https://doi.org/10.17221/147/2022-PSE

Residual effect of straw biochar on grain yield and yield attributes in a double rice cropping system of subtropical China

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Citation: Lv R.J., Wang Y., Wang Q.J., Zeng Y.H., Shang Q.Y. (2022): Residual effect of straw biochar on grain yield and yield attributes in a double rice cropping system of subtropical China. Plant Soil Environ., 68: 328–337.

Abstract: Biochar is considered as a soil amendment for enhancing crop productivity. However, limited information is available on the residual effect of biochar application on rice grain yield and yield attributes. In this study, a fixed field experiment was conducted in a double rice-cropping system from 2017 to 2019. The dynamics of rice grain yield and yield attributes were monitored in the six growing seasons with 0, 20, and 40 t/ha biochar application. The results showed that the averaged grain yields in the first four seasons were increased by 2.56–16.84% and 6.15–10.77% with 20 and 40 t/ha biochar application. The trend of increased grain yield in rice with biochar application during the first seasons was mainly attributable to an increase in total biomass, panicles per m^2 and spikelets per panicle. Nonetheless, the grain yields in the sixth season were not influenced by biochar addition due to decreases in panicles per m^2 and spikelets per panicle. Thus, it can be seen that the positive effects of biochar application on rice yield and yield attributes depend on the duration of biochar application.

Keywords: straw biochar amendment; *Oryza sativa* L.; crop residue; grain weight; multiple season

Rice (*Oryza sativa* L.) is the staple food for nearly 50% of the world's people, mainly in Asia (Tian et al. 2021). Over the past several decades, rice yield has more than tripled in the past 50 years in China (Huang et al. 2019). In recent years, however, the annual growth rate of the rice yield has shown declining or stagnant trends in some regions of China (Fan et al. 2012). Correspondingly, fertiliser nitrogen (N) consumption has increased in a near-linear fashion during the past several decades. Nonetheless, inorganic N inputs do not always ensure high yields, as plant-available N can be reduced by leaching, adsorption and volatilisation (Jaynes et al. 2001). There is an increasingly urgent need to control nitrogen losses while maintaining crop productivity and sustainability.

The incorporation of biochar into soil has been widely recommended for improving crop growth and grain yields. Biochar is a C-rich product derived from pyrolysis of crop residues (Shi et al. 2019, Ibrahim et al. 2022); it typically has a well-developed pore structure, huge surface area, and high degree of stability and great adsorption properties (Zhai et al. 2015, Yuan et al. 2019). Higher crop yields with biochar application have been linked to increased water holding capacity and crop nitrogen uptake, reduced soil acidity and improvements in other soil physical properties (Warnock et al. 2007, Huang et al. 2019, Chen et al. 2021, Liu et al. 2022). In China, approximately 0.7 billion tons of crop residues are produced annually (Yang et al. 2018). Rice straw accounts for most of the crop residues and is commonly burned on site (Zeng et al. 2007), which causes serious global warming potential (Cui et al. 2017, Bhattacharyya et al. 2021, Liu et al. 2021a). One of the approaches to solve these residues is thermally converting these crop residues into biochar.

Supported by the National Natural Science Foundation of China, Grants No. 31601833 and 42167004.

https://doi.org/10.17221/147/2022-PSE

The short-term effects of biochar application on crop productivity have been intensively studied in various cropping systems and environmental conditions (Sarfraz et al. 2020, Zhang et al. 2020). However, there is a lack of field experiments to investigate the effects of biochar over multiple seasons. Soil improvement by biochar is an irreversible decision, so it is important to assess the potential impact of biochar on crop and soil quality, which goes beyond the typical one or two-season experimental cycle. This is particularly important because biochar is not usually used for annual applications, and it is challenging to handle and apply its logistics. Biochar in the soil can last for decades or even centuries after non-pyrogen organic matter, but it also ages over time, changing its interaction and potential effects with plants and the soil environment (Zhang et al. 2019, Futa et al. 2020, Quan et al. 2020). Agricultural management and environmental conditions will affect the ageing of biochar (Verheijen et al. 2010, Jeffery et al. 2011, Wang et al. 2020). Therefore, the field experiment combined with realistic agricultural operation, such as fertiliser application, is valuable to determine the residual effect of biochar on grain yield over time.

