Received: 12 May 2022 | Revised: 7 August 2022 | Accepted: 16 August 2022

DOI: 10.1111/sum.12842

RESEARCH PAPER

Soil Use and Management BRITISH SOCIETY OF SOIL SCIENCE WILEY

High biochar rates may suppress rice (*Oryza sativa*) growth by altering the ratios of C to N and available N to P in paddy soils

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Abstract

Although most studies have indicated that biochar can boost rice (*Oryza sativa*) growth, the material may also suppress it, depending on ratios of carbon (C) to nitrogen (N) and available N to available phosphorous (P). The current study sought to examine the impacts of biochar on rice growth and to identify underlying mechanisms. A pot experiment was conducted using two soils of high (3.05%) and low (0.54%) organic carbon (OC) content, mixed with 0, 1.5, 3, 6, and 12% biochar and planted with rice. Rice growth components, five rice tissue nutrients, and nine soil properties were measured. The results showed that the response of rice growth to biochar rates could be described using an exponential-growth function in high-OC soil but an inverted U-shaped curve in low-OC soil. In high-OC soil, the 12% biochar rate led to the greatest total biomass, increased by 47%, whereas in low-OC soil, the 3 and 6% rates exhibited the highest total biomass, increased by 44%, compared to the no-biochar added soils. Biochar elevated the C:N ratio from 11.5 to 39.1, with an optimal range of 20–30 corresponding to the highest rice growth. Biochar declined the ratio of NH_4-N to Mehlich-1 P, causing N deficiency. In brief, high biochar rates may suppress rice growth when the soil C:N ratio exceeds 30. The applied biochar rate should be considered based on soil properties typically OC and N content to obtain the C:N ratio between 20 and 30 for optimal rice growth.

KEYWORDS

biochar rate, C:N ratio, rice growth, soil nitrogen concentration, soil organic carbon

1 | INTRODUCTION

It is common to know that biochar addition can raise rice growth and yields in various environments, such as field conditions (Chen et al., 2021; Ghorbani et al., 2021; Huang et al., 2018; Liu et al., 2016) and greenhouse conditions (Kamara et al., 2015; Shetty & Prakash, 2020). The rice-improving effects of biochar can be mostly attributed to its influences on soil fertility. For example, Liu et al. (2016) reported that the enhanced grain yield by rice-straw biochar was involved in improved soil nutrient availability, such as available phosphorus (P) and potassium (K). The improved soil fertility by biochar addition that results in enhanced rice growth and yield could be summarized into adsorptive capability, water holding capacity, liming capacity, and nutrient retention (Singh Karam et al., 2021; Vijay et al., 2021). Biochar, on the other hand, being a carbon-rich material, may also lead to (1) nutritional unbalance, (2) a high carbon: nitrogen (C:N) ratio, and (3) a deficiency of some nutrients, such as N. The severity of these adverse consequences could be interactively dependent on the properties of the biochar-added soil. Nevertheless, these negative impacts of biochar have been discussed insufficiently in the literature, necessitating further research.

Many studies have been conducted to apply biochar at various rates, which are commonly <10% by weight (Feng et al., 2012; Knoblauch et al., 2011; Pratiwi & Shinogi, 2016; Singla, Dubey, et al., 2014; Tsai & Chang, 2021), and soil properties were changed relatively proportionally over the biochar rates. Mavi et al. (2018) applied 0, 10, 20, and 40 t ha^{-1}, equal to 0, 0.6, 1.2, and 2.4% by weight (assuming bulk density is 1.1 gcm^{-3}, and soil depth is 15 cm). The authors found that some soil properties such as electrical conductivity, pH, organic carbon (OC), dissolved OC, and available nutrients (N, P, and K) increased over the range of biochar rates. In contrast, Tsai and Chang (2021) applied various biochars at three rates of 0, 2, and 5 (%, wt) and found that the 5% biochar treatments substantially declined the concentrations of inorganic nitrogen (NO_3^--N and NH_4^+-N) in the tested soils. Moreover, in a review and meta-analysis, Nguyen et al. (2017) found that biochar addition can reduce inorganic nitrogen in soils by about 10%. The increase in some elements while depletion in others may lead to soil nutrient imbalance, suppressing rice growth and productivity. A higher rate of biochar application may accentuate the disparity between the two trends. These lead to our first research hypothesis that high biochar rates may suppress rice growth, depending on soil properties.

Nitrogen is a macronutrient for the rice crop, and its abundance is connected to the existence of OC, forming a strong linkage between the two elements (Pérez & Torres-Bazurto, 2020). The ratio of total C to total N has been studied and used as an indicator of soil properties and fertility (Radočaj et al., 2021; Zhang et al., 2016) to measure the mineralization and/or immobilization of soil organic matter. Overall, the C:N ratio can vary from 1 to greater than 50, depending on materials, such as soil, organic matter, plant residue, and animal-derived substances (Brust, 2019). A high C:N ratio may indicate that microbial immobilization could be the predominant process in the soil, lowering the concentration of plant-available N and vice versa. Being a C-rich material, biochar can raise the C:N ratio of the biochar-added soil, resulting in predominantly N microbial immobilization. Consequently, the high C:N ratio by biochar addition may restrict rice growth due to nitrogen deficiency, but this is not reported sufficiently in the literature. This leads to our second hypothesis that biochar addition can elevate the C:N ratio to a level that inhibits rice growth.

In addition, the impact of biochar on the C:N ratio of the tested soil and consequent rice growth could be dependent on soil properties especially OC and N status. Soil with a high-OC content may have a better buffering capacity than that with a low-OC content (Jiang et al., 2018). As a result, the effects of biochar could be weaker in high-OC soil than in low-OC soil. Haefele et al. (2011) found that the fertile and poor soils responded to biochar addition differently. Furthermore, high-OC soil may also have a higher concentration of total nitrogen than low-OC soil. Adding C-rich biochar to low-OC soil may raise the C:N ratio faster than to high-OC soil. These result in our third hypothesis that rice growth responds to the biochar rate more rapidly in low-OC soil than in high-OC soil. Therefore, the current study was conducted using two soils of different organic carbon content and applied with five biochar rates. The current study sought to examine the response of rice growth to biochar application and to identify underlying mechanisms.

2 | MATERIALS AND METHODS

2.1 | Experimental materials

Two soils with different OC concentrations (3.05 and 0.54%) were taken from two paddy fields in Phuoc Thạnh commune, Cu Chi District, Ho Chi Minh City, Vietnam (11°01′N and 106°26′E). The paddy field with a high-OC content was located in a relatively low area and the field with low-OC content was located in an upper area. Soil from these two fields was classified as a Haplic Acrisol (WRB, 2015). The fields had a long history of rice cultivation of more than 10 years, with two rice seasons every year. The fields are located in a tropical monsoon climate with two distinct seasons, the rainy season from May to October and the dry season from December to April. The average annual rainfall in the region is around 1868 mm and the average temperature is around 27.4°C. Soils were taken from 10 sites per field in the surface layer of 0–15 cm depth. The obtained soil from these sites was mixed thoroughly to make two composite samples for the two fields. The collected soil was transferred to a laboratory, air-dried, and sieved to pass through a 2-mm sieve to remove plant residues and gravel before the experiment.

