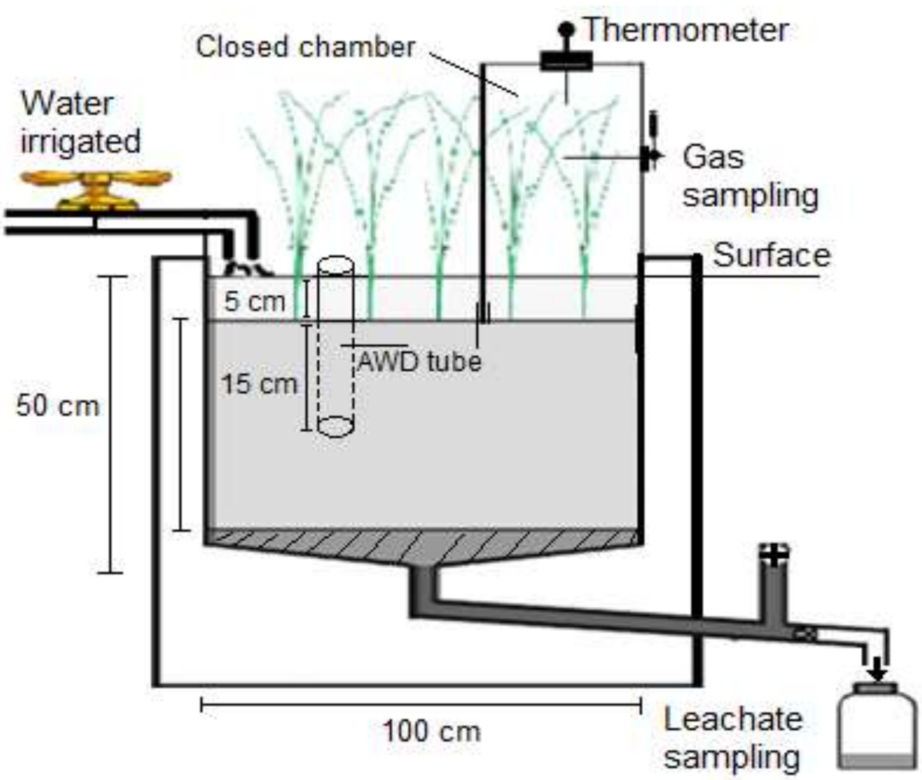

Figure 1. Schematic of free drainage lysimeter bucket with closed chamber method.

to adjust and control the water level. The experiment comprised of five treatments, including control (C), biochar (B), biochar with manure (B+M), biochar with fertilizer (B+F), and biochar with manure and fertilizer (B+M+F). Biochar was produced from a mangrove tree at a lower temperature with low oxygen by a local pyrolysis plant in Thailand. It was crushed to a smaller size by 1 to 3 mm before being applied to the soil at one time per year at rate 10 t ha^{-1} at 20 days before transplanting (DBT). Organic manure was applied one time per crop at 2.5 t ha^{-1} at 20 days after transplanting (DAT). Chemical fertilizer was applied two times, N-P_2O_5-K_2O (15-15-15)

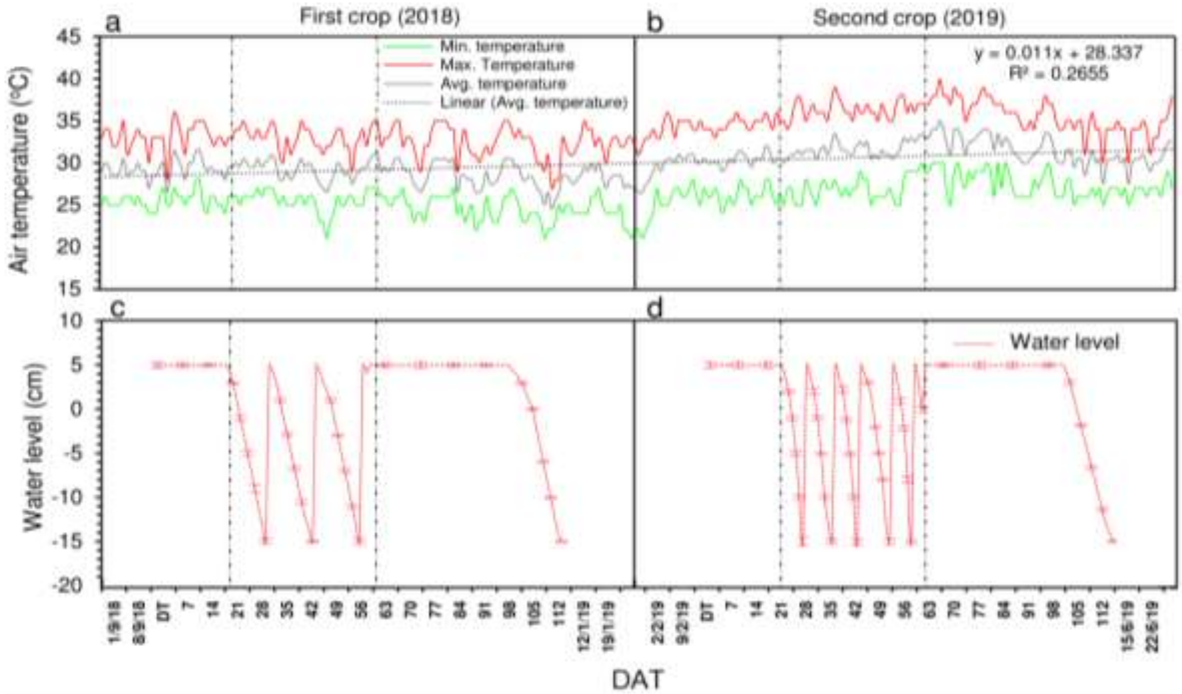

Figure 2. Air temperature (a and b) and water level (c and d) from different treatments throughout two rice cultivation period.

Table 2. Rice cultivation management for both crops under AWD system.

Activities	Wet season (DAT)	Dry season (DAT)
Experimental site preparation	11/08/18	26/01/19
Top soil mixing crop residue	27/08/18	02/02/19
Incorporation biochar	28/08/18	–
Incorporation manure	28/08/18	02/02/19
Cultivar Seedling in trey	29/08/18	03/02/19
Rice transplanting	15/09/18	16/02/19
Basal fertilizer application	05/10/18 (20)	09/03/19 (20)
Top dressing fertilizer application	15/11/18 (60)	20/04/19 (60)
Early vegetation (tillering)	29/09/18 (14)	02/02/19 (14)
Early production (panicle initial)	18/11/18 (63)	23/04/19 (63)
Early flowering (start flowering)	08/12/18 (84)	10/05/19 (83)
100% flowering (complete flowering)	18/12/18 (94)	20/05/19 (93)
100% grain maturing (dry field)	29/12/19 (105)	01/06/19 (105)
Harvesting	06/01/19 (113)	08/06/19 (112)

as a basal fertilizer was applied with a rate of 33 kg N ha^{-1} at 20 DAT and urea (46-0-0) fertilizer as top dressing fertilizer was applied with a rate of 57 kg N ha^{-1} at 60 DAT under the same water management for both crops (Table 2).