Rice yield is formed by spikelets per m^2 (sink size), spikelet filling percentage, and grain weight. Sink size is regarded as a major determinant of rice yield; it can be increased either by increasing spikelets per panicle or panicle per m^2, or both (Kropff et al. 1994). In another approach, rice yield is the function of total aboveground biomass and harvest index (Huang et al. 2019). However, limited information is available on the residual effects of straw biochar on rice grain yield and yield attributes. As a result, grain yield and yield attributes of rice were measured with biochar amendment and N fertiliser application in six continuous seasons in the present study. The objectives of this study were to: (1) evaluate the residual effect of biochar application on rice yield; and (2) investigate the yield attributes that contribute to the yield effect.

MATERIAL AND METHODS

Site description. A field experiment was conducted at Zengjia Village, Shanggao county, Jiangxi province, China (115°09'E, 28°31'N), where the cropping regime is dominated by double rice-cropping systems. The region is characterised by a subtropical humid monsoon climate, with an annual average air temperature of 17.5 °C, precipitation of 1 650 mm, sunshine of 1 500 h, and a frost-free period of 270 days. The paddy soil is classified as stagnic anthrosols developed from Quaternary red clay (IUSS Working Group, WRB 2006). The physicochemical properties of the topsoil measured at the 0~20 cm depth were: pH (soil:water, 1:2.5), 5.4, 32.0 g/kg organic carbon, 3.39 g/kg total N, 245.0 mg/kg available N, 12.3 mg/kg available P, 189.1 mg/kg available K, respectively.

Straw biochar amendment. Rice straw biochar was purchased from Sanli New Energy Co., Ltd. (Shangqiu, China). The biochar was produced by pyrolysis of rice straw at 500 °C with a residence time of 1 h under oxygen-limited conditions. With such a technology, 35% mass of rice straw was expected to be converted to biochar in the form of granular particles of 2 mm in diameter. For our present study, the biochar mass originally in a particulate form was ground to pass through a 1 mm sieve and mixed thoroughly to obtain a fine granular consistency that would mix more uniformly with the soil mass. The biochar had the following characteristics: pH 10.3, 505.1 g/kg total C, 8.56 g/kg total N, 2.37 g/kg total P, 20.37 g/kg total K, and 30.4 $cmol_+$/kg cation exchange capacity.

Experimental design. Three biochar rates (0, 20, 40 t/ha, labelled as B0, B1, and B2 treatments) combined with two N fertiliser application rates (N0 – 0 kg N/ha; N1 – 165 and 180 kg N/ha in the early and late season) were established. Each trial plot was conducted using 30 m^2 (5 m × 6 m) plots, with a 2 m buffer strip between them. All the field plots were laid out in a randomised complete block design with three replications. The individual plots, each with an irrigation and drainage outlet, were separated by protection rows that were 0.4 m in width. The rice straw biochar was spread on the surface of the paddy soil, thoroughly mixed into the topsoil by manually plowing 2~3 times to achieve a thorough mixture of the biochar and soil, and then tilled to a depth of 15 cm on April 15, 2017. To maintain consistency, the B0 plots were treated in the same manner but without the addition of biochar. No further biochar was added for the duration of the experiment. The treatment layout was maintained for the duration of the experiment.

Nitrogen fertiliser was applied as urea, 50% of which was applied as a basal fertiliser before transplanting of rice seedlings, another 20% at the tillering stage and the remaining 30% at the jointing stage. Phosphorus fertiliser as calcium magnesium phosphate was applied before transplanting at the rate of 36 kg P/ha each season. Potassium fertiliser as

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potassium chloride was applied at 70.5 kg K/ha before transplanting and at the jointing stage. Consistent with water management practices in the local double rice-cropping systems, flooding for transplanting and tillering, draining during the midseason, and intermittently irrigating after midseason. In addition, pesticide and herbicide management followed local practices.

In 2017~2019, local rice cultivars (*Oryza sativa* L.), Zhuliangyou 39 and Taiyou 871 were used for the early and late rice-cropping seasons, respectively. Pre-germinated seeds were sown in a seedbed and were transplanted to the field at the seedling age of 30 days and 25 days for the early and late seasons, respectively. Early-season rice seedlings were transplanted at a hill density of 13.2 cm × 23.3 cm with two seedlings per hill, and late-season rice seedlings were transplanted at a 13.2 cm × 26.7 cm hill density with three seedlings per hill. The seedlings were transplanted on 16 April and 19 July in the early and late season in 2017; the corresponding dates were 17 April and 18 July for 2018, and 18 April and 20 July for 2019.