For the current study, rice husk was used to produce biochar using the method by Nguyen, Le, et al. (2018) with a pyrolysis temperature of roughly 350°C. The rice husk was chosen because it is a byproduct of rice production that is widely available in many rice-producing countries, such as Vietnam, which generates an estimated 8.7 million tonnes of rice husk annually (Tong et al., 2018). The rice husk, after being collected from rice milling, was washed with tap water, air-dried for a few days, and oven-dried at 70°C overnight before pyrolysis. The produced biochar was stored until it was used for the experiment. Some main properties of the biochar and examined soils were shown in Table 1 and chemical functional groups of biochar were shown in Figure S1.

2.2 | Experimental setup

In a greenhouse, a pot experiment with two experimental factors and three replicates was set up as a completely randomized design. The first experimental factor consisted of two soils with two levels of OC concentrations (3.05 and 0.54%) (hereafter called high-OC soil and low-OC soil). The second factor included five biochar rates, which were 0, 1.5, 3, 6, and 12% (w:w) biochar. Some other studies used up to 8.3% biochar (Singla, Inubushi, & Environment, 2014), 9.8% biochar (Singla, Dubey, et al., 2014), and 10% biochar-compost amendment (Luo et al., 2017). The current study used the highest biochar rate of 12% because we targeted to increase the C:N ratio of the tested soil over 30, which was obtained in the low-OC soil (Figure 2c). The two experimental factors were crossed to form 10 treatments in total (two soils × 5 biochar rates).

The 10 treatments were established in three replicates by mixing the sieved soils with biochar at 0, 1.5, 3, 6, and 12% biochar and repacking the biochar-soil mixtures (hereinafter referred to as experimental soil) into 30 pots (20 cm × 20 cm, diameter x height) with some gentle tamps to achieve a uniform soil volume in all pots (around 3.6–4.6 kg of experimental soil used for each pot depending on biochar rates and soils). The height of the soil column in each pot was around 15 cm tall, saving around 5 cm from the top of the pot for standing water. The 30 experimental pots were randomly placed in an experimental greenhouse with temperatures varying from 25°C (night) to 36°C (day), no direct rain or wind, and 70–80% humidity. The pots were filled with tap water to a depth of 3–5 cm—water for 10 days before direct seeding of germinated Jasmine 85 rice seeds. The water depth in each pot was maintained between 2 and 5 cm during the 3.5-month life cycle of the rice crop.

2.3 | Measurements

2.3.1 | Rice growth

Rice growth was determined by harvesting rice biomass (the weight of root, stem, and grain) from 30 pots using the procedure applied by Nguyen, Trinh, et al. (2018) after the experiment. The stem weight including the stem, leaves, tillers, and panicles (but not the grain) was determined by collecting the above-ground parts into a plastic bag. Grain weight was determined by collecting the grains from all panicles of individual pots into plastic bags. Water from experimental pots was then decanted, and soil samples were taken from the 0–10 cm surface layer using a soil

TABLE 1 Selected properties of the experimental materials

Materials	Statistics	Clay (%)	Silt (%)	Sand (%)	OC (%)	total N (%)	C:N ratio	NH_4-N (mg kg^{-1})	Mehlich-1 P (mg kg^{-1})	Exch. K (mg kg^{-1})	Exch. Ca (mg kg^{-1})	Exch. Mg (mg kg^{-1})	pH	CEC (mol[c] kg^{-1})
High-OC soil	Mean	21.10	19.25	59.65	3.04	0.22	13.69	53.60	118.15	147.73	426.47	69.61	5.75	11.93
	SE	0.11	0.26	0.35	0.03	0.01	0.27	1.18	2.45	4.58	18.18	0.94	0.08	0.34
Low-OC soil	Mean	7.05	6.06	86.89	0.54	0.05	10.32	33.36	158.21	114.94	158.26	62.32	6.10	6.82
	SE	0.19	0.20	0.06	0.004	0.0005	0.07	0.75	1.98	5.97	12.17	1.91	0.09	0.85
Biochar	Mean				34.36	0.56	63.26	9.51	3363.72	1911.27	164.08	84.69	9.02	23.56
	SE				0.77	0.06	6.69	0.60	46.84	319.31	4.06	1.98	0.11	1.24

Note: Ash content of the biochar was 49.1%. OC, organic carbon; Exch., exchangeable; CEC, Cation exchange capacity; *n* = 4.

sampler for chemical analysis. The residual soil from individual pots was spread out on a plastic sheet and rinsed with tap water to collect root biomass. The collected biomass (grain, stem, and root) was weighed for assessment after being oven-dried at 70°C to a constant weight (Chaimala et al., 2020; Zhang et al., 2019).

2.3.2 | Soil and biochar samples

Before the experiment, the sieved soils and biochar were sampled in four replicates for chemical analysis. After the experiment, about 1 kg of experimental soil (0–10 cm) from all 30 experimental pots was collected, air-dried, and sieved to pass a 2-mm sieve before chemical analysis.

2.3.3 | Chemical analyses of rice tissue

The harvested dried stem biomass was ground to pass through a 20-mesh sieve for chemical analysis for nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), and manganese (Mg) (Kumar et al., 2021; Urmi et al., 2022). The total N in rice tissue was determined using the Kjeldahl method (Horneck & Miller, 1998). The tissue concentration of P, K, Ca, and Mg was determined using the method by Isaac and Johnson (1998).

2.3.4 | Chemical analyses of soil and biochar

All soil and biochar samples were analysed for OC, total nitrogen (N), NH_4^+-N, Mehlich-1 P, exchangeable K, Ca, and Mg, pH, and cation exchange capacity (CEC). The concentration of OC and total N was measured using the dry combustion method with an elemental analyser (Elementar Analysensysteme GmbH, Hanau, Germany) (Knoblauch et al., 2021). The concentration of NH_4^+-N was measured with 2 M KCl, and the concentration of Mehlich-1 P was determined using the Mehlich-1 method (Carter & Gregorich, 2008). The concentration of exchangeable K, Ca, and Mg was measured using the $BaCl_2$ method, and the extract was analysed using an inductively coupled plasma-optical emission spectrometry (Carter & Gregorich, 2008). The pH of all samples was determined using a Thermo Scientific™ Orion™ 3-Star Benchtop pH meter after the samples were added with distilled water in a 1:5 (w/w) ratio for 1 h. CEC was determined using the ammonium acetate method (Nguyen & Lehmann, 2009). In addition, the sieved soils were analysed for particle size distribution (Carter & Gregorich, 2008). The ash content of biochar was determined using the combustion method at 650°C overnight. Fourier transform infrared (FTIR) analysis was applied on the tested biochar to examine their functional groups using the method developed by Li et al. (2020) on a Jasco FT/IR-4700 type A spectrophotometer.

2.4 | Statistical analysis

The OC to total N ratio (C:N ratio) and the NH_4-N concentration to the Mehlich-1 P concentration ratio (available N:P ratio) were computed for the assessment of the impact of biochar on rice growth and soil properties. All data and ratios were statistically analysed, following the procedure of the analysis of variance (ANOVA) of a completely randomized design with two factors, using JMP 16 (SAS Institute Inc, North Carolina, USA). When the ANOVA result showed a significant effect at $p \leq .05$, the Tukey's honestly significant difference test was used to classify treatment means. Linear and nonlinear regression analyses were performed to examine dependent patterns of rice growth and soil properties on biochar rates. The linear and nonlinear models were determined based on the shape of the scatter plot, r^2 value (coefficient of determination), and a 95% confidence level ($p \leq .05$). All figures were established using Sigmaplot 14.0 (Systat Software Inc., San Jose, California).