Leachate sampling and analysis

Leachates were collected from each lysimeter in a plastic container, which was connected through a PVC tube to the drainage outlet at the basal plate of each lysimeter. The content of nitrogen loss through water leaching was collected at a week interval started as a week after transplanting until two weeks before harvesting. The samples were stored at 4°C before analysis and measured using a 50 ml graduated cylinder. The concentration of NO_3^- –N and NH_4^+ –N in the leachate samples were analysed using a Multi-parameter photometer with COD (HI83399) instrument. NO_3^- –N concentration was analysed using the adaptation of the cadmium reduction method in which the reaction between nitrate and the reagent causes an amber tint in the sample (Gapper *et al*., 2004). NH_4^+ –N concentration was analyzed using Nessler Method (Jeong *et al*., 2013). The intensity of the colour was determined by a compatible photometer and the concentration based upon the meter was presented in mg L^{-1} (ppm) of NO_3^- –N and NH_4^+ –N in leachate. The total NO_3^- –N and NH_4^+ –N leaching loss was calculated by multiplying the N concentration by the leachate volume.

Gas sampling and analysis

The closed chamber method recommended by Minamikawa *et al*. (2015) was used to monitor GHGs emissions from rice cultivation starting at two weeks before transplanting until 2 weeks after harvesting. The chamber consisted of two main parts. The first part was fixing foot called the stainless steel square base 30 cm (width) x 30 cm (Length) which was attached 5 cm above the soil surface and 10 cm below the soil surface throughout the growing season. The second part was a closed-top chamber made of acrylic consisting of three heights which were 50, 100 and 150 cm depending on plant height to be placed on a stainless steel base. The gas sampling collected once a week between 9.00 a.m. to 3.00 p.m. with sampling time 0, 5, 10 and 15 minutes using a 20 ml syringe and immediately moved to the evacuated vials. The inside chamber temperature was recorded during the sampling time. CH_4 concentration was analyzed using Shimadzu GC-2014, Japan with a flame ionization detector (FID) and N_2O was analyzed by Gas Chromatography (Shimadzu GC-14B, Japan) with an electron capture detector (ECD) and Porapak column by retention time about 6 mins. The net emission fluxes of CH_4 and N_2O were calculated using a linear fit to the gas concentration variation inside the chamber over the sampling time (Nishimura *et al*., 2008). The seasonal cumulative GHG emissions were assessed by linear interpolation and numerical integration between sampling times (Chidthaisong *et al*., 2018). The sum of 100-year global warming potential (GWP) of CH_4 and N_2O was calculated using factors of 34 for CH_4 and 298 for N_2O (Stocker *et al*., 2013).

Water use measurement

The water used in the present rice cultivation was from irrigation only due to the need to investigate the effects of standard AWD which were managed under the greenhouse. However, the greenhouse facilities were designed to allow sunlight penetration and free airflow into the greenhouse. Water footprint in tons of grain yield m^{-3} water was calculated by the number of grain yields divided by total water inputs. Water was irrigated using the water pump (VENZ VC-200, 2HP) and this recorded the volume of irrigated water used and amount of water measured by water meter (Sanwa, Model SV 15), size of ½ inches throughout the cultivation season. Water leaching in this study was collected once per week and this also recorded the total leach out from each plot.

Rice growth, biomass, and grain yield sampling and analysis

Rice tiller and panicle numbers were measured and randomly collected by the average 9 samplings of rice hills from each replication. Plant height was measured by measuring tape one time per week. Grain yield and biomass (above and below ground) were measured after harvesting and manually collecting from all rice hills in each plot with three replicate plots. They were dried naturally in the sun for two weeks then weighed by weighing machine with 4 positions. To determine the dry matter contents of rice grain and biomass, 1000 kennels of rice grain and 9 randomly rice hills were place in the oven at 80°C for 48 h (Forced Air Convection Drying Oven, redline RF 53, Germany) to a constant weight. The percentage of moisture content calculated from the difference of dried weight and wet weight was divided by wet weight and then multiplied by 100 percent. The remaining part of rice straw aboveground after harvest and root parts were measured and incorporated into the field for the next crop.

Statistical analysis

One Way Analysis of Variance (ANOVA) of SPSS version 20 at confidence level 95% ($P < 0.05$) was used to determine the statistical significance of the treatment effects for two rice cultivation seasons. The value was presented as mean ± standard error (SE). The coefficient of determination (R^2) was used for the analysis to determine the degree of linear correlation that was observed.

RESULTS

Air temperature and AWD system characteristic

As seen in Figure 2a and b, the average of the maximum temperature was 32.7 and 35.7°C in the first crop (wet) and the second crop (dry), respectively. The air temperature in this study affected the alternate wetting and drying cycles in the second crop quicker than the first crop as a result of higher air temperature. The interval of alternate drying cycles was between 10 to 12 days (3-time cycles) in the first crop and 7 days (5-time cycles) in the second crop (Figure 2c and d).

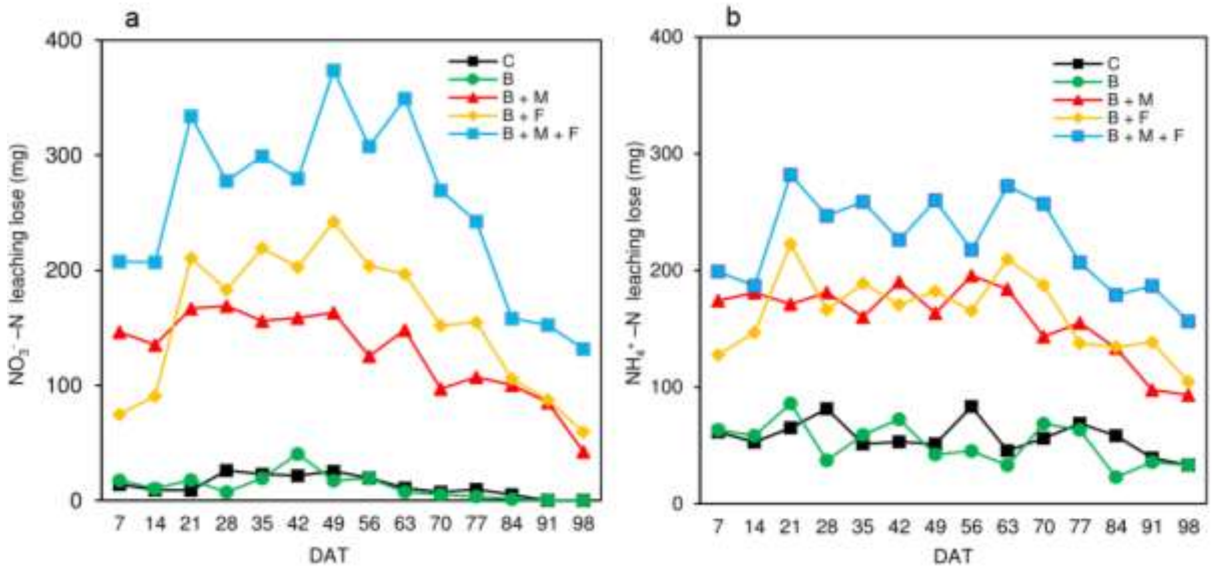

Figure 3. The seasonal dynamic of NO_3^- –N (a) and NH_4^+ –N (b) leaching lose in difference treatments during rice growing period.

