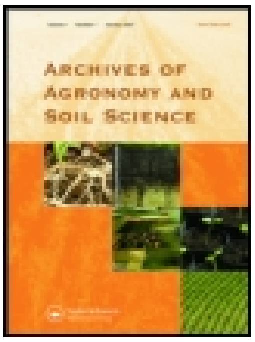

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The interactive effects of biochar and cow manure on rice growth and selected properties of salt-affected soil

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ABSTRACT

Salt-affected soil induces detrimental influences on paddy rice (*Oryza sativa* L.) growth and ameliorating the influences could be done with organic amendments, such as animal manure and biochar. The aims of the current study are: (1) to examine the interactive effects of biochar and cow manure on rice growth and on selected properties of salt-affected soil, and (2) to identify potential mechanisms related to the amendments. Saline-sodic soil was used for a net house experiment with two experimental factors: biochar (no-biochar, rice-husk, and -straw biochar) and cow manure (with and without cow manure). Without the manure, addition of both rice-hush and – straw biochar significantly increased rice growth, whereas a combination of individual biochar with manure did not show a positive synergistic effect. The interactive effect of two factors was not significant on available P and exchangeable K concentrations, but the main effects of the two factors were significant. Biochar addition resulted in higher soil cation exchange capacity (CEC) (28.8 to 29.0 $cmol_c\ kg^{-1}$) than the control (25.6 $cmol_c\ kg^{-1}$), but manure addition did not. Improved nutrient availabilities such as P and K, as well as CEC are among the potential mechanisms accounting for the enhanced rice growth with biochar.

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KEYWORDS
Organic amendment; soil salinity; soil fertility; animal manure; CEC

Introduction

Saline soil is defined as having an electrical conductivity in the saturated soil extract of at least 4 dS m^1, and sodic soil is as having exchangeable-sodium percentage (ESP) greater than 15 (United States Salinity Laboratory Staff 1954). Soils having high concentration of soluble salts (saline soil), or exchangeable sodium (sodic soil) or both (saline-sodic soil) are collectively called salt-affected soil (Saifullah et al. 2017). It is estimated that there are 400 million hectares, equal 6% of the total world land area, classified as salt-affected soil (Arora 2017). Normal soil can become salt-affected due to few basic processes bringing salts to soil layers, including a rise of salt-affected groundwater, seawater intrusion, saline-water irrigation, excessive fertilizer application, and rock weathering (Rengasamy 2010). The salt-affected soil has high concentration of salts such as chlorides, bicarbonates, sodium, and magnesium to a level that may have detrimental effects on plants (Rengasamy 2006). The soil may induce negative

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Supplemental data for this article can be accessed here.

influences on plant growth through osmotic pressure reducing water uptake by plants, toxic effects of ions such as Na^+, and Cl^-, and nutritional imbalance (Munns 2002; Rengasamy 2006). Shereen et al. (2002) found that salinity effects on rice cultivation were a reduction of aboveground biomass (straw and grain) and vigor. A study by Ahmed and Haider (2014) showed that high soil salinity led to a significantly lower rice yield.

Salt-affected soil occupies a considerable portion of the total area in Vietnam, around a million ha (Tran 2015), especially in the Mekong River delta. Out of the total 3.8 million ha of the Mekong River delta in Vietnam, the current rice area occupies about 1.9 million ha (Nguyen et al. 2015). Salt-affected soil occupies 40% of total area in the delta (Minh 1996), mainly due to seawater intrusion and a rise of saline groundwater (CCAFS-SEA 2016). The extent of the problematic soil may increase in the future due to the effects of climate change, e.g., increased drought. A recent report showed that around 1 million ton of rice grain were lost last year because of serious drought and saltwater intrusion in the Mekong river delta (FAO 2016).

Biochar is a residue after incomplete combustion of biomass under a limited supply of oxygen (Lehmann and Joseph 2009). It can be produced from any organic materials, e.g. crop residues at temperature levels below 700°C (Lehmann and Joseph 2009). It is a carbon (C)-rich substrate, typically high in ash, pH, CEC, and surface area. In term of agronomy, biochar amendment is documented to improve (1) available nutrients in the soil, (2) inorganic fertilizer efficiency, (3) soil physical properties, (4) base saturation, (5) microbial populations, and (6) ameliorate saline and drought stresses (Al-Wabel et al. 2017; Saifullah et al. 2017). For salt-affected soil, biochar is documented to improve crop productivity through improving physical, chemical and biological properties of the problematic soil (Amini et al. 2016). More specifically, biochar introduces at least three mechanisms to mitigate the adverse effects of salinity, including (1) temporary salt adsorption to biochar, (2) reduced osmotic effects by improving water availability, and (3) reduced Na uptake by increasing availability of K, P, Ca, and Mg (Akhtar et al. 2015a). As a result, biochar amendment on saline soil improved lettuce growth (Artiola et al. 2012), maize growth and yield (Lashari et al. 2015; Akhtar et al. 2015b), and biomass and mortality rate of haricot bean (*Phaseolus vulgaris)* (Thomas et al. 2013).

However, our up-to-date literature search revealed that studies addressing effects of biochar on rice crop on salt-affected soil are insufficient. The effects of biochar on salt-affected soil could be even improved when the material is co-composted with animal manure due to a positive synergistic effect (Liu et al. 2012). The synergistic effect of the two co-composted materials could be stronger on salt-affected soil because animal manure adds more nutrients to the soil (Glæsner et al. 2012; Dai et al. 2015), while biochar improves the surface area, which adsorbs salts such as Na, ameliorating the salt stresses (Akhtar et al. 2015a). However, the synergistic effect on paddy rice is still poorly known. Therefore, the current study was conducted on saline-sodic soil applied with biochar, cow manure, and their mixture (organic amendments), and planted with rice crop. The aims of the current study were (1) to examine the interactive effects of biochar and cow manure on rice growth and selected properties of salt-affected soil, and (2) to identify potential mechanisms related to the amendments. Aboveground and root biomass of rice crop, and some selected soil properties such as plant – available nutrients, pH, EC, CEC, exchangeable Na^+, Ca^{2+}, Mg^{2+}, and K^+ are measured before and after the experiment.