3 | RESULTS

3.1 | Rice growth and tissue nutrient concentration

In high-OC soil, with an increase in biochar rates from 0 to 12%, the above-ground weight of rice increased from 30.5 to around 48.3 (g DWT pot^{-1}) (Figure 1). In low-OC soil, the above-ground weight of the crop reached its maximum magnitude at biochar rates between 3 and 6%, and a further increase and/or a decrease in biochar rate led to a decline of the above-ground weight (Figure 1a). Similarly, total rice biomass in high-OC soil significantly elevated from 35.3 to 51.7 (g DWT pot^{-1}), corresponding to biochar rates at 0 and 12%, respectively (Figure 1b). In low-OC soil, biochar rates of 3 and 6% produced the highest total rice biomass of around 45.5 (g DWT pot^{-1}). The highest rate (12%) and the lowest rate (0 and 1.5%) resulted in the lowest total rice biomass. Figure 1c and d showed the real image, including the root system and above-ground biomass, of rice after the experiment. In high-OC soil, biochar rates of 6 and 12% exhibited the biggest biomass volume, and in low-OC soil, biochar rates of 3 and 6% had the greatest biomass volume, of the five biochar rates.

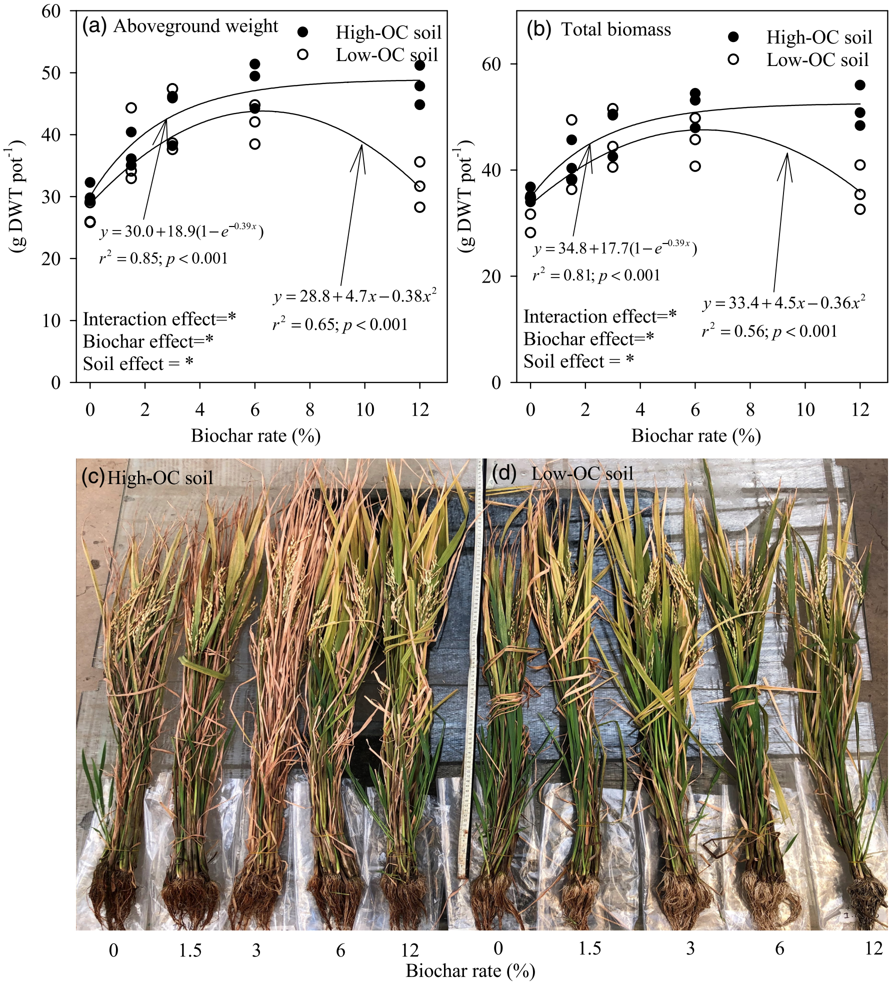

FIGURE 1 Above-ground weight (a), total biomass (b), and real image (c, d) of rice in the two tested soils as affected by biochar addition. * indicates the associated effect is significant

Overall, biochar addition significantly decreased the N concentration in rice tissue, and the decreasing magnitude was dependent on soil (Table 2). In high-OC soil, the tissue N concentration declined from 20.04 to 16.78 (g kg^{-1}), and in low-OC soil, the concentration decreased from 17.58 to 15.32 (g kg^{-1}) at 0 and 12% biochar rates, respectively. The interaction effect of biochar and soil on the concentration of tissue P, K, Ca, and Mg was not significant. The tissue P concentration was significantly enhanced by biochar addition, from 3.2 to 4.1 (g kg^{-1}) in high-OC soil and from 3.1 to 4.4 (g kg^{-1}) in low-OC soil at 0 and 12% biochar rates, respectively. The tissue K and Ca concentration was not significantly affected by soil and biochar rate. The tissue Mg concentration was significantly affected by biochar addition.

3.2 | Soil properties

The interaction effect of biochar and soil on OC and total N concentration was not statistically significant, while that on the C:N ratio and NH_4-N concentration was significant (Figure 2). Biochar addition substantially raised the OC concentration with an increasing rate (the slope of the simple linear regression equation) of 0.28 and 0.22 (%) for every percent of added biochar in high-OC soil and low-OC soil, respectively (Figure 2a). The increasing rate of the total N concentration in the two tested soils was around 0.003 (%) for each percent of additional biochar (Figure 2b). Biochar addition dramatically increased the C:N ratio, and the increasing rate was much faster in the low-OC soil (an increasing rate of 2.2 units for every 1% of added biochar) than in the high-OC soils (an increasing rate of 0.97 units). The NH_4-N concentration rapidly declined from 63.0 to 40.5 (mg kg^{-1}) in high-OC soil and from 35.6 to 23.4 (mg kg^{-1}) in low-OC soil, when the biochar rate was increased from 0% to 6%, respectively. The NH_4-N concentration was levelled off when the biochar rate was further increased to 12% in both soils.