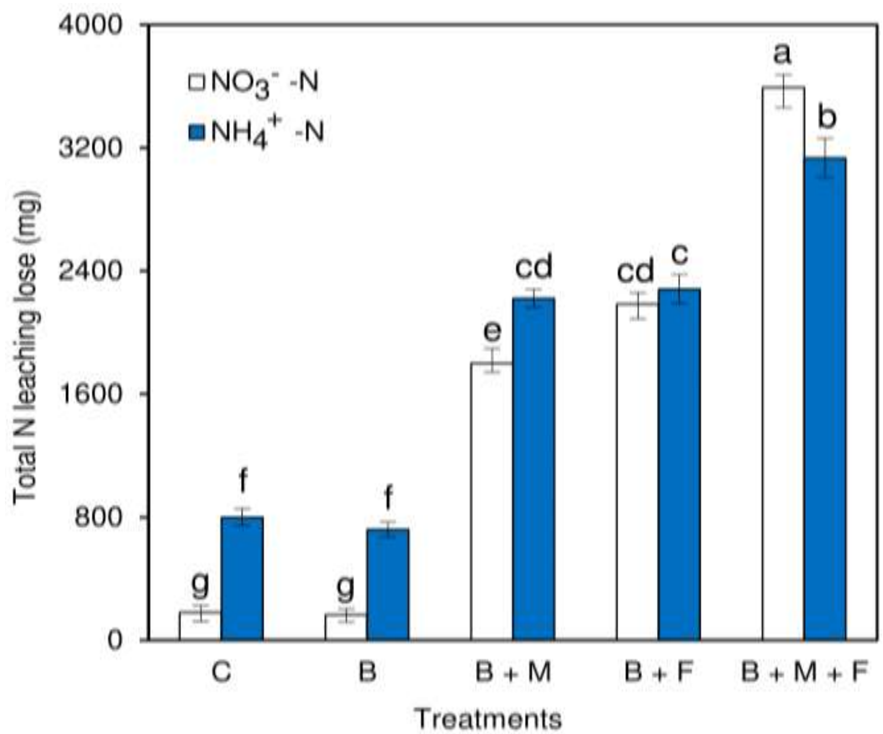

Figure 4. Total mass NH_4^+ –N and NO_3^- –N leaching lose in difference treatments throughout rice growing season.

Nitrogen leaching under different treatments

The seasonal variation of NH_4^+ –N and NO_3^- –N in water leachate loss over the rice-growing period for both crops are shown in Figures 3a and b. The significant difference ($p < 0.05$) was observed in three main periods which are during the early flooded period (7-20 DAT), AWD cycles following basal fertilizer (21-60 DAT), and flooding at the late period following the top dressing fertilizer (61-98 DAT). Both NH_4^+ –N and NO_3^- –N in the leachate occurred after flooding in the early rice-growing period with the low concentration loss then increased after both

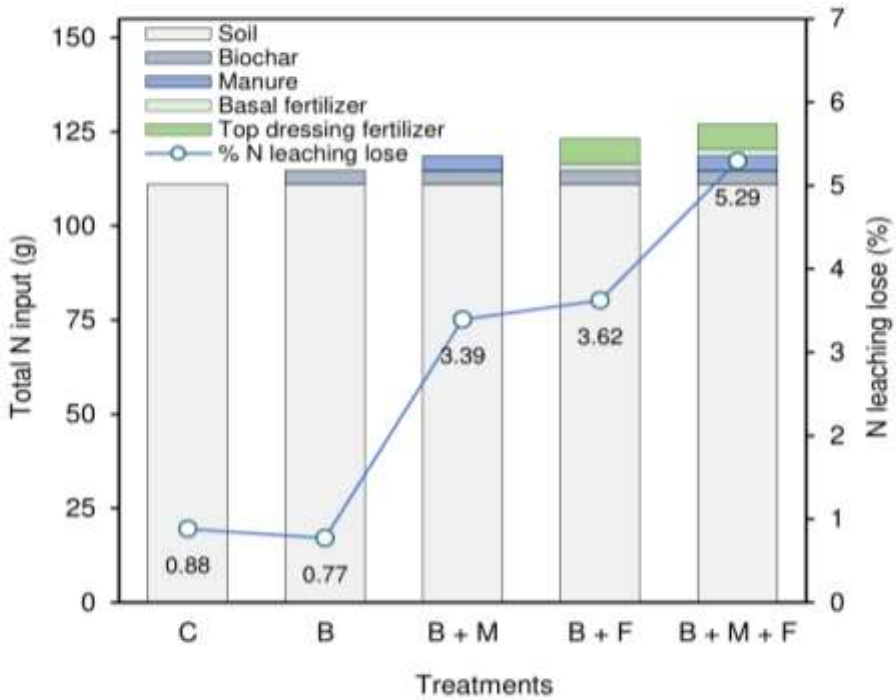

Figure 5. Total nitrogen input and the percentage of N lose from difference treatments.

fertilizations. From mass balance analysis, the total nitrogen losses from each treatment varied as the amount of N applied and the N content was different (Figure 4). We found that only 0.77% of the total N leaching loss in single biochar amended which was not significantly different to control soil treatment (0.88%) (Figure 5). However, the co-application of biochar in B+M, B+F, and B+M+F supplied the higher N input into the soil and as a result high N loss in the leachate was found at the rate of 3.39, 3.62 and 5.29%, respectively. The finding of the study was a non-significant reduction of N leaching in biochar amended alone compared to control. Nitrogen loss varied depending on the amount of N input as shown in the relationship between total nitrogen input and percentage of nitrogen loss (R^2=0.92) in Figure 6a.