Materials and methods

Material preparation for the experiment

The experimental soil was collected from a field in Phu Tan commune, Tan Phu Dong District, Tien Giang province in southwestern Vietnam (located in the Mekong River Delta). The sampling field is located on a shrimp-rice farm, which is few kilometers away from the sea. The shrimp-rice farming system is an alternative cropping of wet-season rice and dry-season shrimp on the same field, and is commonly applied throughout the salt-affected areas in the Mekong Delta (Brennan et al. 2002). The soil is classified as *Haplic Solonchaks* (FAO/UNESCO), which is saline-sodic soil with EC = 5.7 dS m^1, and exchangeable-sodium percentage (ESP) = 42.7. The soil is a silty clay loam with the proportion of clay, silt, and sand of 35.2%, 54.1%, and 10.8%, respectively (more properties of the experimental soil, such as organic carbon, pH, EC, etc. shown in Table 1). The soil sample was collected from 0–10 cm surface layer from ten points over the selected field. After sampling, soil material was transported to the experimental net house, air-dried, and ground to pass a 2-mm sieve before the experiment.

Biochar was produced from rice husk and straw at around 350–400°C using a small biochar producing kiln by Dr. Mai Lan Anh's research group from Thai Nguyen University, Vietnam. Rice husk and straw were collected, air-dried, and cut into 3–5 cm segment (for rice straw) before loading the kiln. Other materials such as packaged dry cow manure and inorganic fertilizers were bought from commercial market located close to the sampling field. Some main properties of the experimental materials were analyzed and shown in Table 1.

Experimental design

A factorial experiment was set up in a net house following a randomized complete block design with four replicates. Two experimental factors included (1) biochar (no biochar, rice straw biochar, and rice husk biochar) and (2) cow manure (no cow manure and cow manure). Combinations of the two factors formed six experimental treatments, including: (1) no manure and no biochar, (2) no manure and rice-husk biochar, (3) no manure and rice-straw biochar, (4) cow manure and no biochar, (5) cow manure and rice-husk biochar, and (6) cow manure and rice-straw biochar. For treatments (5) and (6), biochar and manure were mixed at 1:1 (w/w) ratio and pre-incubated for 45 days to improve quality of the amendments (Glaser et al. 2015); for treatments (2), (3), and (4) biochar and manure separately went through the pre-incubation process similar to that applied to treatments (5) and (6) before the experiment. The pre-incubation process at small scale was conducted based on the principle of composting process used in Glaser et al. (2015). In brief, five kg of each material and their mixture were weighed into a plastic container covered with a lid, and watered to 70%. During the 45-day pre-incubation, the materials were turned seven times and water content was checked by weighing and water was added to maintain the initial moisture.

The organic amendment (biochar, manure, and their mixture after pre-incubation) rate was used at 2.5% (w/w, dry matter of the amendments/dry soil), and around five kg of the mixture (organic amendment and soil) were packed into a 20 cm × 30 cm (diameter x height) plastic pot (total 24 pots). The mixture in the pots was watered to 5 cm depth for ten days before rice seed sowing. Rice seeds (*Oryza sativa* L.) of variety OM 6162 were germinated in a Petri dish lined with two Whatman

Table 1. Selected properties of soil, rice-husk and rice-straw biochar, and cow manure used in the experiment. Ash percentage of rice husk biochar and rice straw biochar is 23.3 and 27.9%, respectively. The numbers in the parenthesis are standard deviations of the mean.

Materials	OC	pH	EC	P*	NH_4^{+*}	NO_3^{-*}	Cl^-	Na^{+**}	K^{+**}	Mg^{2+**}	Ca^{2+**}	CEC
	%		dS m^1	mg kg^{-1}				$cmol_c$ kg^{-1}				
Soil	1.9	6.5	5.7	152	43	14	7921	10.1	3.0	3.36	3.6	23.7
	(0.1)	(0.1)	(0.5)	(0.5)	(11)	(0.2)	(141)	(0.1)	(0.1)	(0.6)	(0.4)	(0.5)
Cow manure	34.8	7.1	1.4	5708	143	20	5140	8.3	23.7	0.10	1.6	36.2
	(0.5)	(0.1)	(0.1)	(242)	(21)	(4)	(50)	(0.3)	(1.0)	(0.04)	(0.3)	(5.8)
Rice husk biochar	36.6	7.6	0.7	582	23	20	638	1.4	30.6	0.13	1.7	35.7
	(1.3)	(0.4)	(0.2)	(23)	(8)	(4)	(100)	(0.02)	(0.3)	(0.03)	(0.2)	(0.10)
Rice straw biochar	38.8	9.4	4.0	6590	23	15	9385	7.7	31.9	0.26	3.5	48.6
	(0.9)	(0.3)	(0.8)	(384)	(6)	(4)	(313)	(0.7)	(0.6)	(0.12)	(0.6)	(2.05)

* available concentration extracted with 2M KCl for NH_4^+ and NO_3^- and with 0.05M H_2SO_4 for available P; ** exchangeable concentration extracted with 1M NH_4^+

No.1 filter papers with 5 ml of distilled water. Individual pots were sown with ten germinated seeds, and five seedlings on each pot were maintained after two weeks from sowing. All pots were applied with same cultivation practices and inorganic fertilizer rate following treatment three in Luu and Nguyen (2006). After ten days, all pots were watered to 5 cm depth, which was maintained constant throughout the experiment duration.

