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RESEARCH ARTICLE

Responses of soil–plant C, N, and P concentrations and stoichiometry to contrasting application rates of biochar to subtropical paddy field

Qiang Jin[1,2], Weiqi Wang[1], Xu Song[1], Jordi Sardans[3,4], Xuyang Liu[1], Shaoying Lin[1], Akash Tariq[5,6], Fanjiang Zeng[5,6] and Josep Peñuelas[3,4]

[1]Key Laboratory of Humid Sub-tropical Eco-Geographical Process of Ministry of Education, Fujian Normal University, Fuzhou, China, [2]College of Resources and Environmental Science and Engineering, Hubei University of Science and Technology, Xianning, China, [3]CSIC, Global Ecology Unit CREAF-CSIC-UAB, 08913 Bellaterra, Catalonia, Spain, [4]CREAF. 08913 Cerdanyola del Vallès, Catalonia, Spain, [5]State Key Laboratory of Desert and Oasis Ecology, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi, China and [6]Cele National Station of Observation and Research for Desert-Grassland Ecosystems, Cele, China

Corresponding authors: Weiqi Wang; Email: wangweiqi15@163.com; Jordi Sardans; Email: j.sardans@creaf.uab.cat

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Summary

Biochar is increasingly used in crop production as a fertilizer; however, its effects on nutrient cycling and stoichiometry in rice paddy soil–plant systems are unclear. We tested for effects of contrasting rates of biochar on soil and rice plant organ carbon (C), nitrogen (N), and phosphorus (P) concentrations and stoichiometry and soil physicochemical properties in early and late paddies. Overall, biochar reduced soil bulk density by an average of 7.4%, while application at 10, 20, and 40 t ha^{-1} increased soil C and N concentrations in early paddies by 31.6, 41.3, and 104.2%, respectively, and by 8.0, 5.0, and 21.8%, respectively; in late paddies, there were increases of 23.0, 94.1, and 117.0%, respectively, and 6.7, 15.4, and 18.0%, respectively ($P < 0.05$). Following biochar application at 10, 20, and 40 t ha^{-1}, soil concentration of P decreased in early paddies by 10.9, 19.0, and 13.9%, respectively, and increased in late paddies by 4.3, 16.4, and 20.1%, respectively. Biochar increased ratios of soil C:N and C:P in early and late paddies ($P < 0.05$), and there was no effect on concentration and stoichiometry of soil available nutrients. Biochar reduced rice plant organ concentration of N and P in early rice and increased leaf N:P ratios. Despite the biochar application improved nutrient status in plant–soil system, we did not observe a significant increase in yield ($P > 0.05$). According to the N:P value of leaves between treatments, it was found that biochar alleviated the current situation of N limitation in paddy fields during the mature period and transformed the N limitation of early rice into a joint limitation of N and P. These results show that the addition of biochar to subtropical paddy soils leads to a short-term reduction in soil bulk density and increases in soil C and N concentrations and soil fertility. Thus, biochar applied at optimal rates is likely to improve the sustainability of subtropical paddy rice production.

Keywords: Biochar; carbon; nitrogen; Paddy field; phosphorus; stoichiometry

Introduction

Biochar is a carbon (C)-rich product formed from the pyrolysis of biomass (Sun *et al*., 2016a), and the application of biochar to soils could increase C fixing, indicating its potential role in the mitigation of climate change (Lehmann, 2007). In addition, biochar may increase levels of soil pH

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and soil organic matter concentration and promote biochemical cycling of soil nitrogen (N) and phosphorus (P) to improve soil fertility and availability of nutrients to plants (Abiven *et al.*, 2015; Hossain *et al.* 2020). However, negative impacts of biochar applications to soils have been reported, including on crop growth. For example, Feng *et al.* (2021) found that the application of 3% biochar restricted growth of rice and nitrogen use efficiency, while field growth of wheat was inhibited following soil applications of 4.5% oak biochar (Aguilar-Chávez *et al.*, 2012), likely as a result of reduced nutrient utilization and release of high levels of salt concentrations and phytotoxic chemicals from the biochar (Aguilar-Chávez *et al.*, 2012). While impacts of biochar on forest and dryland agricultural ecosystems tend to be well studied (Herrmann *et al.*, 2019; Zhang *et al.*, 2019a), impacts on rice paddy field ecosystems, with heterogeneous water and fertilizer management regimes, remain less clear (Zhang *et al.*, 2012). Currently, most of the research is on single-season planting, while the research on double-season planting is insufficient (Das *et al.*, 2020). Thus, there is an urgent need to understand the effects of applications of biochar to paddy soils, as a possible tool to improve the sustainability of rice, as a global staple food crop.

Biological and environmental stoichiometric ratios of elements, such as in organisms and soils (Cleveland and Liptzin, 2007), allow a greater understanding of the influence of plant–soil interactions on nutrient cycling and limitation of geochemical elements (Mooshammer *et al.*, 2014; Zechmeister-Boltenstern *et al.*, 2015). The principal elements of soils, carbon (C), nitrogen (N), and phosphorus (P) drive balances in ecosystem productivity (Elser *et al.*, 2007), as they represent a large proportion of dry matter concentration of plants (C) (Ågren, 2008) and are essential for plant growth (N and P) (Elser *et al.*, 2007). Thus, stoichiometric ratios of soil C, N, and P indicate ecosystem structure and function (Liu *et al.*, 2017). However, soil C, N, and P stoichiometry is affected by complex natural and human factors (Zhang *et al.*, 2013), where soil C:P and C:N reflect the variation in C sequestration capacity of plants with nutrient availability and plant growth rate (Ågren, 2004; Sun *et al.*, 2016b). Given soil–plant C, N, P stoichiometric ratios vary with geographic region (Hu *et al.*, 2018), exogenous inputs (Shen *et al.*, 2019), soil and vegetation type (Yu *et al.*, 2018), and, in agroecosystems, with crop species (Wang *et al.*, 2016a), particularly in the context of climate change (Tian *et al.*, 2019). It is likely that applications of biochar to agricultural soils affect soil–plant concentrations and stoichiometry of C, N, and P.

Paddy rice is a staple food for more than 60% of the global population; paddy rice cultivation in China covers an area of 28.4 million hm^2, accounting for nearly 30% of global paddy production (IRRI, 2009).While the application of biochar as a soil improver may improve the soil–plant N-P cycle, effects on paddy soil–plant C, N, and P are unclear (Li *et al.*, 2017) and impacts on wider paddy soil physicochemical properties, such as soil bulk density, soil salinity, nutrient availability, stoichiometry, and rice yields, are required exploration. For example, following the application of biochar to paddy soils, concentration of soil organic matter has been shown to increase, with no effect on soil available P (Chen *et al.*, 2020), along with increases in crop yields, due to higher levels of soil pH (Wang *et al.*, 2018a). But analysis of impacts on stoichiometry has tended to focus on the separate components of plants and soils (Shen *et al.*, 2019; Zhang *et al.*, 2019b), rather than the soil–plant system. Therefore, the objectives of this study were to quantify the responses of subtropical paddy soil properties and soil–plant C, N, and P concentrations and stoichiometry to contrasting biochar application rates as a potential tool to improve the sustainability of paddy rice production.