The concentration of Mehlich-1 P and exchangeable Ca was significantly affected by the interaction of biochar and soils (Figure 3a, c). The increasing rate of Mehlich-1 P concentration in low-OC soil was 32.6

TABLE 2 The elemental concentration in rice tissue (g kg^{-1})

Soil	Biochar rate (%, w:w)	N	P	K	Ca	Mg
High-OC soil	0	20.04^{ab}	3.20^{ab}	14.82	1.53	0.99a
		(0.59)	(0.24)	(0.87)	(0.06)	(0.02)
	1.5	21.13^{a}	3.34 ^{ab}	15.85	1.56	0.77^{ab}
		(0.39)	(0.29)	(0.12)	(0.09)	(0.02)
	3	18.40^{bc}	3.55 ^{ab}	16.52	1.60	0.78^{ab}
		(0.56)	(0.25)	(0.21)	(0.07)	(0.02)
	6	18.42^{bc}	3.70 ^{ab}	16.50	1.55	0.68^{ab}
		(0.33)	(0.18)	(0.78)	(0.03)	(0.06)
	12	16.78^{cde}	4.15^{ab}	17.28	1.59	0.71^{ab}
		(0.27)	(0.17)	(0.89)	(0.09)	(0.03)
Low-OC soil	0	17.58^{cd}	3.10^{b}	14.71	1.76	0.87^{ab}
		(0.14)	(0.23)	(0.47)	(0.14)	(0.06)
	1.5	16.46^{cde}	3.52 ^{ab}	16.45	1.71	0.74^{ab}
		(0.11)	(0.23)	(0.72)	(0.29)	(0.08)
	3	15.20^{e}	3.32 ^{ab}	15.90	1.71	0.75^{ab}
		(0.47)	(0.15)	(1.03)	(0.18)	(0.11)
	6	15.76^{de}	4.07 ^{ab}	17.46	1.75	0.65^{b}
		(0.41)	(0.58)	(0.74)	(0.18)	(0.06)
	12	15.32^{e}	4.16^{a}	16.59	1.82	0.61^{b}
		(0.42)	(0.49)	(1.22)	(0.2)	(0.07)
Effect tests						
Soil × biochar interaction		*	NS	NS	NS	NS
Soil effect		*	NS	NS	NS	NS
Biochar effect		*	*	NS	NS	*

Note: The numbers in the parenthesis are the standard deviation of the mean, $n = 3$. Within a column, data attached with the same letter (a, b, c, d, and e) were not significantly different from each other. * and NS indicate the associated effect is significant and not significant, respectively.

(mg kg^{-1}) for each percent of added biochar which was much greater than that (7.3 mg kg^{-1}) in high-OC soil (Figure 3a). The exchangeable Ca concentration in high-OC soil decreased exponentially but that in low-OC soil did not change significantly over the range of tested biochar rates (Figure 3c). Biochar addition greatly enhanced the exchangeable K concentration by 39 (mg kg^{-1}) for each percent of biochar addition in high-OC soil and 36 (mg kg^{-1}) in low-OC soil. Biochar and soil had no impact on the exchangeable Mg concentration (Figure 3d). An increase in biochar rate from 0 to 12% led to an enhanced pH from 6.0 to 6.6 in high-OC soil and from 6.2 to 6.9 in low-OC soil. CEC rose from 8.3 to 12.3 (cmol[c] kg^{-1}) in high-OC soil and from 5.6 to 8.6 (cmol[c] kg^{-1}) in low-OC soil, when the biochar rate was raised from 0 to 12%, respectively. While the pH was much greater in low-OC soil than in high-OC soil, the CEC was much lower in low-OC soil than in high-OC soil.

3.3 | Inter-relationships

Figure 5 showed that total rice biomass was positively correlated with OC, total N, C:N ratio, Mehlich-1 P, exchangeable K, pH, and CEC, while inversely correlated with NH_4-N and exchangeable Ca in high-OC soil. The biomass also had a positive connection with P but a negative connection with N and Mg in rice tissue. In low-OC soil (Figure 6), total rice biomass had a significantly inverted U-shaped relationship with some soil properties such as OC, C:N ratio, Mehlich-1 P, exchangeable K, pH, and CEC, which was best described using a quadratic polynomial model. Total rice biomass was inversely linked to the tissue N and NH_4-N concentration in low-OC soil while having a positive correlation with tissue P and K.

With an increase in the C:N ratio from 13.6 to 19.8, total rice biomass in high-OC soil rose from 35.3 to 51.8 (g DWT pot^{-1}), and a further increase in the C:N ratio did not lead

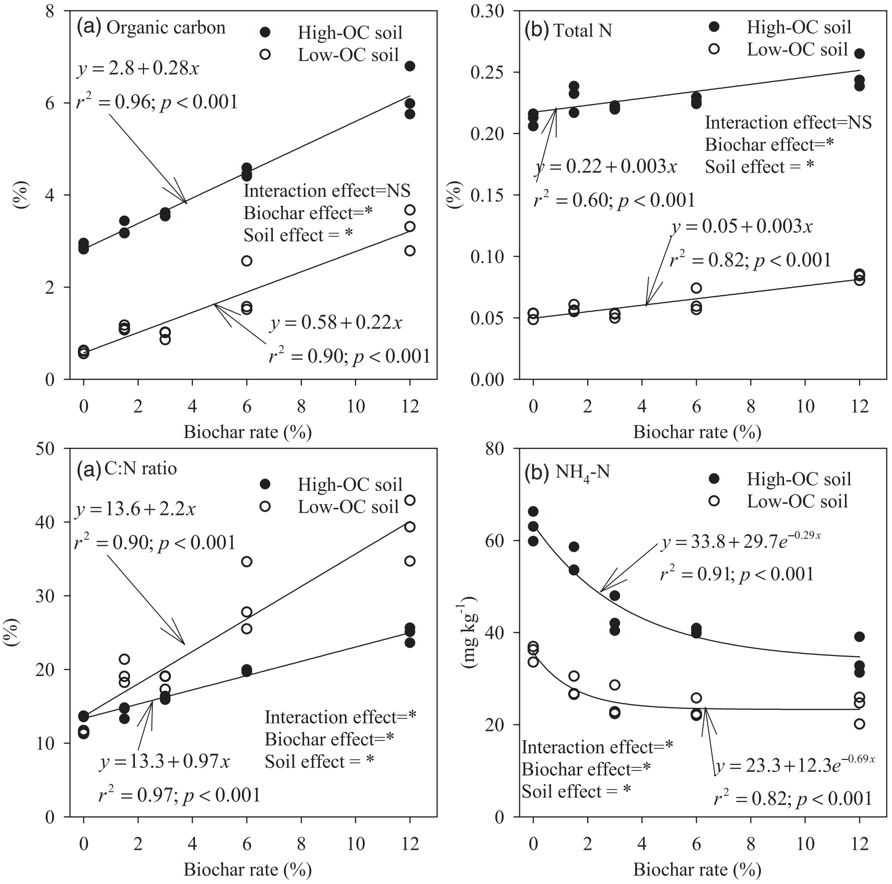

FIGURE 2 Soil OC (a), total N (b), C:N ratio (c), and NH_4-N concentration (d) in the two tested soils after the experiment as affected by biochar addition. * and NS indicate the associated effect is significant and insignificant, respectively