Observations

Rice growth

After the experiment, aboveground biomass, and root biomass were harvested from individual pots. Aboveground biomass including stem, leaves, tillers, and panicles (the rice grains not ripen) was cut to the ground level into a plastic bag. Water from experimental pots was then decanted and soil samples were collected from 0–10 cm surface layer for chemical analyses. The pots were then laid down and the soil in the pots was pulled out carefully to maintain the pot shape and a knife was used to cut the top soil layer (0–10 cm). The top soil was then spread out on a plastic sheet and washed out with tap water to collect root biomass. The collected biomass was oven dried at 70°C to a constant weight. The dried biomass of individual pots was weighed again and used for the assessment.

Experimental soil, biochar, and cow manure

Before experiment the ground soil, biochar, and cow manure were sampled in three replicates for chemical analyses. After the experiment, the soil from each pot was sampled separately for the 0–10 cm surface layer. Around 1 kg soil for each pot was taken, air-dried, and ground to pass a 2-mm sieve before analyses.

Chemical analyses: All soil, biochar, and manure samples were analyzed for pH, electrical conductivity (EC), organic carbon (OC), plant available nutrients (available P, NH_4^+, and NO_3^-), Cl^-, exchangeable cations (Na^+, K^+, Mg^{2+}, and Ca^{2+}), and cation exchange capacity (CEC). In addition, the ground soil was analyzed with particle size distribution (Carter and Gregorich 2008). The materials were added with distilled water at 1:2 (w/w) ratio and the extract was measured for pH and EC. OC was measured using Walkley – Black method. Concentrations of NH_4^+ and NO_3^- were measured using 2M KCl (Carter and Gregorich 2008). Available P was based on (Murphy and Riley 1962), shaking 1 g of ground soil with 25mL H_2SO_4 (0.05M) for 5 min. Concentrations of exchangeable Na^+, K^+, Mg^{2+}, Ca^{2+}, and potential CEC were measured using ammonium acetate method as used in (Nguyen and Lehmann 2009). Cl^- concentration was measured using titration method (Hajrasuliha et al. 1991). In addition, to examine the portion of potential CEC occupied by other exchangeable cations (not Na^+), the concentration of total exchangeable cations, excluding exchangeable Na (ECENa) was calculated by subtracting the Na^+ concentration from the CEC.

Statistical analyses

All post-experimental data were tested for differences using Analysis of Variance (ANOVA) following a two-factor randomized complete block design, using JMP 10 (SAS Institute Inc, North Carolina, USA). The overall ANOVA model is $\gamma_{ijek} = \mu + \beta_i + \alpha_j + \beta\alpha_{ij} + \delta_e + \in_{ijek}$, where γ_{ijke} is the response of individual treatment; μ is overall mean; β_i is a fixed effect of the ith level of manure factor; α_j is the fixed effect of the jth level of biochar factor; $\beta\alpha_{ij}$ is the interactive effect of manure and biochar factors; δ_e is fixed effect of eth block; and $\in_{ijek}$ is the random error with mean zero and having normal distribution (Ott and Longnecker 2011). From the ANOVA and effect test results, if the interactive effect was significant with $P \le 0.05$, Tukey honestly significant difference test was applied to classify the 6 treatments. If not, the main effects of biochar and manure were examined.

If any of the two main effects is significant (P ≤ 0.05) Tukey honestly significant difference test for biochar factor or Student's t test for manure factor was applied to classify the 3 treatments of biochar factor, or 2 treatments of the manure factor. If there was no significant effect shown, neither Tukey HSD test, nor Student's t test was applied and the data of the tested parameter was not shown but was described in the result section. Linear regression fittings were performed to examine dependent patterns of biomass on selected soil variables. The regression analysis and figures were performed using Sigmaplot 12 (Systat Software Inc.).

Results

Rice growth

Three months after sowing, rice growth was visualized with some typical morphological symptoms such as stunted growth, burning of leaf tips and edges, and leaf senescence. In the control treatment applied with no biochar and no manure, rice crop died back from order leaves, and some tillers, stem height, and leaf area were seriously affected (data not shown). In addition, rice crop from all treatments except for the control (unamended soil) had panicles. The aboveground biomass including stem, leaves, tillers, and panicles were harvested and shown in the Figure 1(a) and Supplementary data Table 1. The combined addition of biochar and cow manure resulted in a significant interactive effect on aboveground biomass of rice crop. For three treatments applied without manure, treatments amended with rice-husk and -straw biochar had significantly higher aboveground biomass (10.4, and 12.9 g of dry matter pot^{-1}, respectively) than unamended soil (3.7 g of dry matter pot^{-1}). In contrast, three treatments applied with cow manure but different biochar (no biochar, rice-husk and – straw biochar) had similar aboveground biomass, varying from 4.9 to 8.5 g of dry matter pot^{-1}. Different from aboveground biomass, root biomass was not affected by the interaction of the two experimental factors, biochar and manure (Figure 1(b)). While cow manure addition did not result in significant difference in root biomass (Table within Figure 1 (b)), the addition of rice-husk and – straw biochar gave significantly higher root biomass (1.3 and 1.2 g of dry matter pot^{-1}, respectively) than no-biochar addition (0.5 g of dry matter pot^{-1}).