Materials and Methods

Study sites

The experimental paddy field study site was located at the Rice Research Institute of the Fujian Academy of Agricultural Sciences at Wufeng (26.1°N, 119.3°E), where the climate conditions are subtropical maritime monsoon, with an annual average temperature and precipitation of 19.6 °C and 800–1500 mm, respectively, and an annual frost-free period of 330 d (Fig. S1). The study site is located on an alluvial plain, where surface soils (0–15 cm) comprise 12, 28, and 60% clay, sand, and silt, respectively, with a pH of 6.5, organic C, total N, and total P concentration of 18.16, 1.93, and 1.80 g kg^{-1}, respectively, and soil available P, NH_4^+-N, and NO_3–N concentration of 87.48, 24.91, and 4.51 mg kg^{-1}, respectively (Wang *et al.*, 2016b). Rice production in the region is characterized by an early-late paddy-vegetable rotation, and we cultivated 'Hesheng No. 10' (conventional) and 'Qinxiangyou 212' (sterile hybrid) from April 21, 2016 to July 6, 2016 and from July 28, 2016 to October 31, 2016, respectively. Rice plants were machine-inserted into soils at 14 × 28 cm spacings, with nursery periods of early and late paddies of about 1 month and 15 days, respectively. Compound fertilizers ($N:P_2O_5:K_2O = 16\%:16\%:16\%$) were applied prior to transplantation at 42, 40, and 40 kg ha^{-1}, respectively, at the splitting stage, 1 week after transplantation at 35, 20, and 20 kg ha^{-1}, respectively, and then at panicle formation (8 weeks after transplantation) at 18, 10, and 10 kg ha^{-1}, respectively (Wang *et al.*, 2017). The water management in the rice-growing period is to implement flooding management in the early stage of rice and implement a combination of roasting field, flooding, and moist irrigation after the tillering period.

Experimental design

We established three replicate 10-m^2 plots of three biochar treatments (10, 20, and 40 t ha^{-1}) and an untreated control that were arranged at random and each surrounded by 0.5 cm thick and 30 cm high PVC boards; plots were separated by a 1-m wide buffer. The biochar was prepared by slow pyrolysis at 600 °C for 90 min from rice straw, average temperature rise rate was between 3 and 5 ° C min−1, and the biochar particle size was 0.8 and 1.0 mm. Biochar nutrient information is provided in Table S1. Before application, a small amount of water was added to mix well biochar and soil. The biochar was screened through a 2-mm sieve before application. Further, it was added to 0–15 cm of soil on the first day before early and late rice transplanting in the same studied area.

Soil sampling and analysis

Soil samples from the 0 to 15 cm layer were collected at the splitting, jointing, flowering, and mature growth from three locations within each plot using a corer. Samples were bulked in a ziplock bag to form a composite sample per plot before they were taken to the laboratory in a portable incubator where plant residues and impurities were removed; then, soil samples were divided into two, with one portion stored at 4 °C and the other air-dried prior to analysis.

Soil total C (TC) and N (TN) concentrations were determined using a CN element analyzer (ElementarVario MAX CN, Hanau, Germany), and soil total P (TP) concentration was analyzed, following digestion using the $HCIO_4$-H_2SO_4 method, in a continuous flow analyzer (San++, Skalar Corporation production, Breda, Netherlands). Dissolved organic C (DOC) was extracted using deionized water (water-to-soil ratio of 4:1); after centrifugation and shaking, the solution was filtered through a 0.45-μm filter membrane and DOC was measured using a TOC analyzer (TOC-VWP; Shimadzu, Kyoto, Japan). Available N (AN) was extracted using 2 mol L^{-1} of KCl and measured using a continuous flow analyzer (San++, Skalar Corporation production, Breda, Netherlands), while available P (AP) was extracted from a Mehlich III extract and measured using a continuous flow analyzer (Carter and Gregorich, 2007).

Soil salinity and temperature were measured in the field using a salinity/temperature meter (2265FS, Spectrum Technologies Inc., Paxinos, USA). Soil pH was determined using a water-to-soil mass ratio of 2.5:1, shaken for 30 min and left for 30 min with a pH meter (STARTER 300, Parsippany, USA). Bulk density was measured using three 15 × 3 cm cores (Wang *et al.*, 2016a) and was estimated by core mass dry weight divided by core volume and represent the averaged bulk density of 0–15 cm. We measured iron (Fe) concentration, following leaching with 0.5 mol L^{-1} of HCl for 24 h, using o-phenanthroline colorimetry; then, Fe^{3+} was reduced to Fe^{2+} using hydroxylamine hydrochloride (Lu, 1999).

Plant sampling and analysis

Mature rice plants (three plants were collected for each treatment) from the early and late paddies were collected at 92 and 106 days after transplantation, stored in a portable refrigerator, and taken to the laboratory. Then, the root, stem, and leaf material of the rice plants were dried at 70 °C to a constant weight, milled using a grinder, passed through a 100-mesh sieve, and sealed in a plastic bag prior to analysis. Concentrations of C and N of the plant parts were measured using an elemental analyzer (CHNOS, Elemental Analyzer Vario EL III, Germany), and P concentration was measured, following digestion with $HClO_4$-H_2SO_4, using a continuous flow analyzer (San++, Skalar Corporation production, Breda, Netherlands).

Statistical analyses

We tested for treatment differences using one-way ANOVA, and associations between soil–plant nutrients and soil properties were tested using Pearson's correlation analysis in SPSS 20.0 (SPSS Inc., Chicago, IL, USA). We mainly conducted repeated measurement analysis of variance on nutrient factors and growth period of rice and regression fitting relationship of soil nutrient factors. Redundancy analysis (RDA) was performed on each indicator and environmental factor using Canoco 5.0 software (Microcomputer Power, Ithaca, USA). We used Pearson's correlation analysis in the corrplot packages of R to test for associations between soil element concentrations and stoichiometry and soil environmental factors.

Results

Soil physicochemical properties

The application of biochar at 40 t ha^{-1} increased soil salinity in early and late paddies ($P < 0.05$), and there were contrasting effects of biochar on pH between early and late paddies; overall, there were no within-season effects of biochar on soil temperature, bulk density, or pH (Fig. 1). Biochar application can increase soil Fe^{2+} concentration and decrease soil Fe^{3+} concentration (Fig. 2). The application of biochar at 40 t ha^{-1} increased soil Fe^{2+} concentration in rice flowering period ($P < 0.05$). We found growing season differences in soil temperature, salinity, pH, bulk density, and concentrations of Fe^{2+}, Fe^{3+}, and total Fe ($P < 0.05$, Table S2).