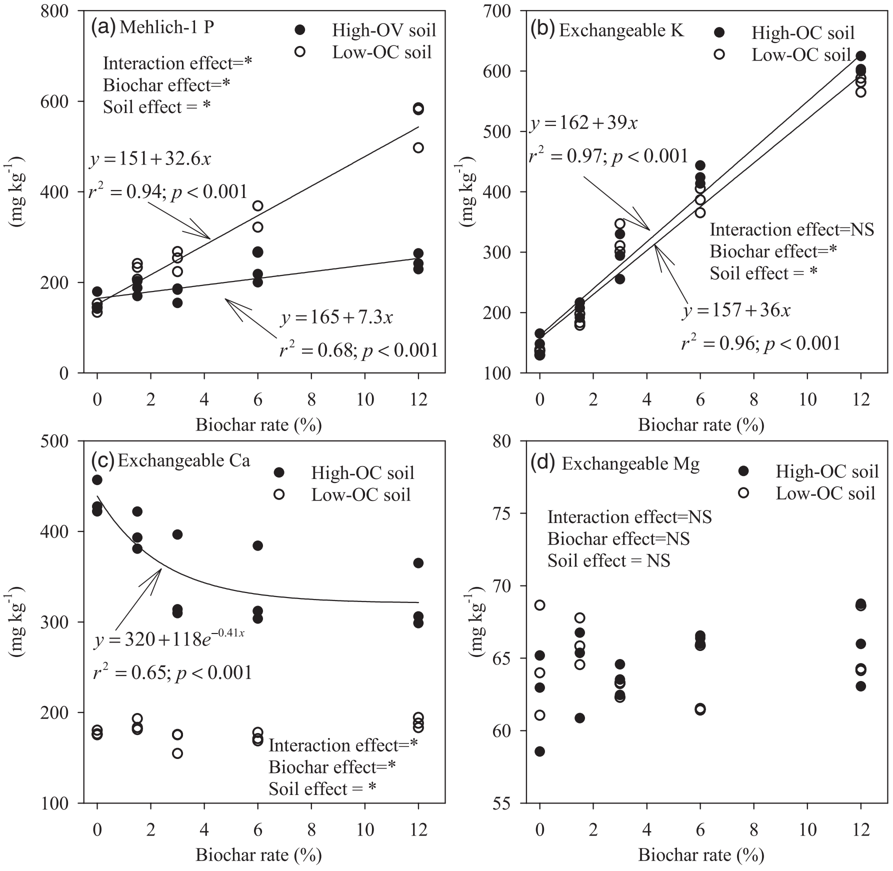

FIGURE 3 The concentration of Mehlich-1 P, exchangeable K, Ca, and Mg of the two tested soils after the experiment as affected by biochar addition. * and NS indicate the associated effect is significant and insignificant, respectively

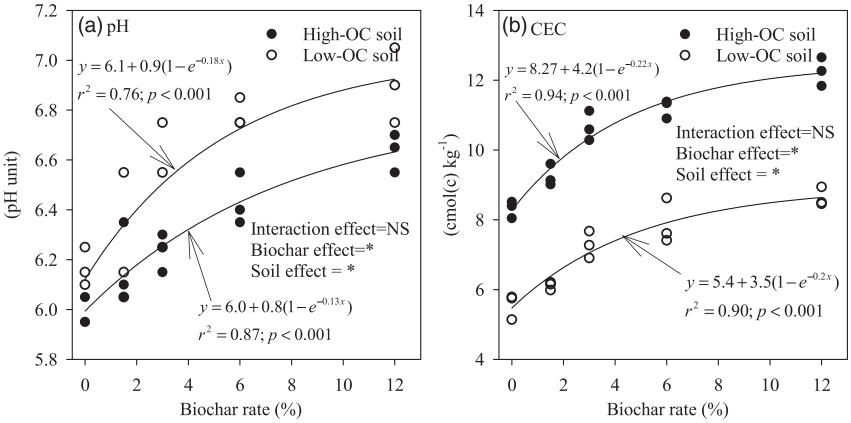

FIGURE 4 The value of pH and CEC of the two tested soils after the experiment as affected by biochar addition. * and NS indicate the associated effect is significant and insignificant, respectively

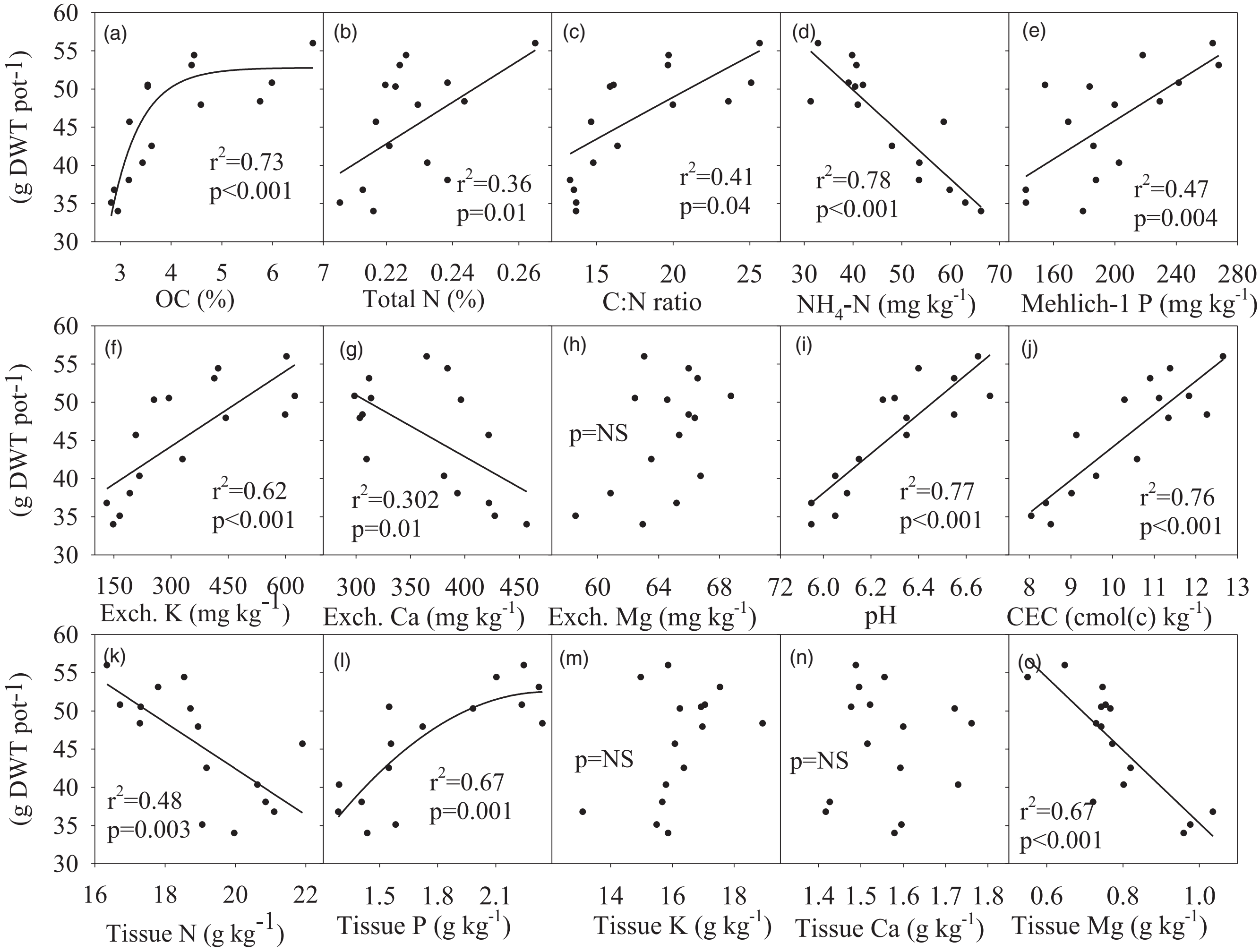

FIGURE 5 Relationship between total biomass and measured parameters of soil and rice tissue in the high-OC soil. Exch., exchangeable; *p* = NS indicates the relationship is not significant

to a considerable enhancement of the biomass (Figure 7a). In contrast, a reduction in the available N:P ratio from 0.41 to 0.14 led to improved total rice biomass from 35.3 to 51.8 (g DWT pot^{-1}). In the low-OC soil (Figure 7b), rice reach its highest magnitude of total rice biomass, when the C:N ratio increased from 11.5 to 18.5 and 29.3. A further rise in the C:N ratio resulted in a reduction of total rice biomass. When the available N:P ratio was around 0.08–0.10, total rice biomass exhibited the greatest value of around 45 (g DWT pot^{-1}).