GHG emissions under different treatments

The seasonal pattern of CH_4 and N_2O emission associated with each treatment was measured in both crops 2018 and 2019 (Figures 7a to d). Total CH_4 and N_2O emission over the rice growing seasons was dramatically affected by N fertilization, AWD cycles, and biochar amendment. As the field water flooded, CH_4 initially increased after transplanting and decreased dramatically during the AWD cycles. CH_4 emission increased rapidly during 3 weeks after fertilization and emissions decreased sharply until harvest. Similarly, N_2O fluxes were high peaked after basal fertilizer (20 DAT) and top dressing fertilizer at flowering stages (60 DAT). But, N_2O fluxes peaked during the AWD cycle and reduced after field water flooded. N_2O emissions varied depending on the total N input as the result shown in Figure 6b. Total N_2O emissions had a positive correlation with the total N input (R^2=0.69). However, the biochar amendment significantly increased total N to the soil but emitted a lower amount of CH_4 and N_2O emissions from the rice paddy studied compared to control (Figure 8a and c). In biochar amended alone, soil CH_4 emissions reduced by 20.92 and 25.75% and N_2O emissions reduced by 6.84 and 13.46% in the first and second crop, respectively, compared to control. This is the annual combined GHG emissions as GWP varied from 5.03 to 14.33 t CO_2 eq. ha^{-1} $year^{-1}$ (Table 3). As shown in Figure 8b, there was also a significant decrease in annual GHG emissions as GWP in a single biochar application but increased in biochar co-application as compared to control.

Water use and grain yields under different treatments

Water used varies from 55.36 to 72.32 m^3 ha^{-1} day^{-1} in the first crop and 88.39 to 110.71 m^3 ha^{-1} day^{-1} in the second crop (Table 3). The result showed that biochar application did not significantly affect seasonal water used in rice cultivation for both crops. The relationship between the amounts of water input into the field and the result of water leaching were estimated by correlation were shown in Figure 6d. The seasonal grain yield of different treatments was significantly different as shown in Table 3. Biochar application alone significantly ($P<0.05$) promoted yield 9.49% in the first crop and 14.10% in the second crop

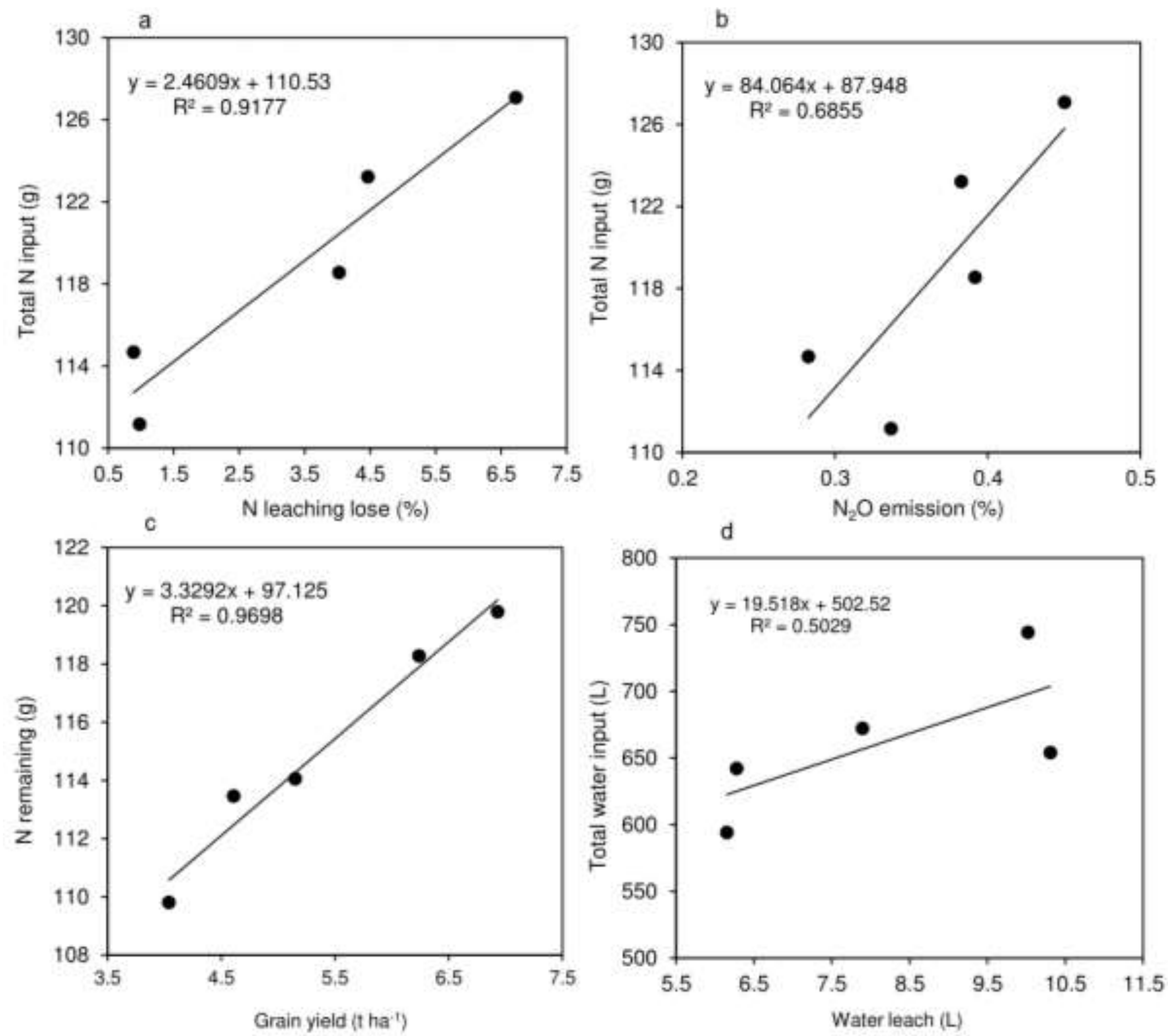

Figure 6. Relationship between total N input with N leaching lose (a), N input with N_2O emissions (b), N remaining with grain yield (c), and total water input with water leaching per plot (d).

higher than control. The result of higher grain yield due to the combined effects of manure or fertilizer increased the amount of panicle per hill and kernel per panicle higher than biochar application alone and control significantly. The enhancing of grain yield depended on the total nitrogen available and remaining in the soil. The relationship between total remaining nitrogen in the soil and the total grain yield showed a good trend line R^2=0.97 of positive correlation as shown in Figure 6c. Water productivity (water footprint) in terms of the annual amount of water used per grain yield was significantly different between treatments varying from 1508.95 to 2194.25 m^3 t^{-1} (Table 3). The highest ratio of annual water per grain yield was in control treatment. Similarly, the ratio of annual GHG emissions per rice yield (carbon footprint) was significantly affected under different treatments varying from 0.56 to 1.05 t CO_2 eq. t^{-1} yield. A single biochar treatment significantly emits less GHG emissions by producing a higher grain yield compared to the control while the combined application was the highest.