Soil carbon, nitrogen, and phosphorus concentrations

In early paddy of the growth period, concentration of TC and TN was greater following addition of biochar, while concentration of TP was decreased. In late paddy of the growth period, concentration of TC, TN, and TP was greater following the application of 20 and 40 t ha^{-1} of biochar ($P < 0.05$, Fig. 3). Effects of biochar treatment on soil TC and TP concentrations varied with rice growth stage in early and late paddies and on soil TN in late paddies ($P < 0.05$, Table S3).

There were no overall effects of biochar on DOC, AN, or AP, with the exception of lower levels of AP following the application of 10 t $ha^{-1}$of biochar ($P < 0.05$; Fig. S2). Effects of biochar on soil AN varied with rice growth stage in late paddies ($P < 0.05$, Table S3).

There were positive associations between soil TN and TC in early and late paddies ($P < 0.01$), and for soil TP with TC ($P < 0.01$) and TN ($P < 0.05$) in late paddies; soil TP was negatively associated with TC ($P < 0.05$) and TN in early paddies ($P < 0.01$) (Fig. 4).

Soil nutrient stoichiometry

The application of biochar at 20 and 40 t ha^{-1} tended to increase C:N and C:P ratios in early and late paddy soils and increased N:P ratios in early paddy soils ($P < 0.05$, Fig. 5). With the exception

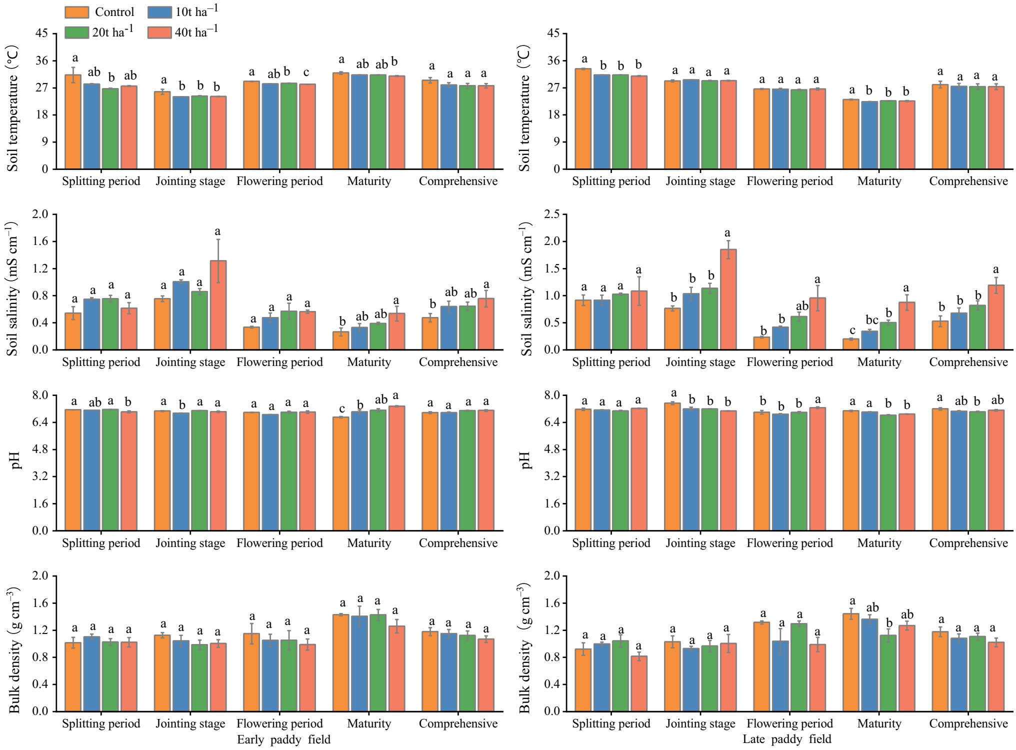


Figure 1. Effects of rate of biochar on soil physicochemical properties within rice growth stages in early and late paddies. Data are means ±SE; different lowercase letters indicate within-growth stage treatment differences at $P < 0.05$.

of effects on N:P ratios in late paddy, effects of biochar on nutrient ratios varied with rice growth stage ($P < 0.01$, Table S4).

With the exception of ratios of DOC:AP in early paddy soils, where the application of biochar at 20 t ha^{-1} reduced DOC:AP ratios in early paddy soils ($P < 0.05$), there were no effects of biochar on ratios of soil DOC, AN, and AP in early or late paddy soils (Fig. S3). Effects of biochar on ratios of AN:AP varied with rice growth stage in late paddy soils ($P < 0.05$, Table S4).

Association between environmental conditions and soil nutrient stoichiometry

There was a greater number of associations between environmental variables and soil nutrients in paddy fields (Fig. 6). Soil concentration of TC was positively correlated with salinity ($P < 0.05$) and Fe^{2+} ($P < 0.01$) and negatively correlated with Fe^{3+} ($P < 0.05$), while soil concentration of TN was negatively correlated with pH ($P < 0.05$) and soil concentration of TP was positively correlated with salinity, Fe^{3+}, and total Fe ($P < 0.05$); ratios of soil C:N were positively associated with salinity and Fe^{2+} ($P < 0.01$) and negatively correlated with Fe^{3+} ($P < 0.05$), while ratios of soil C:P were positively associated with Fe^{2+} ($P < 0.01$) and negatively correlated with Fe^{3+} ($P < 0.05$), and ratios of soil N:P were negatively correlated with salinity, Fe^{3+}, and total Fe ($P < 0.05$).

We know from RDA that Fe^{2+} and Fe^{3+} were the main environmental factors associating with soil nutrients and their stoichiometric ratios in paddy fields ($P < 0.05$, Fig. 6).