4 | DISCUSSION

An interesting finding from the current study was shown in Figure 1 that total rice biomass increased to a maximum

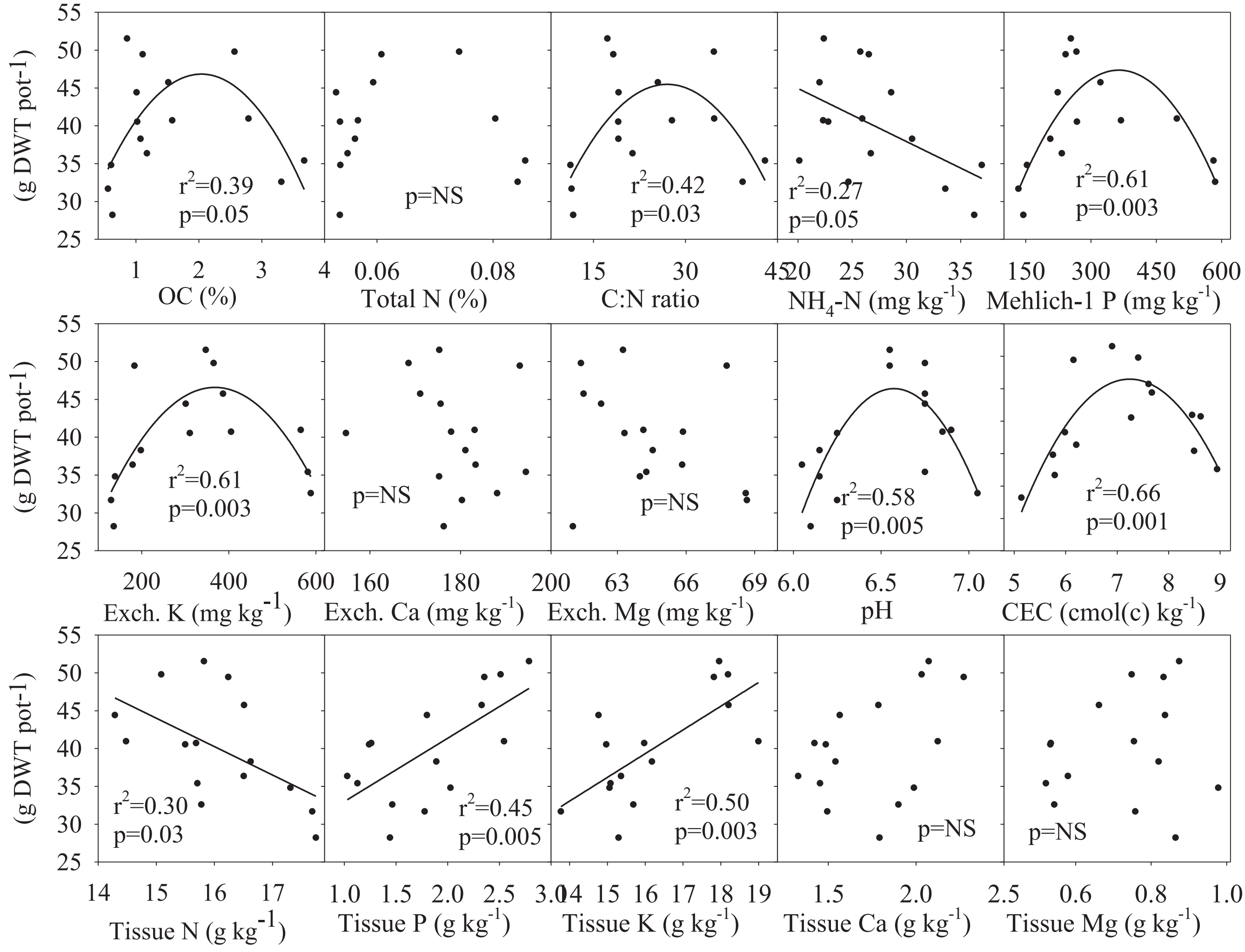

FIGURE 6 Relationship between total biomass and measured parameters of soil and rice tissue in the low-OC soil. Exch., exchangeable; p = NS indicates the relationship is not significant

level of around 46 (g DWT pot^{-1}) at biochar rates of 3 and 6% in low-OC soil, but it increased exponentially to about 52 (g DWT pot^{-1}) at the highest biochar rate of 12% in high-OC soil. The finding confirms our first hypothesis that the highest biochar rates may suppress rice growth, especially in low-OC soil. Many studies reported that biochar addition promoted rice growth in various environments (Chen et al., 2021; Ghorbani et al., 2021; Huang et al., 2018; Shetty & Prakash, 2020). We were unable to find any other study that showed similar results to the current study. The reason may be involved in the used biochar rates, which were much lower in the previous studies than in the current study. The difference in the responsive pattern of total rice biomass over the range of biochar rates in both soils could be determined by the C:N ratio and the available N:P ratio, which are discussed in the following parts.

It was obvious that the addition of high-C biochar (34.36% of C, Table 1) greatly enhanced OC concentration in both tested soils, compared to the no-biochar addition (Figure 2a). Another notable finding from the current study was an improvement in total N concentration (Figure 2b) but a reduction in soil NH_4-N concentration (Figure 2d) and rice tissue N concentration (Table 2) in both soils following biochar addition. An increase in total N in soils is in line with earlier studies (Zhang et al., 2017; Zhang et al., 2020) and could be attributed to the N amount contained in the added biochar. Biochar addition raised the total N concentration while decreasing the NH_4-N concentration, suggesting that a large portion of the increased total N could be inaccessible for rice. The reduced NH_4-N concentration in soils may be mainly attributed to the biochar's NH_4-N adsorption with its maximum capacities found to vary from 4.7 to 44 (mg N g^{-1}) (Kizito et al., 2015; Mathurasa & Damrongsiri, 2018; Pratiwi et al., 2016). Biochar can adsorb NH_4-N on its surface through chemical functional groups such as carboxylic, hydroxyl, lactone, and lactol groups which were demonstrated to be the cause of the reduced NH_4-N concentration in the biochar-added soils (Nguyen et al., 2017; Tsai & Chang, 2021). Some chemical functional groups such as carboxylic, hydroxyl, aliphatic CH, and aromatic were also