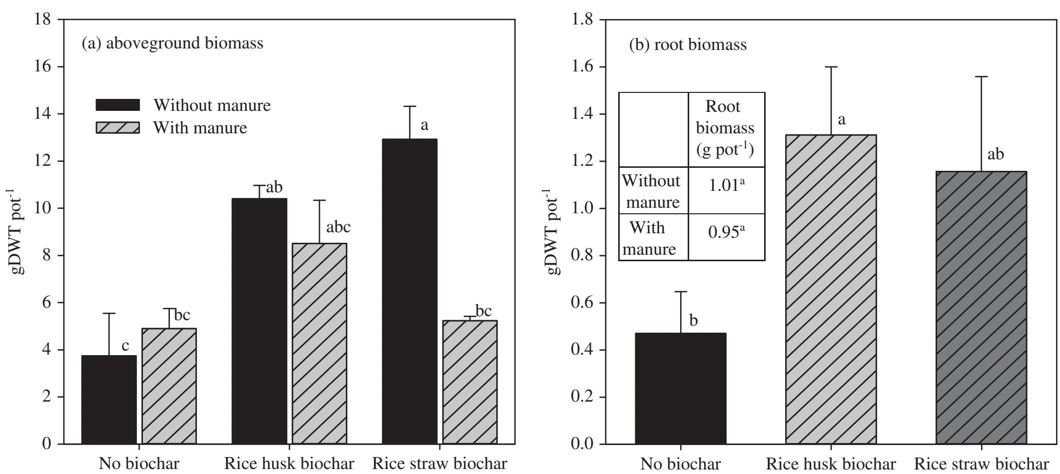

Figure 1. Interactive effect of biochar and manure on aboveground biomass (a), and main effect of biochar on root biomass (b). The Table within the Panel b is the root biomass from treatments added with and without cow manure. Error bars are the standard deviation of the mean. Bars attached with the same letters are not significantly different from the others at P ≤ 0.05. (DWT = dry weight; SD = standard deviation of the mean). (Note: the interactive effect of biochar and manure was significant on aboveground biomass, and only main effect of biochar on root biomass was significant).

Nutritional and chemical properties of the experimental soil

After experiment, soil EC varied from 4.10 to 4.70 with a mean of 4.5 dS m^{-1} and soil pH was from 6.9 to 7.1 with a mean of 7.0 for six treatments. NO_3^- concentration varied from 9.9 to 19.9 with a mean of 14.6 mg kg^{-1} and NH_4^+ concentrations changed from 37 to 65 with a mean of 52.6 mg kg^{-1}. Concentrations of exchangeable Na^+, Ca^{2+}, and Mg^{2+} were 9.2 (varying from 8.6 to 10.0), 4.3 (4.0 to 4.9), 1.8 (1.7 to 1.9) $cmol_c$ kg^{-1}, respectively. The concentration of Cl^- varied from 4004 to 4686, with a mean of 4383 mg kg^{-1}. Because these concentrations were not significantly affected by either interactive effect or main effects (Supplementary data Tables 4–11), their values were not showed in the main article, but in the Supplementary data Tables 1 and 2).

Statistical analyses showed that there was no interactive effect of biochar and manure on concentrations of available P and exchangeable K^+ (Supplementary data Tables 12–13), and thus data of each of six treatments were not shown. Nevertheless, the main effects of biochar and cow manure on concentrations of available P and exchangeable K^+ were significant (Figure 2(a,b,c, and d)). Addition of rice-husk biochar resulted in significantly higher available P concentration (47.5 mg kg^{-1}) than no biochar addition (32.9 mg kg^{-1}, Figure 2(a)). Soil added with cow manure had higher available P concentration (48.5 mg kg^{-1}) than that added

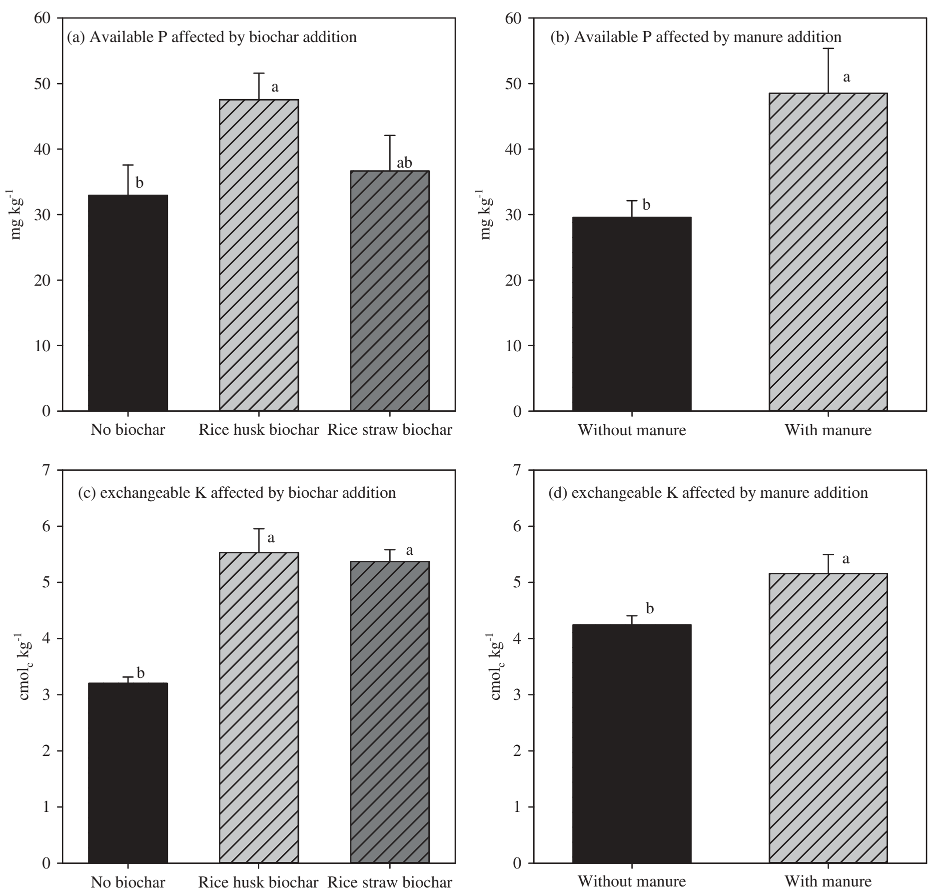

Figure 2. Main effects of biochar and cow manure on available P concentration (a and b) and on exchangeable K concentration, (c and d). Error bars are the standard deviation of the mean. Bars attached with the same letters are not significantly different from the others at P ≤ 0.05. (Note: only main effects of biochar and manure were significant, and the interactive effect was not significant).