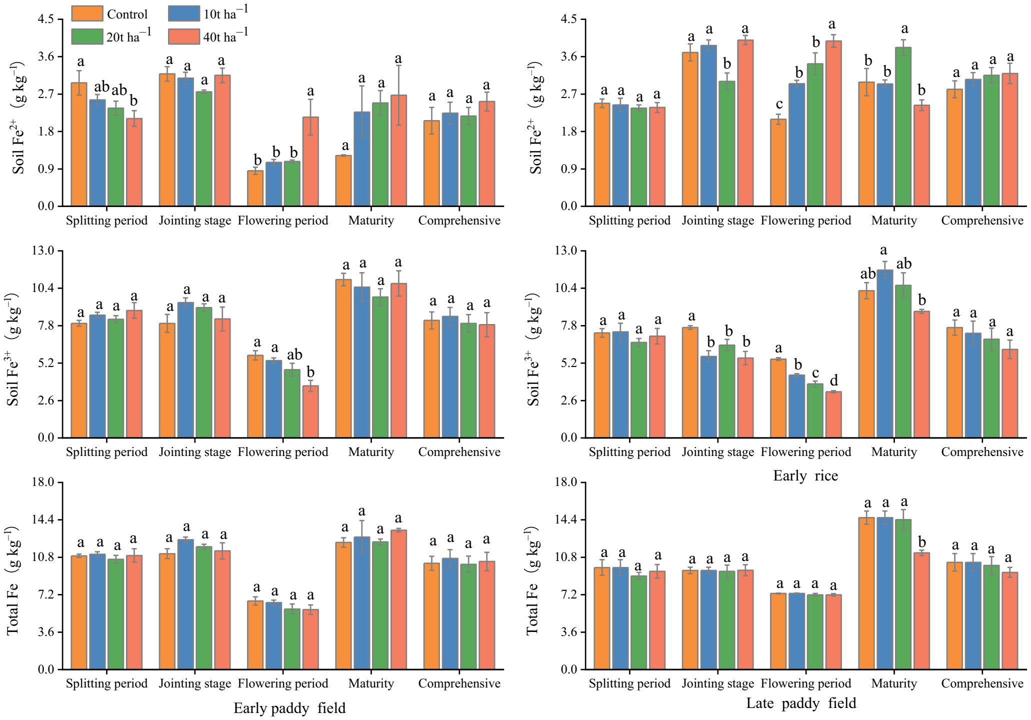


Figure 2. Effects of rate of biochar on soil Fe^{2+}, Fe^{3+}, and total Fe within rice growth stages in early and late paddies. Data are means ±SE; different lowercase letters indicate within-growth stage treatment differences at $P < 0.05$.

Plant nutrient concentrations and stoichiometry

Effects of rate of biochar on nutrient concentration and stoichiometry of plant organs were inconsistent between early and late paddies (Table 1). In early paddy, 20 t ha^{-1} of biochar reduced N concentration of stem and leaf material and increased P concentration of root and leaf material ($P < 0.05$), whereas in late paddy, this treatment increased concentration of N and P of stem material and P concentration of leaf material. In late paddy, 40 t ha^{-1}of biochar increased stem and leaf concentration of N and root and stem concentration of P ($P < 0.05$).

In early paddy, the application of biochar at 20 and 40 t ha^{-1} increased root and stem ratios of C:N, while application at 10 t ha^{-1} increased stem C:N, C:P, and N:P ratios ($P < 0.05$). In late paddy, the application of biochar at 20 and 40 t ha^{-1} reduced stem and leaf ratios of C:N and the application of each of the three rates of biochar reduced stem ratios of C:P; the application of 10 and 20 t ha^{-1} of biochar reduced stem N:P ratios ($P < 0.05$).

Relationship between plant and soil nutrient stoichiometry

Soil TC was positively correlated with root TP and root C:N ratio, stem TP, and leaf TN ($P < 0.05$) and negatively correlated with stem TC ($P < 0.05$) and leaf C:N ratio ($P < 0.01$), while soil TN was positively correlated with root C:N ratio ($P < 0.01$) and soil TP was positively correlated with stem TN and TP and leaf TN ($P < 0.05$) and negatively correlated with leaf C:N ratio ($P < 0.05$) (Table 2).

Ratios of soil C:N were positively correlated with stem TP and leaf TN ($P < 0.05$) and negatively associated with stem TC ($P < 0.01$), stem TC:P ratios ($P < 0.05$), and leaf C:N ratios ($P < 0.01$); soil C:P ratios were positively correlated with root C:N ratios ($P < 0.01$) and negatively

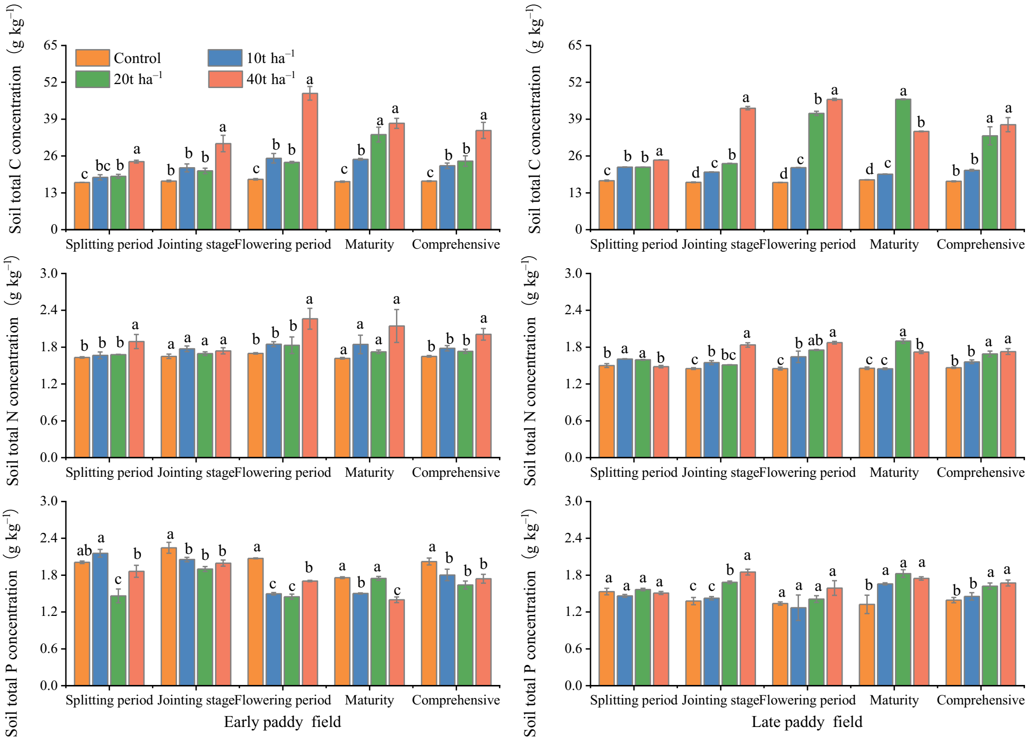


Figure 3. Effects of rate of biochar on concentrations of soil C, N, and P within growth stages in early and late paddies. Data are means ±SE; different lowercase letters indicate within-growth stage treatment differences at $P < 0.05$.

correlated with leaf TC and leaf C:N ratios ($P < 0.05$), and soil N:P ratios were positively associated with root C:N ratios ($P < 0.01$) (Table 2).

Rice yields

The application of biochar at 10 t ha^{-1} increased early and late rice yields by 15.7 and 16.9%, respectively, while application at 40 t ha^{-1}reduced yields by 17.3 and 3.8%, respectively ($P > 0.05$, Fig. 7).

Discussion

Effects of biochar rate on paddy soil physicochemical properties

In this short-term study, the application of biochar reduced soil bulk density, consistent with previous research (Herath *et al.*, 2013), likely due to its low bulk density (Bhogal *et al.*, 2009). Iron is a trace element necessary for plant growth and development (Hussain *et al.*, 2019). We found that the soil concentration of Fe^{3+} gradually decreased from the splitting growth stage to flowering, before increasing during mature rice growth, likely reflecting the transition from wet to dry paddy conditions. Under flooded conditions, Fe^{3+} reduces to Fe^{2+} that is fixed as iron oxide and released into soil pore water. In contrast under dry, aerobic conditions, Fe^{2+} is gradually oxidized to Fe^{3+} (Sun *et al.*, 2019).