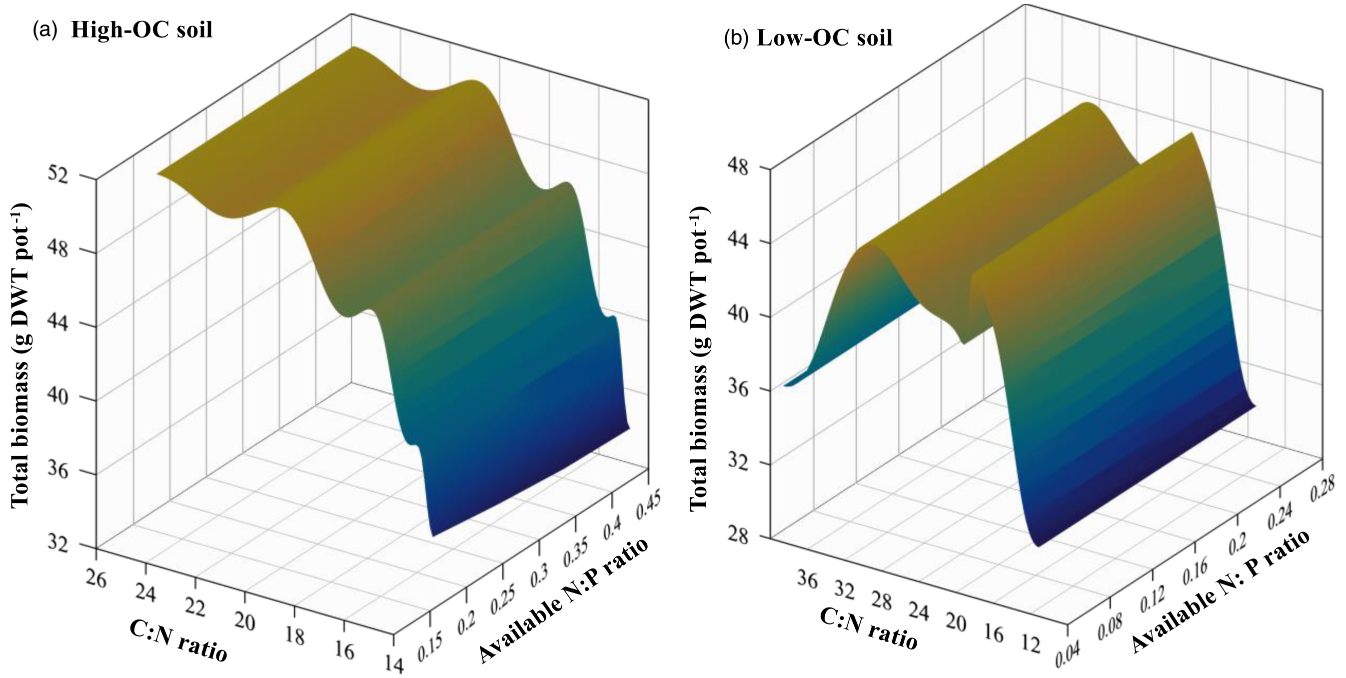

FIGURE 7 Total rice biomass in relation to the two ratios of C:N and NH_4-N: Mehlich-1 P (available N:P ratio)

found in the biochar used for the current study (Figure S1). The adsorptive capacity of biochar-added soil could additionally be enhanced by the increased pH (Figure 4a), which may contribute to the elevation of CEC due to the formation of more negative charges (Hailegnaw et al., 2019). In addition, the reduction in NH_4-N could be attributable to N microbial immobilization (Nguyen et al., 2017) as a result of the increased C:N ratio (Figure 2c). On the other hand, soil with higher OC concentration and clay content should have greater buffering capacity than soil with lower OC concentration. High-OC soil with a greater buffering capacity may replenish more NH_4-N into the available pool, which was depleted by biochar's adsorption, making the changing pattern of NH_4-N concentration over the range of biochar rates different between the two tested soils (Figure 2d). The tissue N concentration was proportionally linked with the NH_4-N concentration but insignificantly connected with the total N concentration in the two tested soils (data not shown), indicating that the NH_4-N concentration in soils may determine the N uptake of the rice crop. This might explain why biochar addition drastically lowered the N concentration in rice tissue, as shown in Table 1. These findings suggest that the improved rice growth by biochar addition in the two tested soils could be involved in the C and N elements.

Many studies have shown that biochar addition increased the concentration of plant-available P (Glaser & Lehr, 2019; Yang et al., 2021) and K (Farrar et al., 2021; Rasuli et al., 2021), which were comparable to the current study (Figure 3a, b). Moreover, the current study also showed that the enhanced rate of Mehlich-1 P in the low-OC soil was substantially greater than that in the high-OC soil (Figure 3a) and two possibilities could be responsible for the difference. The first one may be involved in the greater buffering capacity, which was found to be strongly correlated with soil organic carbon (SOC) and clay content (Curtin & Trolove, 2013), of the high-OC soil than the low-OC soil. Consequently, the added biochar-P could be more immobilized in the high-OC soil than in the low-OC soil. The second one may be involved in the elevated pH caused by biochar addition due to the alkaline nature of the material (Fidel et al., 2017). The increased soil pH value may lead to an enhancement of the Mehlich-1 P concentration because a considerable portion of P, which was fixed by aluminium and iron, could be solubilized, when pH rises (Ch'ng et al., 2014; Johan et al., 2021). Consequently, with a greater enhancement in pH (Figure 4a), low-OC soil exhibited a greater improved Mehlich-1 P concentration than the high-OC soil as a result of biochar addition. The increased magnitude of exchangeable K in the biochar-added soil could be primarily derived from the added biochar, which contained a greater K content than the two tested soils (Table 1).

The increase in tissue P (Table 2) could be the consequence of the enhanced Mehlich-1 P concentration in soil, which showed a strong and positive relationship with tissue P (data not shown). Consequently, there was a strong linkage between biochar rate, plant-available P in soils, and tissue P. While the exchangeable Mg concentration in the two tested soils was unaffected by biochar addition, the Mg concentration in rice tissue decreased with biochar rates, which was not clearly understood. One possibility may be related to the antagonistic effects between K and Mg (Ding et al., 2006); the increased exchangeable K concentration in soils (Figure 3b) may diminish the uptake of Mg by rice, leading to a reduction of Mg in rice tissue. These findings additionally revealed that the available P content in soil could be one of a few factors contributing to the determination of rice growth in the current study.