without manure (29.6 mg kg^{-1}, Figure 2(b)). Similarly, rice-husk and -straw biochar resulted in significantly higher exchangeable K^+ concentration (5.5 and 5.4 cmol$_c$ kg^{-1}, respectively) than no biochar (3.2 cmol$_c$ kg^{-1}, Figure 2(c)) and cow manure gave higher K^+ concentration (5.2 cmol$_c$ kg^{-1}) than no cow manure (4.2 cmol$_c$ kg^{-1}, Figure 2(d)).

Likewise, because statistical analyses showed that the interactive effect of biochar and manure on soil CEC and the concentrations of ECENa (total exchangeable cations, excluding exchangeable Na) was not significant (Supplementary data Tables 14–15), the data of each of six treatments were not shown. Of the two experimental factors tested, biochar addition resulted in significantly higher CEC and concentration of ECENa than no-biochar addition (Figure 3(a and b)). Rice-husk and -straw biochar gave significantly higher soil CEC (29.0 and 28.8 cmol$_c$ kg^{-1}, respectively) than no-biochar treatment (25.6 cmol$_c$ kg^{-1}) and higher concentrations of ECENa (19.4 and 19.8 cmol$_c$ kg^{-1}, respectively) than no-biochar treatment (16.3 cmol$_c$ kg^{-1}). Treatments applied with and without cow manure had similar CEC and concentration of ECENa (Tables within the Figure 3(a and b)).

Relationship between rice growth and examined properties of the soil

Aboveground biomass of rice crop was significantly correlated with soil CEC (r^2 = 0.29 and p = 0.006) and the concentration of ECENa (r^2 = 0.24 and p = 0.02), but not with exchangeable Na concentration (Figure 4(a,c,b), respectively). For every one cmol$_c$ of CEC aboveground biomass increased 0.79 g of dry matter pot^{-1}. The increase rate of aboveground biomass was 0.83 g of dry matter pot^{-1} for every one cmol$_c$ of ECENa.

Figure 5 showed that exchangeable Na occupied a considerable portion of CEC, varying from 30 to 37% of CEC, and was not significantly different among three treatments (no-biochar, rice-husk and -straw biochar treatments). The greater portion (63 – 70%) of soil CEC occupied by ECENa was significantly affected by biochar addition. Rice husk- and -straw biochar addition gave a significantly higher concentration of ECENa, CEC, aboveground and root biomass than no biochar addition.

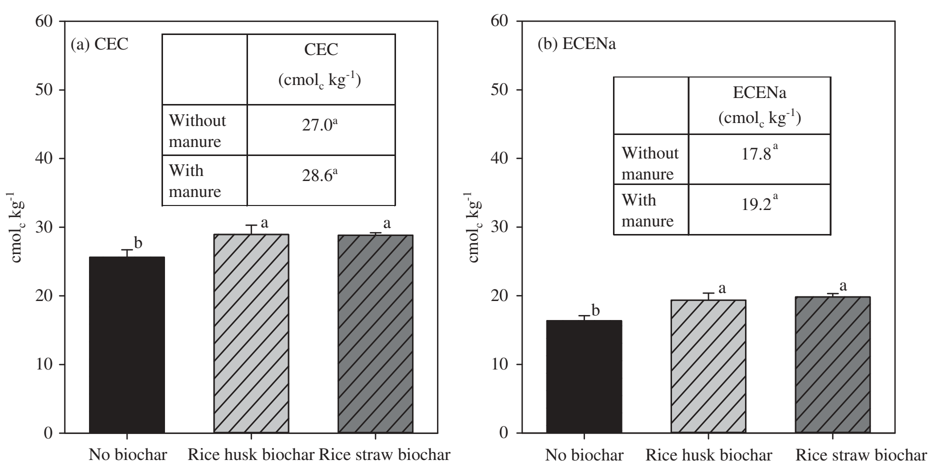

Figure 3. Effect of biochar addition on cation exchange capacity (CEC) (a) and concentration of ECENa (total exchangeable cations, excluding exchangeable Na). The Tables within the Panels a and b are CEC and concentrations of ECENa of the treatments added with and without cow manure. Error bars are the standard deviation of the mean. Bars attached with the same letters are not significantly different from the others at P ≤ 0.05. (SD = standard deviation of the mean). (Note: only main effect of biochar was significant, and the interactive effect and the main effect of manure were not significant).