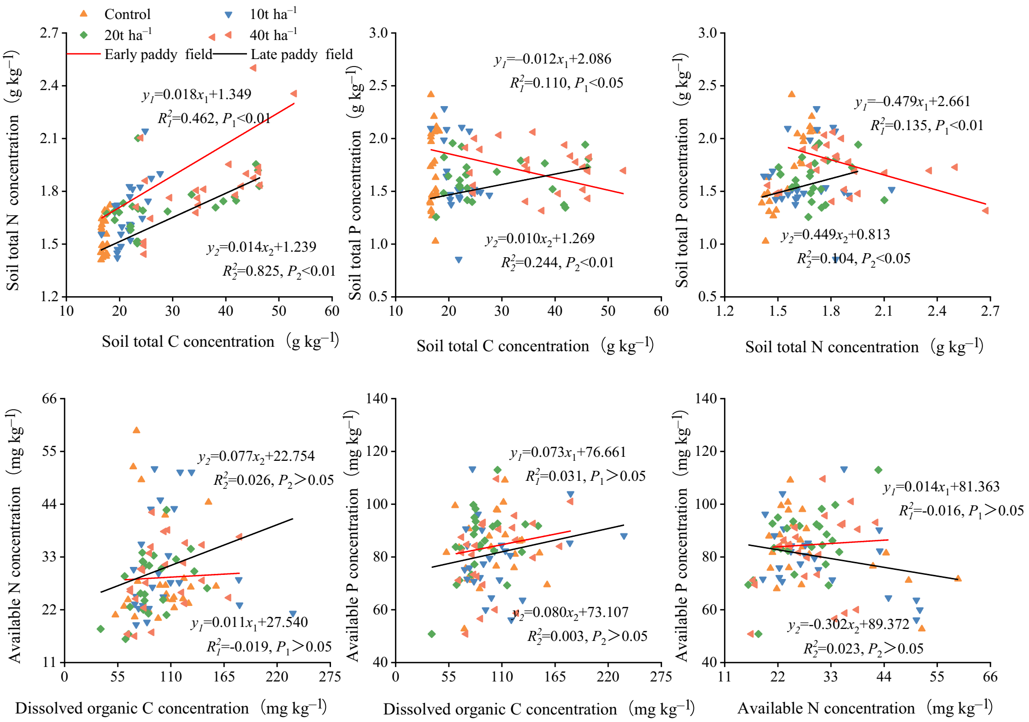


Figure 4. Regression analysis between soil C, N, P, DOC, available N, and available P in early and late paddies.

Effects of biochar rate on paddy soil nutrient concentrations

In this study, the application of biochar increased paddy soil concentration of C and N due to its concentration of unstable C and N that is subsequently converted to soil organic C and N (Liang *et al.*, 2014). The concentration of N produced from low-nutrition lignocellulosic raw materials leads to short-term increases in soil N (Gul and Whalen, 2016; Luo *et al.*, 2011). In addition, the large number of carbonaceous bonds with complex cross-linking networks in biochar that represents a more persistent form of C than preexisting organic C (Bhaduri *et al.*, 2016; Knicker *et al.*, 2013). We found that effects of biochar on soil P concentrations contrasted between early and late paddies, where P concentration was decreased in early paddy soils and increased in late paddy soils. This may be due to incomplete digestion of P by $HClO_4$ after biochar application and possible sorption of P by residual biochar (Mukherjee and Zimmerman, 2013; Takaya *et al.*, 2016), the timing of application and soil concentration of coexisting anions and other nutrients (Qian *et al.*, 2013). The chemical composition and surface characteristics of the biochar, as alkaline biochar is known to convert P from mobile to recalcitrant pools (Chintala *et al.*, 2014), and can absorb the from rock weathering and leaching (Lü *et al.*, 2015) that are particularly prevalent in subtropical regions.

Soil available nutrients are easily absorbed by plants during growth and indicate soil quality (Dong *et al.*, 2012; Su *et al.*, 2019). In this study, there was no effect of biochar on soil concentration of DOC, AN, or AP (Fig. 4), showing that, although biochar fixes C (Lehmann, 2007), it does not increase the short-term availability of soil nutrients but suppose the short-term increase of N and P soil storing capacity. In addition, the pore structure of biochar provides a good habitat for soil microorganisms, increases nutrient availability, and promotes nutrient absorption by rice (Hossain *et al.*, 2020). Biochar may decelerate the short-term release of available nutrients,

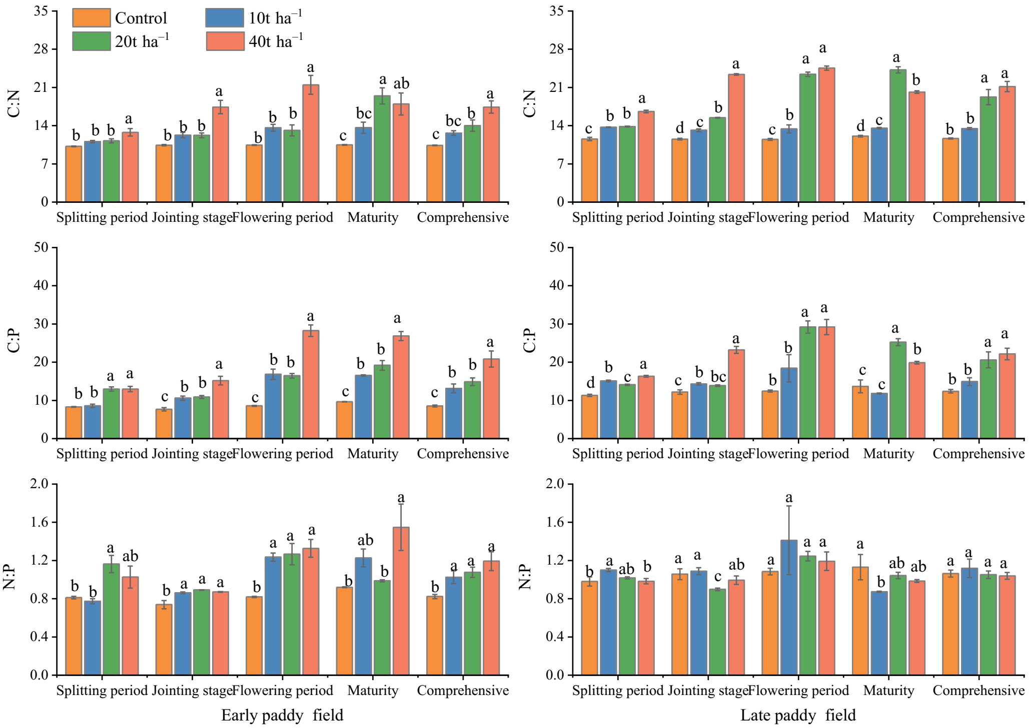
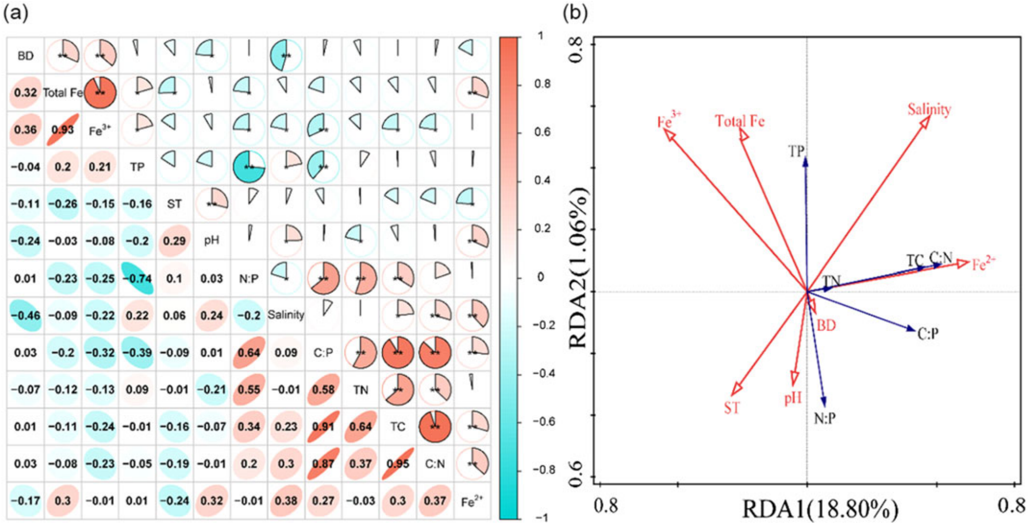