The above findings and discussion strongly suggest that the effects of biochar on rice growth could be involved in elemental N (total N and NH_4^+-N) and available P (Mehlich-1 P) in the biochar-added soils. This argument was further reinforced by strong inter-relationships between total rice biomass and total N, NH_4-N, Mehlich-1 P, tissue N, and tissue P in the high-OC soil (Figure 5) and NH_4-N, Mehlich-1 P, tissue N, and tissue P in the low-OC soil (Figure 6). Furthermore, OC, an important organic factor in regulating soil functions, fertility, and productivity (Schjønning et al., 2018), exhibited a substantial inter-relationship with total rice biomass in the two tested soils (Figures 5a, 6a). Therefore, the C to N ratio (total C to total N) and available N to P ratio (NH_4-N to Mehlich-1 P) could be key factors in determining the impacts of biochar on rice growth. The current study found that the averaged C:N ratio increased from 13.6 to 24.8 in high-OC soil and from 11.5 to 39.1 in low-OC soil when the biochar rate was increased from 0 to 12% (Figure 2c). The range of C:N ratio of 20–30 was attributed to being the balancing state of mineralization and immobilization (Brust, 2019), which may result in balanced N nutrient for rice to grow. The exponential growth pattern of total rice biomass over the C:N ratio range from 13.5 to 24.8 in high-OC soil (Figure 7a) could be attributable to a relatively low C:N ratio (<30) in the biochar-added soil. Likewise, the reduced rice biomass in the low-OC soil applied with 12% biochar compared to that applied with 3 and 6% (Figure 7b) might be explained by an increase in the C:N ratio from 30.3 to 39.1, which confirmed our second hypothesis. The highest C:N ratio (39.1) could be characterized by a dominant process of microbial immobilization of inorganic N. Consequently, the NH_4-N concentration decreased as the C:N ratio rose (data not shown). These findings imply that the C:N ratio plays a critical role in determining biochar's influence on rice growth from paddy soils and that the optimal C:N ratio for rice growth could range from 20 to 30.

After the experiment, the high-OC soil exhibited a C:N ratio varying from 13.6 to 24.8 while the low-OC soil had a ratio ranging from 11.5 to 39.1. This indicates that the C:N ratio of low-OC soil changed more greatly than that of high-OC soil. On average, total rice biomass changed at a rate of 2.42 (g DWT pot^{-1}) for 1% of added biochar in the high-OC soil, but at a rate of 2.71 (g DWT pot^{-1}) in the low-OC soil. These results support our third hypothesis that rice growth in low-OC soil responds more quickly to biochar addition than in high-OC soil. The rationale for the finding could be related to the buffering capacity, which could be greater in high-OC soil than in low-OC soil (Jiang et al., 2018). This also implies that the effects of biochar on rice growth may be interactively determined by soil properties, particularly SOC status.

As total rice biomass was substantially connected with NH_4-N, Mehlich-1 P, and tissue P in the two tested soils, total rice biomass could be also determined by the ratio of available N (NH_4-N) to available P (Mehlich-1 P) (available N:P ratio) (Figure 7a, b). The available N:P ratio in soil was found to influence soil microbial communities and enzyme activities from a long-term experiment applying N fertilizer (Shen et al., 2019). The available N:P ratio in the current study was relatively low, varying from 0.14 to 0.41 in the high-OC soil and 0.05 to 0.25 in the low-OC soil when compared to the other soils (Griffiths et al., 2012). Rice crop, in general, requires balanced N and P nutrients in tissue, and the N:P ratio in rice tissue could vary from 3.1 to 7.8 (Wang et al., 2019; Ye et al., 2014). The current study found that the tissue N:P ratios ranged from 4.1 to 6.3 in the high-OC soil and 3.5 to 5.7 in the low-OC soil, both of which were lower than the mean values reported by (Wang et al., 2019; Ye et al., 2014). The N and P concentrations in rice tissue in the current study were from 15.2 to 21.1 and 3.1 to 4.4 (g kg^{-1}), respectively. The tissue N range in the current study was much lower than that reported by (He et al., 2017), while the tissue P range in the current study was comparable to those reported by Ye et al. (2014). These findings suggest that soil nitrogen may be a deficient nutrient in the two tested soils, more seriously in the low-OC soil and that the available N:P ratio in soils played an important role in influencing total rice biomass.

The findings from the current study suggest that total rice biomass can reach its maximum level when the C:N ratio falls between 20 and 30, which could be considered an optimal range for rice growth. Biochar addition can elevate the C:N ratio, and if the elevated ratio exceeds this optimal range, rice growth may be suppressed. Biochar addition, on the other hand, lowered the available N:P ratio in the two tested soils, leading to a decline in the N:P ratio in the rice tissue. The findings from the current study also suggest that the applied biochar rate should be based on soil characteristics, typically SOC, in order to gain the soil C:N ratio within the optimal range. Soil with a great concentration of N can be applied with high biochar rates because the high N content in soil may lower the increasing effects of biochar on the C:N ratio, compared to the soil with lower N concentration. Furthermore, inorganic N fertilizer combined with biochar can be added to the paddy soils to reduce the C:N ratio while raising the N:P ratio for better rice growth. In addition, the 6 and 12% biochar treatments in the current study may be impractical to apply in paddy fields due to the great quantity of biochar required. Nonetheless, the inclusion of these two highest biochar rates may have some scientific significance that the responsive pattern of rice growth could be determined based on a wide range of biochar rates, including the two highest rates. High biochar rates may induce great costs while resulting in a relatively small increase or even decrease in rice growth when compared to lower biochar rates. This suggests that, for the practical application, it may require more studies to examine the optimal biochar rates, which would lead to the highest rice yield but the lowest biochar costs. Based on the findings of the current study, the optimal rate is estimated to fall within the range of 2–3% biochar.

5 | CONCLUSIONS

The current study demonstrated that biochar addition had a significant influence on rice growth and the influential magnitude and pattern were strongly dependent on soil and biochar rate. In high-OC soil, the effects of biochar addition on rice growth may be modelized using an exponential-growth function with a maximal increase in total rice biomass by 47% obtained at the highest biochar rate of 12%. Meanwhile, in low-OC soil, total rice biomass was raised following an inverted U-shaped curve over the biochar rates with a maximal increase by 44% at biochar rates of 3 and 6%, compared to the no-biochar added soils. The 12% biochar rate applied to low-OC soil exhibited a reduction in rice growth, compared to the 3 and 6% biochar rates. The primary mechanisms behind these effects could be strongly involved in the C:N ratio and available N:P ratio (NH_4-N to Mehlich-1 P ratio) in the two tested soils. In high-OC soil, biochar addition raised the C:N ratio to a maximum of 24.5, while in low-OC soil, biochar elevated the ratio to a value of 39.1. The optimal C:N ratio for rice growth was identified to be between 20 and 30. Biochar addition also declined the NH_4-N concentration while raising the Mehlich-1 P concentration, leading to a reduction in the available N:P ratio in the two soils. These findings indicate that the effects of biochar addition on rice growth can be determined by the C:N ratio and available N:P ratio in paddy soils, and that high biochar rates may cause N deficiency for rice growth. The selected biochar rate to apply should vary with soil properties, typically SOC, or be supplementarily combined with appropriate inorganic N fertilizer rates to achieve the optimal C:N ratio for better rice growth.

ACKNOWLEDGEMENTS

This research is funded by the Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 105.08-2019.341. The authors would like to thank the Institute of Environmental Science, Engineering, and Management (IESEM), Industrial University of Ho Chi Minh City (IUH) for supporting the current study. Many thanks are given to the staff and students of IESEM for their assistance with the field trip and lab work.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Nguyen, B. T., Nguyen, V. N., Nguyen, T. X., Nguyen, M. H., Dong, H. P., Dinh, G. D., Van Nguyen, N., & Pham, T.-V. (2022). High biochar rates may suppress rice (*Oryza sativa*) growth by altering the ratios of C to N and available N to P in paddy soils. *Soil Use and Management*, *00*, 1–14. https://doi.org/10.1111/sum.12842