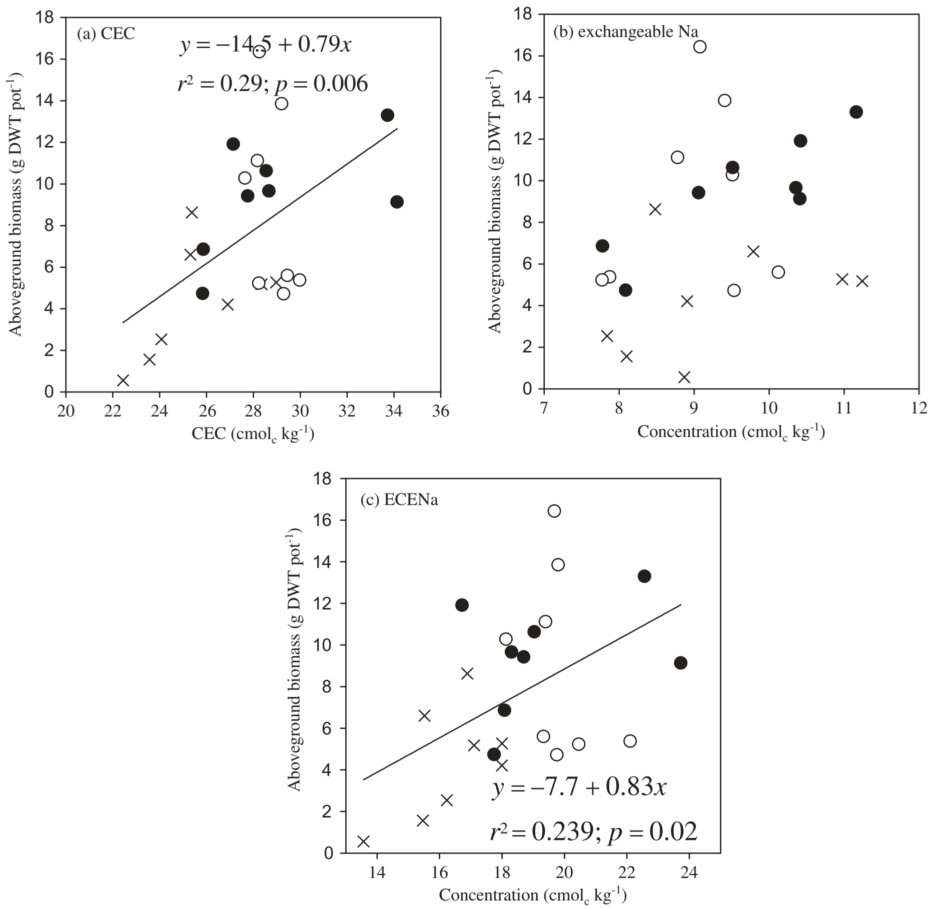

Figure 4. The relationship between CEC (a), exchangeable Na (b) and concentration of ECENa (b) with aboveground biomass. Thin Xs denote for without-biochar treatments; open circles are for rice-straw biochar treatments, and solid circles are for rice-husk biochar treatments.

Discussion

Effects of saline-sodic soil on rice growth

In the Mekong River Delta in Vietnam, salinity is due to the intrusion of seawater and rising of saline groundwater (CCAFS-SEA 2016). Soil used in the current study is high in electrical conductivity (5.7 4 dS m^{1}) and exchangeable-sodium percentage (42.7), classified as a saline-sodic soil (United States Salinity Laboratory Staff 1954), and high in sodium concentration of 10.1 $cmol_c$ kg^{-1} (Table 1). Additionally, the soil is characterized by high CEC of 23.7 ± 0.5 cmol-$_c$ kg^{-1}, of which a considerable portion is occupied by exchangeable Na (42.7%, Table 1). These resulted in high EC value and high pH value.

Previous studies showed that salinity reduced growth and vigor (Shereen et al. 2002) and yield (Ahmed and Haider 2014) of the rice crop. Some detrimental effects of salt-affected soil on plant growth could include osmotic effects reducing water uptake by crops, toxic effects of ions such as Na^+, and Cl^-, and nutritional imbalances (Munns 2002; Rengasamy 2006). Some morphological symptoms such as stunted growth, burning of leaf tips and edges, and leaf senescence visualized on the rice crop in the current study was similarly reported by other authors on salt-affected soils (Shereen et al. 2002; Mahmood et al. 2009). In the other treatments especially these applied with

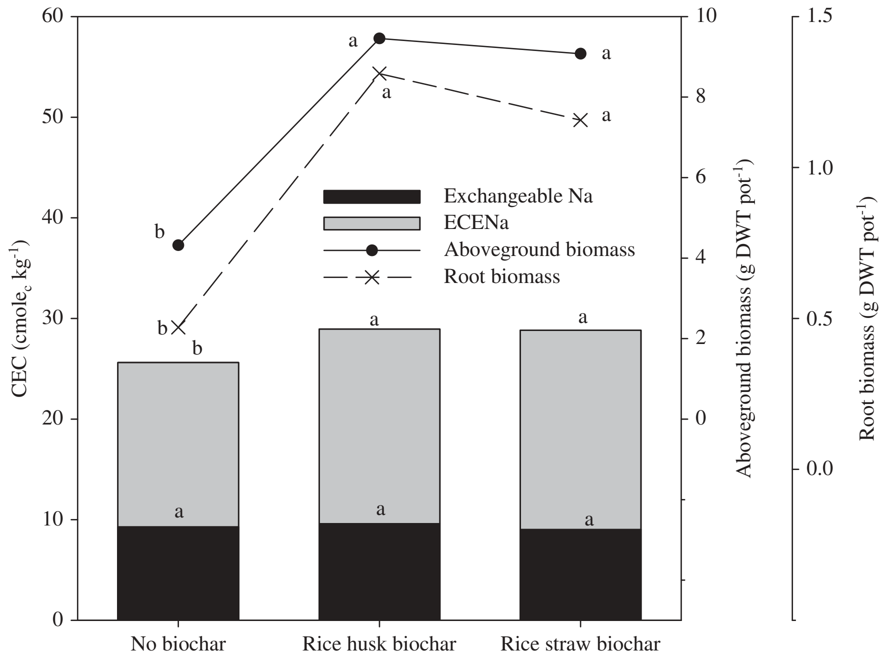

Figure 5. Soil CEC and its components, aboveground and root biomass of biochar-added soil. In the same parameter, data attached with the same letter are not significantly different from the other. (Note: ECENa = total exchangeable cations, excluding exchangeable Na).

biochar, these symptoms were not as strong as seen in the no-biochar and no-manure treatment. The aboveground biomass of the treatments applied with rice-husk and –straw biochar was significantly higher (210–219%) than that of unamended treatment (Figure 1(a)), indicating an improved rice growth by biochar amendment.