Figure 5. Effects of rate of biochar on soil C:N, C:P, and N:P ratios within growth stages in early and late paddies. Data are means ±SE; different lowercase letters indicate within-growth stage treatment differences at $P < 0.05$.



BD												
0.32	Total Fe											
0.36	0.93	Fe^{3+}										
−0.04	0.2	0.21	TP									
−0.11	−0.26	−0.15	−0.16	ST								
−0.24	−0.03	−0.08	−0.2	0.29	pH							
0.01	−0.23	−0.25	−0.74	0.1	0.03	N:P						
−0.46	−0.09	−0.22	0.22	0.06	0.24	−0.2	Salinity					
0.03	−0.2	−0.32	−0.39	−0.09	0.01	0.64	0.09	C:P				
−0.07	−0.12	−0.13	0.09	−0.01	−0.21	0.55	−0.01	0.58	TN			
0.01	−0.11	−0.24	−0.01	−0.16	−0.07	0.34	0.23	0.91	0.64	TC		
0.03	−0.08	−0.23	−0.05	−0.19	−0.01	0.2	0.3	0.87	0.37	0.95	C:N	
−0.17	0.3	−0.01	0.01	−0.24	0.32	−0.01	0.38	0.27	−0.03	0.3	0.37	Fe^{2+}

Figure 6. Pearson's correlation (a) and redundancy (b) analysis of association between soil nutrients and soil properties. (ST: soil temperature; BD: bulk density; * $P < 0.05$; **$P < 0.01$).

Table 1. ANOVA of biochar differences in rice plant organ nutrient concentration and ratios in early and late paddies. Data are means ±SE; different lower case letters indicate differences at $P < 0.05$

		Early paddy			Late paddy		
	Treatment	Root	Stem	Leaf	Root	Stem	Leaf
C	CK	93.62 ± 19.89a	336.32 ± 1.70a	301.22 ± 9.42a	126.84 ± 24.04a	300.73 ± 6.80a	291.71 ± 12.60a
	10 t ha^{-1}	91.64 ± 19.44a	312.10 ± 6.36a	311.12 ± 11.53a	75.64 ± 14.87a	298.75 ± 5.21a	300.46 ± 3.22a
	20 t ha^{-1}	101.21 ± 12.39a	311.17 ± 11.46a	288.90 ± 6.33a	118.91 ± 34.63a	271.80 ± 18.80a	274.07 ± 21.03a
	40 t ha^{-1}	151.37 ± 36.91a	318.16 ± 7.27a	278.83 ± 12.39a	160.74 ± 31.95a	293.22 ± 11.05a	305.43 ± 8.49a
N	CK	3.87 ± 0.45a	8.69 ± 0.17a	10.63 ± 0.26a	4.65 ± 0.88a	6.66 ± 0.38c	8.58 ± 0.39b
	10 t ha^{-1}	3.33 ± 0.45a	6.90 ± 0.15b	10.26 ± 0.81ab	3.07 ± 0.45a	7.91 ± 0.18bc	9.49 ± 0.77b
	20 t ha^{-1}	3.23 ± 0.15a	5.98 ± 0.03b	9.05 ± 0.16b	4.11 ± 0.82a	8.73 ± 1.10ab	13.00 ± 0.13ab
	40 t ha^{-1}	4.38 ± 0.94a	6.48 ± 0.58b	10.28 ± 0.14ab	5.42 ± 0.38a	10.06 ± 0.27a	15.08 ± 2.45a
P	CK	1.39 ± 0.05b	1.16 ± 0.04ab	0.94 ± 0.06a	1.42 ± 0.08b	0.95 ± 0.09b	1.16 ± 0.18b
	10 t ha^{-1}	1.84 ± 0.09a	0.60 ± 0.13c	0.69 ± 0.07ab	1.62 ± 0.12b	1.73 ± 0.13a	1.08 ± 0.07b
	20 t ha^{-1}	1.81 ± 0.07a	0.96 ± 0.07b	0.67 ± 0.12b	1.65 ± 0.05b	2.08 ± 0.20a	1.57 ± 0.07a
	40 t ha^{-1}	1.67 ± 0.14ab	1.27 ± 0.09a	0.71 ± 0.04ab	2.05 ± 0.15a	1.65 ± 0.05a	1.30 ± 0.06ab
C:N	CK	23.56 ± 2.64b	38.71 ± 0.69b	28.33 ± 0.72ab	27.33 ± 1.34a	45.37 ± 2.38a	34.01 ± 0.62a
	10 t ha^{-1}	27.01 ± 1.92ab	45.34 ± 1.88a	30.59 ± 1.83ab	24.28 ± 1.28a	37.81 ± 1.48ab	30.63 ± 2.67a
	20 t ha^{-1}	31.15 ± 2.25a	52.06 ± 1.69a	31.98 ± 1.28a	27.95 ± 2.38a	32.56 ± 5.98b	21.06 ± 1.51b
	40 t ha^{-1}	33.96 ± 1.85a	49.66 ± 3.10a	27.10 ± 0.83b	29.18 ± 1.62a	29.13 ± 0.73b	21.38 ± 3.59b
C:P	CK	67.45 ± 13.69a	291.47 ± 10.21b	323.24 ± 28.91a	88.64 ± 15.13a	321.14 ± 22.67a	265.43 ± 47.61ab
	10 t ha^{-1}	49.45 ± 9.39a	571.57 ± 111.84a	456.22 ± 34.94a	48.63 ± 13.49a	174.42 ± 13.58b	281.02 ± 18.37a
	20 t ha^{-1}	55.56 ± 5.28a	324.71 ± 13.67b	454.52 ± 69.91a	72.08 ± 20.06a	134.49 ± 20.76b	175.29 ± 17.01b
	40 t ha^{-1}	93.12 ± 25.56a	253.56 ± 18.42b	393.49 ± 22.40a	77.05 ± 11.58a	177.92 ± 11.58b	237.51 ± 19.25ab
N:P	CK	2.80 ± 0.33a	7.54 ± 0.33b	11.39 ± 0.88a	3.27 ± 0.62a	7.10 ± 0.51a	7.82 ± 1.41a
	10 t ha^{-1}	1.80 ± 0.21a	12.48 ± 2.18a	14.89 ± 0.42a	1.96 ± 0.45a	4.62 ± 0.38b	9.26 ± 0.69a
	20 t ha^{-1}	1.78 ± 0.06a	6.27 ± 0.47b	14.18 ± 2.05a	2.50 ± 0.48a	4.33 ± 0.86b	8.30 ± 0.31a
	40 t ha^{-1}	2.71 ± 0.72a	5.13 ± 0.39b	14.52 ± 0.71a	2.62 ± 0.27a	6.10 ± 0.28ab	11.56 ± 1.52a