Rice growth and biomass yields under different treatments

As shown in Figures 10a and b, the minimum and maximum rice height varied from 84.13 to 102.75 cm in the first crop and 89.33 to 109.47 cm in the second crop. Biochar did not significantly affect rice height as it showed the difference as been only 1.90% in the first crop and 1.72% in the second crop compared to the control soil. Rice growth was enhanced under the combined application of biochar with manure, fertilizer, and application of them together compared to biochar application alone and control. The significant effects of

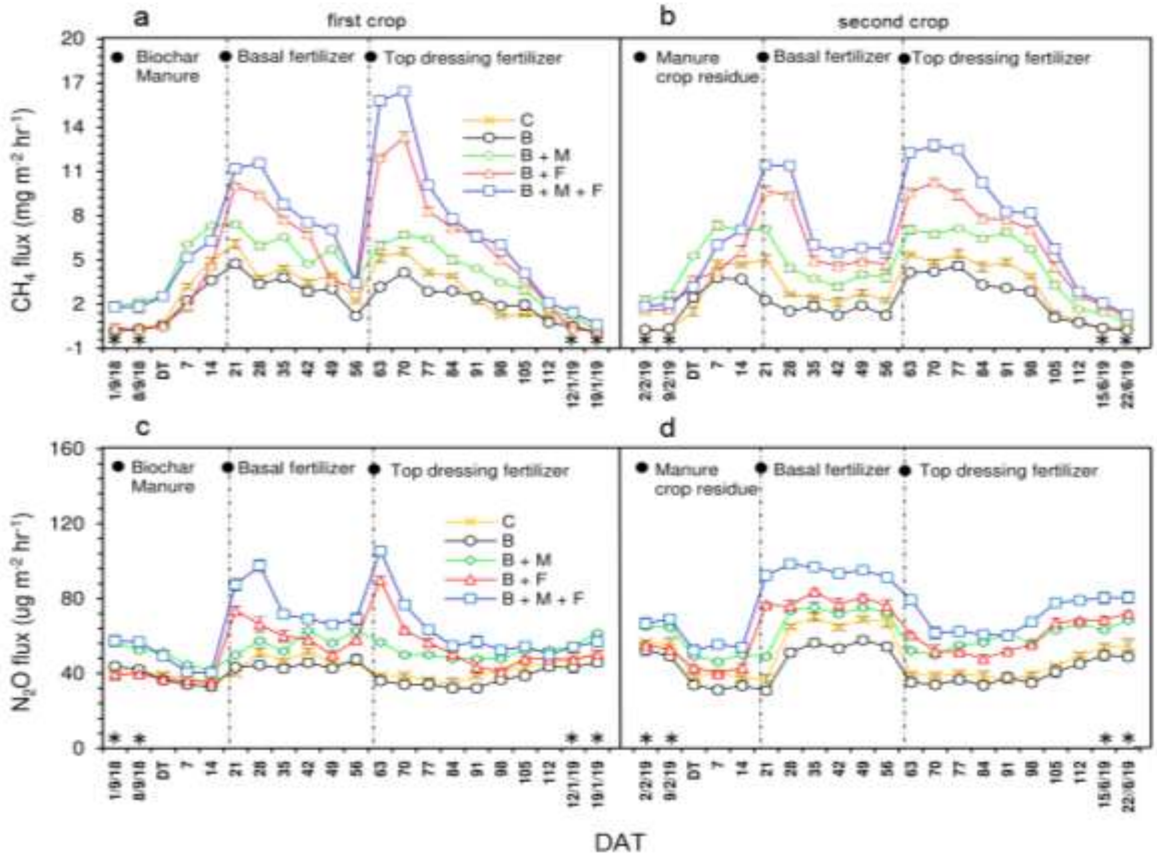

Figure 7. CH_4 fluxes (a and b) and N_2O fluxes (c and d) under different treatments over two cropping seasons. Biochar was applied two weeks before transplanting for B, B+M, B+F, and B+M+F plots, while manure was added to B + M plot. Basal fertilizer was applied at 20 DAT and top dressing fertilizer at 60 DAT for B+F and B+M+F plots. Error bars for CH_4, N_2O concentration, and water lever (n = 3) showed the standard error.
* recognized the fallow period before and after rice cultivation.

between treatments on tiller number per hill are shown in figures 10c and d. The amount of tiller number increased and this led to more panicle numbers and grain yield increase. The minimum and maximum tiller number per hill at the end season varied from 24.78 to 36.43 tiller hill^{-1} in the first crop and 26.89 to 40.82 tiller hill^{-1} in the second crop. Biochar significantly increased the tiller number by 10.81% in the first crop and 8.64% in the second crop. Integrated with manure was significantly added 25.02 to 25.27%, fertilizer added 31.43 to 36.33%, and all combined added 38.19 to 44.20% to single biochar. Increasing rice growth promoted the total biomass yield (above and below ground). The dried weight of biomass varies depended on rice high and the number of rice steam. The result showed that the total dried biomass weight under biochar treatment was higher than control, while the highest were found under the integration of biochar with manure and with fertilizer.

DISCUSSION

Our findings are consistent with a previous report that NH_4^+ –N and NO_3^- –N under biochar application alone, there was no significantly reduction of soil N leaching loss compare to control (Jin *et al*., 2016). This result can be explained due to biochar accelerating the nitrification rate in the soil, resulting in more NH_4^+ –N and NO_3^- –N being available for leaching. Moreover, soil percolation slightly increases during the period of the dry cycle, the available N at the root zone could leach after the new wetting cycle. In addition, the result is consistent with Tan *et al*. (2013), who reported that AWD cycle significantly increased NH_4^+ –N and NO_3^- –N concentrations from the rice paddy field. Our finding also found that the supplementation of chemical fertilizer during the period of AWD was likely to induce more NO_3^- –N and NH_4^+ –N leaching loss. However, a small reduction of N loss in single biochar amended may be cause by biochar properties which have been defined by Zheng *et al*. (2013) and Zhao *et al*. (2014). Biochar had good adsorption properties (Singh *et al*., 2010) and related to increased aggregation as the result in higher water holding capacity and enhanced N mobilization (Yoo *et al*., 2014). Ding *et al*. (2010) illustrated that biochar could adsorb NH_4^+ –N predominantly through its high cation