Properties of saline-sodic soil as affected by organic amendments

The higher concentrations of available P and exchangeable K in biochar-added and cow manure-added treatments in the current study could be controlled by a few mechanisms. Rice-husk and -straw biochar, as well as cow manure, had higher availble P and K^+ concentrations than the original soil (Table 1). Consequently, the addition of these organic amendments mechanically added P and K, resulting in higher available P and exchangeable K^+ concentrations of the amended soil than those of the unamended soil after the experiment, as shown in Figure 2. Likewise, Biederman and Harpole (2013) reviewed the literature and concluded that biochar addition increased soil phosphorus significantly. Jin et al. (2016) reported that application rate of manure-derived biochar at 1.2% increased Olsen P from 21 to 141 mg kg^{-1} on clay loam soil and from 12 to 110 mg kg^{-1} on silt loam soil. In addition, biochar amendment was reported to introduce both negative and positive priming effects on mineralization of native soil organic carbon (SOC) (Fang et al. 2015). This means that biochar addition may stimulate decomposition of native SOC (positive priming), which was reported by Wardle et al. (2008). Because soil organic matter (SOM) contains a considerable portion of P, mineralization of SOM likely increases plant-available P in soil. Another common mechanism to increase soil P availability is a rise of soil pH as a result of biochar addition (Ding et al. 2016). The change in soil pH as a result of biochar addition may affect P sorption and desorption and consequently available P concentration in soil (Xu et al. 2014). However, such a mechanism could hardly exist in the current study because initial soil pH was within the neutral levels and was not significantly affected by biochar addition.

Animal manure was reported to contain high P content (Glæsner et al. 2012; Dai et al. 2015). Therefore, it is not surprising that cow manure used in the current study is high in available P concentration (Table 1), providing a considerable amount of inorganic P to the amended soil, compared to the unamended soil. Cow manure-added soils were reported to have a higher available P concentration after the experiment (Anwar et al. 2017). Similar to biochar, animal manure was reported to produce significant priming effect on native SOM (Ma et al. 2013), which may solubilize soil organic P, further increasing plant-available P concentration. Mechanisms related to the priming effects are complicated and reviewed by Kuzyakov et al. (2000). The priming effects of biochar and cow manure could be present in the current study to increase available P derived from soil organic P that necessitates more study.

Biochar itself is a high – CEC organic substrate, depending on feedstock and charring conditions (Nguyen et al. 2010). The current study found that CEC of rice-husk and – straw biochar was 36 and 47 $cmol_c\ kg^{-1}$ dry matter, respectively (Table 1). For comparison, Gamage et al. (2016) found that CEC of rice-husk biochar was 19.54 $cmol_c\ kg^{-1}$, and Kamara et al. (2015) reported a value of 44 $cmol_c\ kg^{-1}$ from rice-straw biochar. In addition, Zheng et al. (2013) reported CEC of rice -straw and -husk biochar were 38 and 3.6 $cmol_c\ kg^{-1}$. Variation in CEC values could be due to the difference in pyrolysis conditions (charring temperatures and charring rate) to produce biochar from different studies.

It is repeatedly reported that biochar increases soil CEC (Liang et al. 2006; Abrishamkesh et al. 2015; Gamage et al. 2016), which is similar to the finding of the current study (Figure 3). There could be a few mechanisms of biochar to increase soil CEC after soil addition as found in the current study (Figure 3). Biochar was higher in CEC than soil alone in the current study (Table 1). As a result, the addition of biochar to soil physically increased soil CEC per kg of mixture. Biochar oxidation, which increased the density of negative surface charges (CEC) of biochar particles (Nguyen et al. 2010), is another important process to enhance CEC of the biochar added soil. For example, Liang et al. (2006) found that charge density of biochar-rich soil was higher than that of the counterpart that was attributed to biochar oxidation. Similarly, oxygen concentration and negative surface charges of biochar were reported to increase after a 12 – month incubation at different temperatures (Cheng and Lehmann 2009).

The effect of biochar on the exchangeable Na concentration in soil was reported in two opposite directions. On the negative direction, Wu et al. (2014) found that biochar addition significantly reduced soil exchangeable sodium percentage (ESP) after a four-month incubation. Similarly, the reduced exchangeable Na as a result of saw-dust biochar addition was reported by Sappor et al. (2017). On the positive direction, Chan et al. (2007) found that exchangeable Na concentration in soil significantly increased with biochar application rates. Sappor et al. (2017) reported that the effect of biochar on soil exchangeable Na concentration was not significant, compared to the control. Likewise, the current study found that organic amendments (biochar and cow manure) did not significantly affect exchangeable Na concentration of the amended soil, compared to the unamended soil. Soil used in the current study was high in EC (5.7 $dS\ m^{-1}$) and ESP (42.7), and was finely textured (silty clay loam), compared to those used in the studies by Wu et al. (2014) (ESP = 27, loamy sand) and by Sappor et al. (2017) (ESP = 18 and EC = 3.7 $dS\ m^{-1}$). The real reason accounting for the reduced exchangeable Na concentration in soil by the amendments is still unclear that needs more study. Nevertheless, a possibility could be that the total negative surface charges derived from the amendments could absorb exchangeable Na from soil (Kim et al. 2016) and thus reduce exchangeable concentration. If it could be the case, the application rate of biochar in the current study was not high enough to provide the designed total negative charges to significantly reduce the exchangeable Na concentration in soil by the exchangeable sorption.