Table 2. Pearson's correlation analysis of associations between soil and plant nutrient concentration and stoichiometry

	SC	SN	SP	SC:N	SC:P	SN:P
RC	0.284	0.358	−0.045	0.202	0.328	0.297
RN	0.126	0.161	0.03	0.094	0.121	0.101
RP	0.421*	0.347	0.214	0.394	0.358	0.111
RC:N	0.493*	0.593**	−0.178	0.35	0.610**	0.550**
RC:P	0.105	0.2	−0.156	0.04	0.182	0.247
RN:P	−0.118	−0.041	−0.094	−0.132	−0.086	0.029
STC	−0.451*	−0.007	−0.172	−0.536**	−0.354	0.111
STN	0.131	−0.167	0.408*	0.23	−0.027	−0.363
STP	0.459*	0.049	0.425*	0.505*	0.308	−0.193
STC:N	−0.167	0.213	−0.392	−0.289	0	0.392
STC:P	−0.334	0.069	−0.32	−0.411*	−0.231	0.204
STN:P	−0.336	−0.012	−0.213	−0.377	−0.28	0.078
LC	−0.403	−0.139	0.081	−0.397	−0.450*	−0.147
LN	0.439*	0.178	0.438*	0.462*	0.283	−0.133
LP	0.256	−0.145	0.232	0.343	0.127	−0.221
LC:N	−0.615**	−0.308	−0.451*	−0.601**	−0.470*	0.049
LC:P	−0.152	0.181	−0.187	−0.231	−0.051	0.221
LN:P	0.139	0.367	−0.019	0.045	0.198	0.253

SC: soil total C concentration; SN: soil total N concentration; SP: soil total P concentration; SC:N: soil C:N ratio; SC:P: soil C:P ratio; SN:P: soil N:P ratio; RC: root organic C concentration; RN: root total N concentration; RP: root total P concentration; RC:N: root C:N ratio; RC:P: root C:P ratio; RN:P: root N:P ratio; STC: stem organic C concentration; STN: stem total N concentration; STP: stem total P concentration; STC:N: stem C:N ratio; STC:P: stem C:P ratio; STN:P: stem N:P ratio; LC: leaf organic C concentration; LN: leaf total N concentration; LP: leaf total P concentration; LC:N: leaf C:N ratio; LC:P: leaf C:P ratio; LN:P: leaf N:P ratio. * ($P < 0.05$), ** ($P < 0.01$).

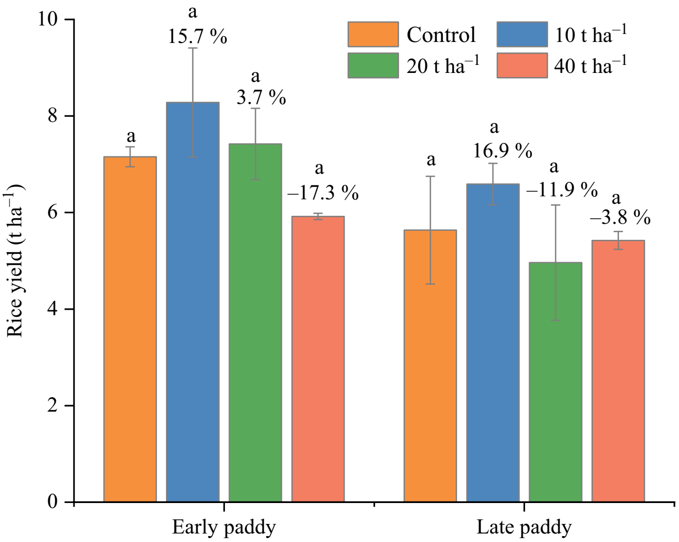


Figure 7. Effects of biochar application rate on rice yield in early and late paddies.

improving the sustainability of paddy rice production, due to high chemical recalcitrance and resistance to biodegradation (Diatta *et al*., 2020).

In addition, biochar contains a high nutrient concentration, and we found that biochar application increased total soil N and P concentration, suggesting that an increase in total concentration in the short term may be translated to increases in available forms at longer time (Bhaduri *et al*., 2016).

Effects of biochar rate on stoichiometry of paddy soil nutrients

Studies have shown that microbial activity and decomposition of organic matter are enhanced at soil C:N ratios <25 (Mooshammer *et al.*, 2014). In this study, soil C:N ratios increased from <25 in the untreated control paddies to >25 in the mid- and later growth stages of early and late rice following the application of biochar, indicating higher levels of microbial activity and increased decomposition of organic matter. Soil C:P ratios reflect the availability of P, and soil N:P ratios reflect the supply of soil nutrients during plant growth (Wang and Yu, 2008). We found that biochar increased soil C:P ratios that tended to increase during the early and vigorous rice growth stages (splitting and jointing) under irrigated conditions, before decreasing due to storage in senescent leaf material during the mature stage. We found that C:P ratios of late paddy soils were higher than for early paddy soils, due to differences in temperature, as indicated by the negative association between soil C:P ratios and soil temperature. Activity of microorganisms is positively associated with decomposition of C in biochar, and rises in temperature lead to increases in soil phosphatase activity (Sardans *et al.*, 2006), thereby enhancing the absorption of P by plants and further reducing soil P concentrations.