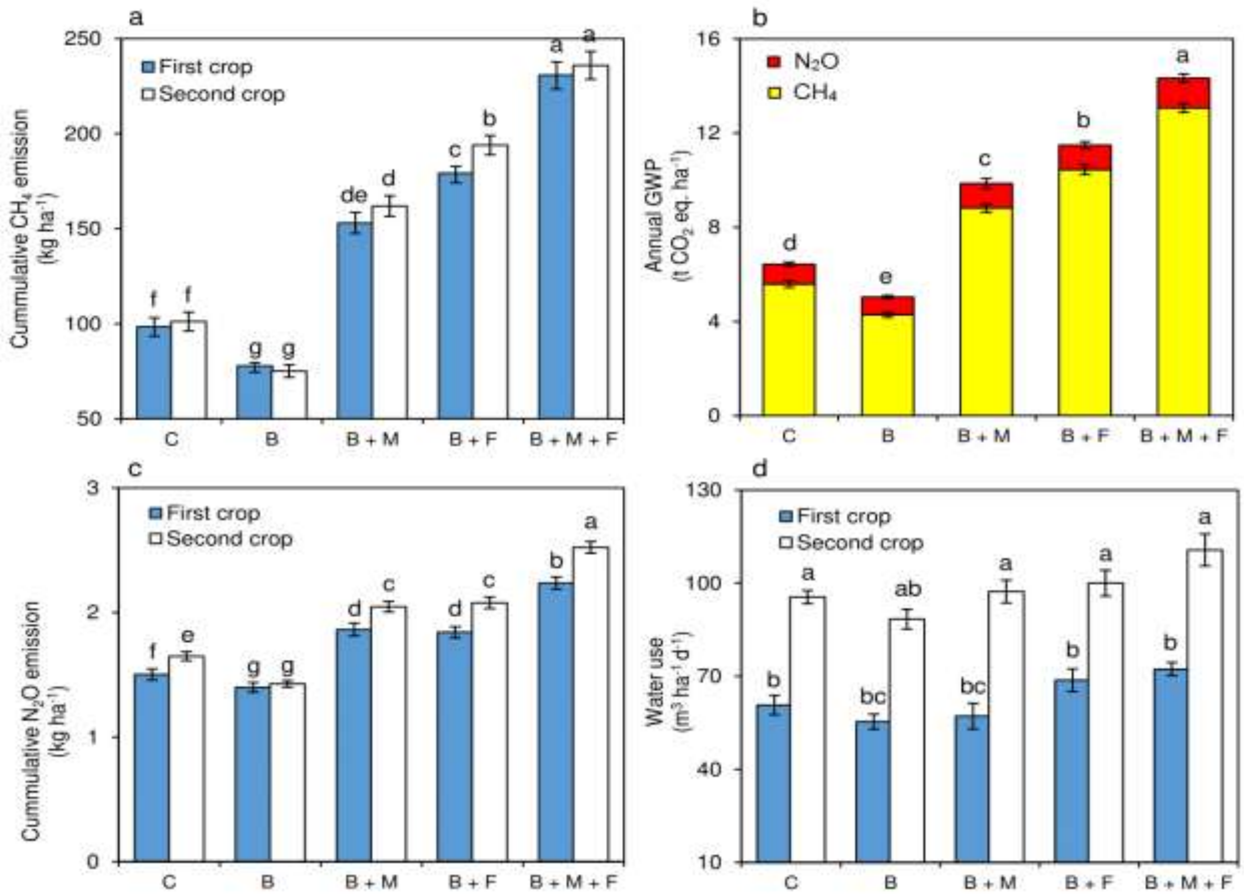

Figure 8. Seasonal cumulative CH_4 emissions (a), N_2O emissions (c) Annual GWP (b), and water use in rice cultivation (d) for both crops under the AWD system. Error bars indicated the standard error (n = 3).

Table 3. The annual water use, grain yield, GWP, water footprint, and carbon footprint.

Treatments	Annual water use (m^3 ha^{-1} yr^{-1})	Annual grain yield (t ha^{-1} yr^{-1})	Annual combined GWP emissions (t CO_2 eq. ha^{-1} yr^{-1})	Water footprint (m^3 t^{-1})	Carbon footprint (t CO_2 eq. t^{-1})
C	17560.71±142.88^c	8.00±0.19^e	6.42±0.18^d	2194.25±752.00^a	0.80±0.12^d
B	16155.36±125.13^d	8.95±0.21^d	5.03±0.12^e	1805.27±595.85^b	0.56±0.10^e
B + M	17357.14±173.92^c	10.16±0.28^c	9.85±0.24^c	1708.39±621.14^c	0.97±0.24^b
B + F	18968.75±155.94^b	12.24±0.20^b	11.52±0.29^b	1549.82±764.41^d	0.94±0.25^{bc}
B + M + F	20572.32±167.77^a	13.63±0.32^a	14.33±0.21^a	1508.95±524.28^{de}	1.05±0.34^a

The values indicated by mean ± standard error (SE). The different alphabet represented the significant differences ($P < 0.05$) compare to different treatments of rice cultivation.

exchange capacity, resulting in a 15.2% reduction in cumulative NH_4^+ –N losses. The significant effect may also be due to the enhanced nitrification rate induced by biochar, rendering it less available for leaching. We suggested that NH_4^+ –N and NO_3^- –N leaching peaked after N fertilizer (21 and 63 DAT) and sharply decreased at two weeks after. Adding N Fertilizer to the soil, NH_4^+ –N was converted into a natural process resulting from the activity of the enzyme and oxidizing bacteria. Then, the NH_4^+ –N is immediately transformed to NO_3^- –N through nitrification (Ghaly and Ramakrishnan, 2013). The result by Xu *et al*. (2016) reported that NH_4^+ –N was reduced by 19.1 to 28.1%, NO_3^- –N by 16.0 to 19.3%, and total N by 18.8 to 20.2% from the soil respective to control due to biochar application. The additional input of manure or fertilizer as co-application increased the total nitrogen loss significantly (P < 0.05) compared to biochar application alone and control. From Figure 5, the total N