Biochar addition, while increasing soil CEC and unaffecting the exchangeable Na concentration, improved the concentration of ECENa, compared to the non-biochar treatment (Figure 3, 5). Silva et al. (2017) found that concentrations of exchangeable Ca, Mg, and K increased with an application rate of biochar. The same findings were reported by Chan et al. (2007) and Houben et al. (2013). Ash is rich in dissolved metals especially oxides of alkali metals (example, rice straw ash having SiO_2 = 75%, K_2O = 10%, MgO = 1.9% and CaO = 1.5%) (Liu et al. 2011). Therefore, the increase in concentrations of exchangeable Ca, Mg, and K could be attributed to the ash existing in the added biochar. Because K_2O concentration was highest among the alkali metals in rice straw ash (Liu et al. 2011), the addition of biochar could explain the higher exchangeable K concentration in the amended soils than unamended soil in the current study. Besides, exchangeable sites originally derived from or newly created on biochar particles could absorb and thus dissolve some loosely fixed portions of soil bases such as Ca^{2+}, Mg^{2+}, K^{+}, and others such as NH_4^+, H^+, and Al^{3+}, improving the concentration of ECENa. Although this mechanism might occur in the current study, it needs more study to clarify.

Improved rice growth on saline-sodic soil by biochar

There are some studies reporting positive effects of biochar on crop growth on saline soil. Lettuce growth was found to increase significantly on biochar-added alkali soil than its control (Artiola et al. 2012). Maize growth was significantly higher in biochar-amended saline soil than unamended soil, and the associated mechanisms included reduced sodium concentration while improved K concentration in xylem sap (Akhtar et al. 2015b). Better maize grain yield was observed on saline soil applied with biochar than without biochar and associated mechanism related to the applied biochar included decreases in soil EC, sodium adsorption ratio (SAR), and exchangeable sodium ratio (ESP) (Lashari et al. 2015). As a result of biochar addition, soil salinity reduction or salt sorption on biochar particles was common and central mechanisms demonstrated to ameliorate the adverse effects of salt-affected soil on crop growth (Lashari et al. 2013; Thomas et al. 2013; Hammer et al. 2015). Similar to those findings, the current study showed that without cow manure, biochar addition significantly increased rice growth on saline-sodic soil. However, the reason for the improved rice growth by biochar in the current study is quite different from those reported. In details, improved CEC, especially the concentration of ECENa such as K, by biochar as well as the enhanced available P concentration (Figure 2, 3, 4, and 5) could be the potential primary reason. Other studies reporting similar mechanisms included Silva et al. (2017) on bean plant, Chan et al. (2007) on radish vegetable, Houben et al. (2013) on rapeseed, and Kim et al. (2016) on maize growth. As discussed in section above, biochar addition did not affect the exchangeable Na concentration due to a relatively low application rate of biochar and high exchangeable Na concentration in the original soil. As a result, the relationship between exchangeable Na concentration in soil with aboveground biomass of the rice crop was not significant.

One interesting, but unexpected, finding from the current study is that the combination of cow manure and biochar did not show a positive synergistic effect on aboveground biomass (Figure 1(a)). Similarly, Trupiano et al. (2017) found that addition of biochar alone improved total biomass and soil concentrations of N and P compared to its control, while a combination of biochar and a compost resulted in lectuce growth similar to the biochar alone. Additionally, Agegnehu et al. (2015) showed the same finding that biochar, compost, and a combination of the two did not result in significant difference in peanut seed yield and total biomass. For the current finding, although the real reasons are unknown, some possibilities to explain the non-synergistic interaction of the combination could be that by pre-incubating with cow manure biochar particles could adsorb organic substances (Liang et al. 2006, 2013), which were originally derived from the manure during the pre-incubation period. The capacity of biochar to adsorb organic compounds from the environment was proven from a study by Pietikäinen et al. (2000). As a result, the adsorbate could either block a portion of holes and surface area of biochar particles or occupy the exchangeable sites on the biochar and thus (1) prevent soil salts such as Na from being adsorbed on the pre-incubated biochar particles, and (2) prevent base cations such as calcium and magnesium originated from the biochar materials from releasing to the tested soil. These could restrict the enhanced effects of biochar especially that derived from rice straw on rice aboveground biomass on the experimental soil. However, these mechanisms might not affect the development of the root system because root growth could be influenced by (1) soil Na concentration and (2) some other soil nutrients, such as soil K concentration (Rahman et al. 2001; Acda et al. 2017). In salt-affected soil, rice crop could restrict root development and increase K uptake to optimize Na^+/K^+ ratio that are among adaptive responses of the crop (Reddy et al. 2017). More importantly, Backer et al. (2017) concluded that biochar tended to foster root growth in the early stage. These may explain the non-interactive effect of the two tested amendments, but significant effect of biochar on root biomass of rice crop (Figure 1(b)). Nevertheless, such the mechanisms are possible hypotheses and thus necessitate more studies.

Conclusions and implementation

Without cow manure, biochar addition to saline-sodic soil improved rice growth significantly than no-biochar addition, whereas a combination of biochar and cow manure through pre-incubation did not show an expected positive synergistic effect. Blocking and/or exchangeable-site occupancy could be the mechanisms involved in the non-synergistic effect. Improved soil P availability, exchangeable K, and CEC, especially the concentration of ECENa could be potentially responsible for the increased rice growth with biochar. The current study did not observe a reduced exchangeable Na concentration in the amended soil as a result of biochar and manure addition. Given that there are insufficient studies addressing the effects of biochar on rice growth on salt-affected soil and that the nature of a net-house pot experiment is within a short-term period, more studies are needed both in net-houses and on fields to fully clarify the interactive effect of biochar and animal manure on rice growth and yield on salt-affected soils.

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