High soil N:P could indicate some degree of P deficiency in soil and plants (Guo *et al.*, 2019; Du *et al.*, 2020). In this study, we found that there was a possible shift from N to P deficiency in rice growth following the application of biochar, likely as a result of short-term net N fixation and greater soil N concentrations (Gul and Whalen, 2016; Luo *et al.*, 2011) and P migration to the root system (Zhao *et al.*, 2021). In this study, there was no effect of biochar on soil available nutrient stoichiometry (Fig. S3), perhaps as a result of the complex environmental conditions of the paddy soils. For example, soil available nutrient stoichiometry is affected by changes in fractional mass and is closely related to levels of soil salinity, temperature, and pH. Given the effective release of nutrients from biochar may be short term, further research on longer-term release mechanisms is required to improve the efficiency of biochar as a fertilizer.

Effects of biochar rate on rice yield and plant organ nutrient stoichiometry

Despite the biochar application improved nutrient status in plant–soil system, we did not observe a significant increase in yield production ($P > 0.05$). Anyway, a moderate application on 10 t ha^{-1} could be tested in future studies as also a potential source to improve rice yield. In addition, studies have shown that variation in level of reduction in plant organ N concentration with rate of biochar may be related to inhibitory effects on plant growth at higher application rates (Kammann *et al.*, 2011). Supporting our finding that increases in TN concentration of early paddy soils, but not AN following biochar application, possibly due to increases in inert N, with limited effects on plant nutrient concentration. Limitation of N occurs when plant leaf N:P ratios are <14 and P limitation occurs at plant leaf N:P ratios of >16. Limitation of N and P limitation occurs at plant leaf ratios of $14 < \text{N:P} < 16$ (Güsewell *et al.*, 2003). In this study, we found that the application of biochar to early paddies (during the mature period) shifted N limitation to N and P co-limitation (Fig. 8) due to lower plant P concentration. However, there were contrasting responses of plant organ nutrient concentrations and stoichiometry to biochar applications in early and late paddies. This may be due to the indirect effects of biochar on plants (Wu *et al.*, 2019) mediated by factors, such as temperature, precipitation, light, and growth stage (Wang *et al.*, 2018b). Our results suggest that biochar has a higher capacity for retaining N than P, indicating a more efficient biochar–soil N exchange, as previously suggested. Biochar produced through the pyrolysis of organic wastes tends to be acidic (pH range of 4.6–6.1). Under near-neutral soil conditions, such as the ones in our study, biochar has a greater capacity to absorb positively charged, low-mass soil components, such as NH_4^+, than negatively charged ones, such as PO_4- (Li *et al.*, 2017). As a result, biochar is more efficient in the retention and control of the release and biochar–soil exchange of N than P.

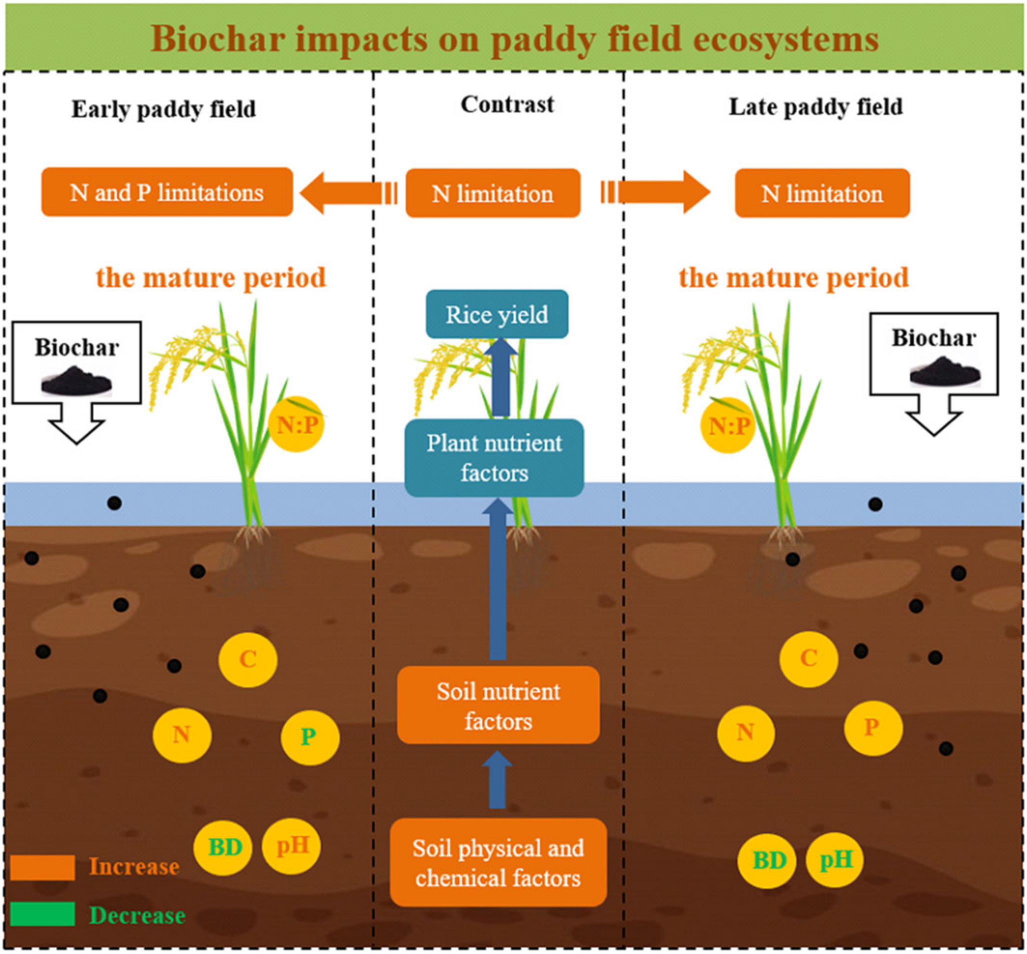


Figure 8. Conceptual model diagram of effects on soil and plant stoichiometry of biochar in early and late paddies. (BD: bulk density. pH = soil pH).

Conclusions

Short-term experiments of biochar in subtropical rice paddies reduced bulk density, with smaller effects on dynamics of soil temperature and soil iron. Biochar increased levels of soil C and N concentration in early and late paddies, reduced soil P concentration of early paddies, and increased P concentration of late paddy soils; biochar increased C:N and C:P ratios of early and late paddy soils. The application of biochar (10 t ha^{-1}) reduced rice plant organ concentration of N in early paddy. According to the N:P value of leaves between treatments, it was found that biochar alleviated the current situation of N limitation in paddy fields during the mature period and transformed the N limitation of early rice into a joint limitation of N and P. Based on these findings, we suggest that biochar with high capacity for P and overall N adsorption in neutral soils, and further capacity to supply these nutrients more gradually, reducing their leaching risk, should be applied to subtropical rice paddy soils. We recommend additional research on the mechanisms of nutrient release from biochar to improve soil fertility levels and rice yields.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0014479723000108

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