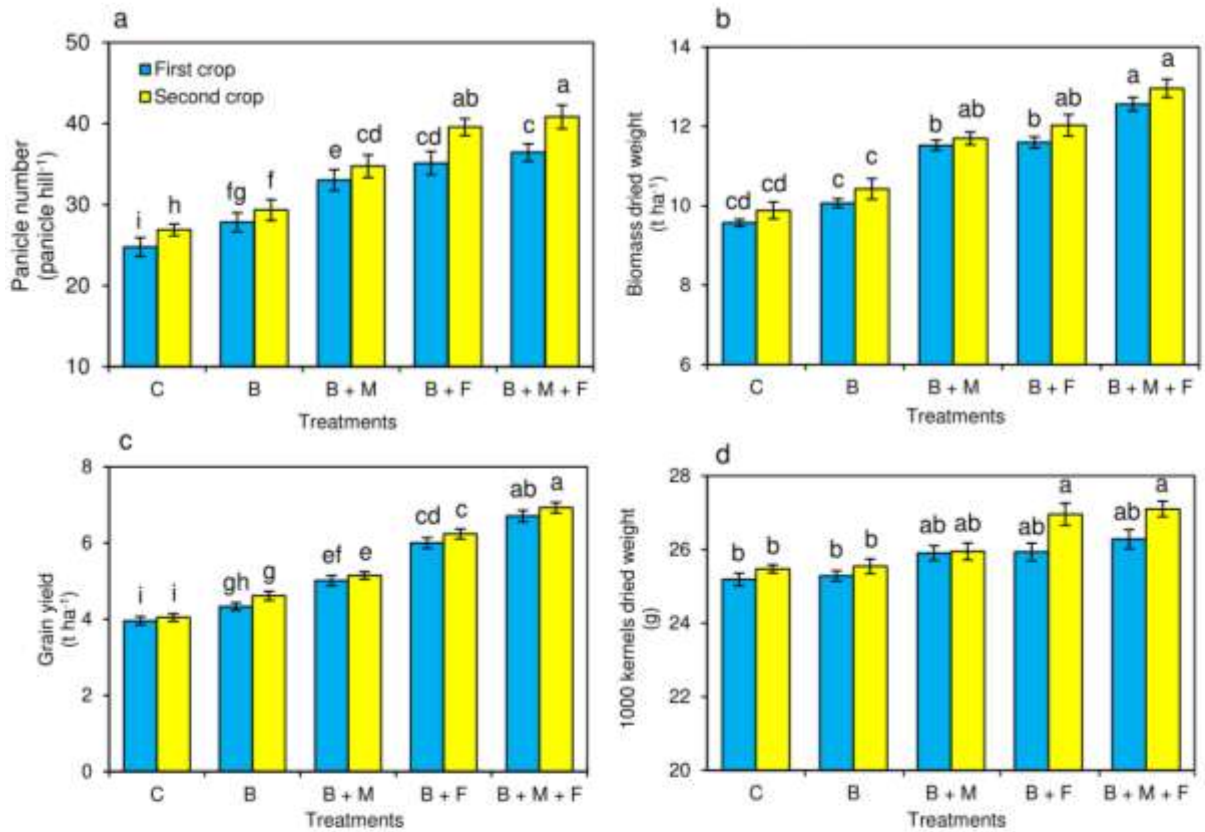

Figure 9. Total panicle number (a), total biomass dried weight (b), seasonal grain yields (c), and 1000 kernel dried weight (d) for both crops under the AWD system. Error bars indicated the standard error (n = 9).

input in B+F was higher than B+M but the total N loss was a small difference. This finding showed that the total mass of inorganic applied in B+F may reduce by biochar application either more sorption by the rice plant for enhancing higher grain yield as compared to B+M.

Regarding the result of GHG emissions, we found that biochar used in this study significantly reduced cumulative CH_4 and N_2O emissions from the soil but the biochar co-application with manure, fertilizer, combined of them significantly increased compared to control. CH_4 production occurred under anaerobic conditions, while N_2O promoted by the shift from anaerobic to aerobic conditions (Cai *et al*., 1997). Soil microbial biomass activities were enhanced after manure application, thus decomposition of organic matter in rice paddies offered the predominance source of methanogenic substrates (Das *et al*., 2011) which would promote CH_4 productions and emissions (Zheng *et al*., 2007). Increased N_2O emissions following available N source fertilizer and manure applications were reported due to simply offered mineral N for natural cycle through nitrification and denitrification (Das and Adhya, 2014). Cui *et al*. (2012) stated that annual N_2O emission increased by 73% when N-fertilizer was added in flooded rice fields. Application of the biochar in the flooded paddy field reduces CH_4 emission due to biochar can improve the water holding capacity of the soil (Karhu *et al.*, 2011) and stimulated the formation of soil structures and improved the soil aeration which oxidized the CH_4 in the soil and rice plant absorption. Biochar application enhanced soil aeration (Yanai *et al*., 2007), altered ammonia-oxidizer and denitrifier activity (Van Zwieten *et al*., 2014), sorption of N by the presence of inhibitory complexes such as ethylene has been suggested as mechanisms to explain the reduction in N_2O flux with biochar amendment. A small reduction of NH_4^+ –N and NO_3^- –N accessibility would decrease the total denitrified N and would reduce the ratio of N_2O (Cayuela *et al*., 2013). In our study, the biochar amendment reduced just a small amount of the soil NH_4^+ –N and NO_3^- –N leaching lose and this may due to the accelerating ammonia oxidation and stimulating total N use for enhancing rice growth and grain yield. The findings agreed with results reported by Bruun *et al*. (2012) that biochar application reduced CH_4 emission from the soil. Liu *et al*. (2011) also reported that biochar reduced CH_4 emission from waterlogged paddy soil because biochar reduced methanogenic activity. Moreover, Zhang *et al*. (2012) reported similar results that biochar application emitted CH_4 emission less than biochar co-applications with nitrogen fertilizer. AWD cycle decreased CH_4 emissions because increased O_2 in the soil led to anaerobic conditions that inhibited CH_4

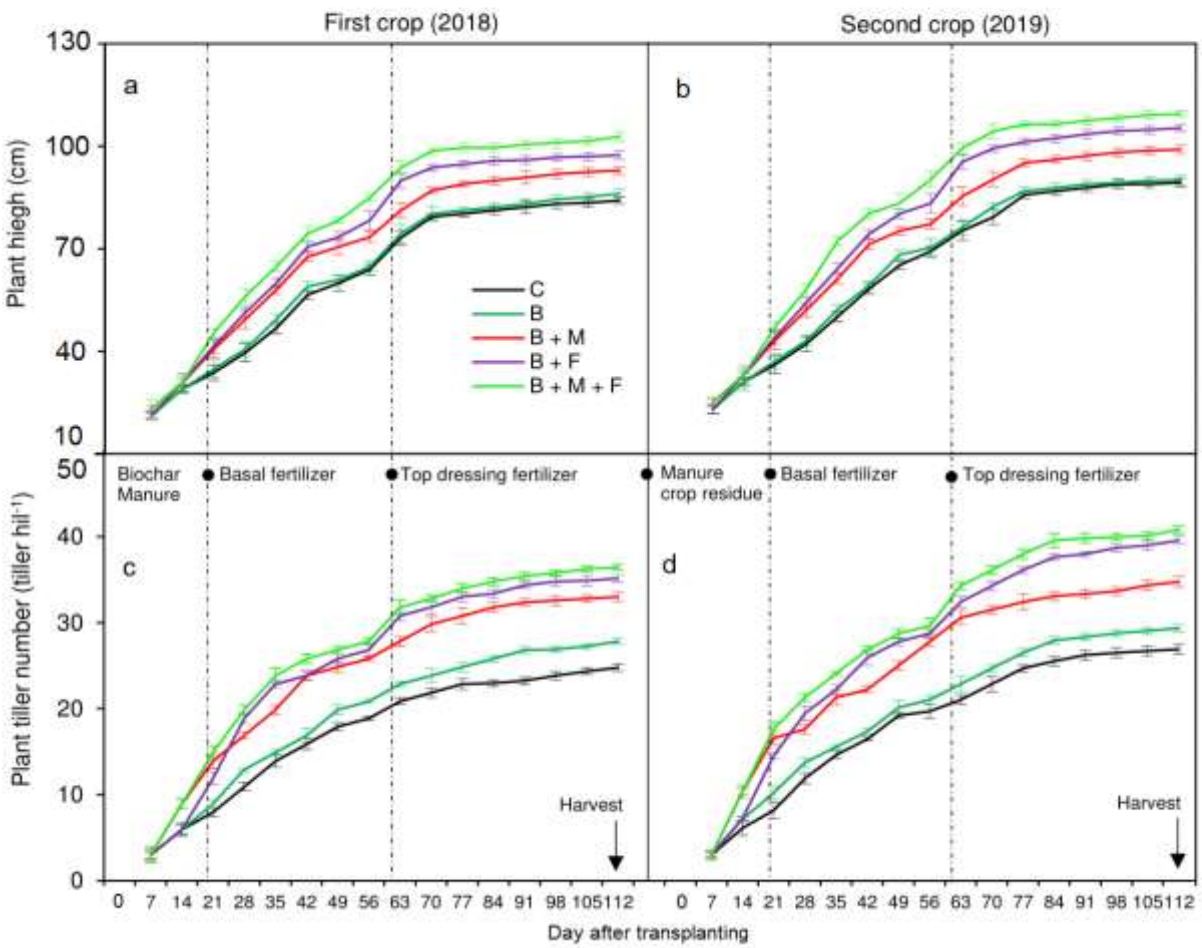

Figure 10. Plant height (a and b) and plant tiller number (c and d) for both crops under the AWD system. Biochar was applied two weeks before transplanting for B, B+M, B+F, and B+M+F plots, while manure was added to B+M plot. Basal fertilizer was applied at 20 DAT and top /dressing fertilizer at 60 DAT for B+F and B+M+F plots. Error bars indicated the standard error (n = 9).

production and emission (Linquist *et al*., 2015). Effectively mitigation of CH_4 emissions in paddy field through the AWD system has been suggested by many consistent studies (Chidthaisong *et al*., 2018; Sriphirom *et al*. 2019). Sriphirom *et al*. (2019) reported that the AWD system reduced CH_4 by averaging about 10.52% in the first crop and 26.20% in the second crop and increased N_2O by averaging about 7.37% in the first crop and 21.84% in the second crop compared to the conventional system. Our result revealed that the N_2O emissions were observed during the dry cycle of AWD system and after N fertilizer application, while CH_4 emissions were reduced.

On the other hand, we found that grain yields were increased in biochar application alone compared to control (Figure 9). The results were consistent with the finding reported by Zhang *et al*. (2010) who defined that the biochar applied at 10 t ha^{-1} and 40 t ha^{-1} significantly increased rice yield by 12 and 14% compared to soil without biochar, respectively. Biochar application increased N availability to the soil (Chan *et al*., 2008) and improve soil organic carbon accumulation (Pan *et al*., 2009). Our study agreed with Rahman *et al*. (2009) who reported the grain yields significantly increased due to the application of organic manure and chemical fertilizers. Biochar applied with manure or fertilizer increased more rice yields because biochar had positive effects on fertilizer use efficiency (Blackwell *et al*., 2010; Zhang *et al.*, 2010), increased nutrients utilization, particularly nitrogen from the applied organic and inorganic fertilizer (Steiner *et al*., 2008). Ye *et al.* (2013) reported that AWD irrigation with and without urea increased grain yield by 5.9 and 5.5% in 2010 and 6.3 and 5.4% in 2011 compared to continuous flooding, respectively. The biochar application alone did not significantly reduce the water used in this study. But we demonstrated that biochar application under AWD system might enhance water use efficiency which has been reported by Yang *et al.* (2018) who suggested that biochar enhances water used efficiency by 15.10 to 42.5% under AWD, respectively. However, our study agreed that AWD can reduce water use significantly if compared to CF due to AWD reduce the amount of irrigation.

Our findings indicated that rice growth and biomass yield was enhanced under the co-application of biochar with manure, fertilizer, and application of them compared together to biochar application alone and control. There was an improvement in crop performance due to manure and fertilizer application into the rice paddy. Additional nitrogen from fertilizer immediately available for the plant nutrient uptake to develop the plant height and tiller number led to more biomass yield (Chaturvedi, 2005; Siavoshi *et al*., 2011). Fertilizer was stronger influenced to the height of the plant over manure (Yosef Tabar, 2012). Biochar inspires the plant growth and increases fertilizer used efficiency, especially when biochar combined with fertilizer (Zhang *et al*., 2010; Steiner *et al*., 2008) and organic amendment. However, biochar application alone showed a small effect on rice height but significantly enhanced rice tiller number and biomass yield found in this study. Yang *et al*. (2009) reported that AWD makes up greater access to water and nutrients at depth in the soil profile. Thus, the placement of nitrogen into the soil delivers a slow release of fertilizer near the root system of rice plants. The field dried during the tillering stage can accelerate the stronger root growth (Thakur *et al*., 2011), which resulted in enhancing more effective tiller (Howell *et al*., 2015). Thus, improving the efficiency of nutrient uptake can limit nitrogen losses and promoted more rice growth and grain yield.

CONCLUSION

A significant (P<0.05) response of soil CH_4 emissions, N_2O emissions, and grain yield parameters to biochar and biochar combined with manure or nitrogen fertilizer application compared to control were reported in this study. However, the result showed no effect of single biochar on the reduction of soil NO_3^- –N and NH_4^+ –N leaching loss compared to control. Moreover, the total N loss is highly dependent on the total nitrogen input. In the case of biochar co-application with manure, fertilizer, and the combination of them was increased both nitrogens to the soil and higher nitrogen loss. As compare between combined manure with combined fertilizer treated, the co-application of biochar had reduced higher amount of inorganic nitrogen loss in combined fertilizer treated rather than organic nitrogen from manure. The cumulative CH_4 and N_2O emissions potentially reduced by biochar application alone compared to control. However, biochar co-application led to additionally increase GHG emissions. Biochar application alone increased yield contribution and crop yield viz., rice tiller number, rice height, biomass yield, and grain yield, while there was no effect on water retention compared to control. Introducing AWD during the flowering stage can reduce CH_4 emissions, but was offset by the increase of N_2O emissions due to wet and dry cycle. The full cycle of AWD was a good condition for paddy soil to improve more oxygen to oxidizing the nutrients in the root zone for plant uptake to develop on rice growth but also induces leaching loss.

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