Sampling and measurement. Grain yields were measured at physiological maturity by hand harvesting 2.5 m × 2.0 m per plot of the centre several rows, avoiding edge rows on each plot end. The grain yield was adjusted to a moisture content of 13.5% using a moisture detector (GAC2100AGRI, Dickey-john, Minneapolis, USA). At the time of harvest, the number of spikes was counted manually for five rows within 4 m. Fifty spikes from each plot were taken in succession, and the number of kernels per spike was recorded. For 1 000-kernels weight, 1 000 kernels were randomly counted and weighed.

Statistical analyses. Analysis of variance (ANOVA) was used to test the significance of the interaction between biochar and N rates on each parameter in each season (SPSS 19.0, IBM Corp., Armonk, USA). Seasons and their interaction by Tukey's honestly significant difference (*LSD*) test, the significance was set at the 0.05 probability level. The *t*-test was used to compare the difference between the mean values of the two groups. To make interpretation easier, parameters without significant interaction effects in most seasons were presented as means across the two N rates. Percentage changes comparing B1 to B0 and B2 to B0 and their 95% confidence intervals were calculated for all parameters in each season. The change is considered statistically significant if the 95% confidence interval does not overlap with zero.

RESULTS

Biochar and N rates had no significant interaction effects on grain yield, panicles per m^2, spikelets per panicle, spikelets per m^2, grain weight and harvest index in the early and late seasons from 2017 to 2019 (Table 1). The interaction effects of biochar and N rates were insignificant on spikelet filling in five of the six seasons and total biomass in four of the six seasons, respectively. Therefore, only the means for all of the parameters of the two N rates were shown in subsequent figures.

Changes comparing B1 to B0 (4.58% and 16.84%) for yield were significant in the early season in 2017 and late season in 2018 (Figure 1A – B1). In contrast, grain yield was significantly lower under B1 than under B0 in the early season of 2019. In the first four seasons, grain yields of B2 were higher than that of B0 by 6.15–10.77%, and changes comparing B2 to B0 for yield were significant (Figure 1A – B2). Changes in yield comparing B2 to B1 decreased slightly in the early and late seasons in 2019.

In the early season in 2019, changes comparing B1 to B0 (–3.99%) and B2 to B0 (–4.84%) for panicles per m^2 were significant, but there was no significant change comparing B1 to B0 and B2 to B0 for panicles per m^2 in other seasons (Figure 1B). In the early and late seasons of 2017, spikelets per panicle in B1 were higher than that of B0, and changes comparing B1 to B0 (6.26% and 4.48%) were significant (Figure 1C – B1). In addition, changes comparing B2 to B0 (9.97, 10.06 and 5.06%) for spikelets per panicle were significant in the early and late seasons in 2017 and early season in 2018. The change comparing B1 to B0 (–4.70%) for spikelets per m^2 was significant in the early season of 2019. However, spikelets per m^2 were significantly richer in B2 compared to B0 (6.10, 6.12 and 8.85), and changes comparing B2 to B0 were significant in the early and late seasons in 2017 and early season in 2018 (Figure 1D – B2).

The change comparing B1 to B0 for spikelet filling was significant (3.48% and 1.27% in the late season in 2017 and the early season in 2018) (Figure 2A – B1). However, spikelets filling in B2 were greater than that of B0 by 2.09% and 3.48% in the early and late seasons in 2017, and changes comparing B2 to B0 were significant (Figure 2A – B2). Grain weight was higher in B1 than in B0 (0.84%) in the late season in 2019 (Figure 2B – B1), and the change compared B1 to B0 was significant. In addition, the change comparing B2 to B0 (4.23%) was significant in the early season of 2017 (Figure 2B – B2).

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Table 1. Grain yield (t/ha), panicles per m^2, spikelets per panicle and spikelets per m^2 (× 10^3) under different nitrogen fertiliser (N) and biochar rates (B) in the first and second seasons from 2017 to 2019

Nitrogen rate	Biochar rate	First season			Second season		
		2017	2018	2019	2017	2018	2019
Grain yield							
N0	B0	5.04 ± 0.29^b	3.47 ± 0.08^b	4.57 ± 0.14^c	6.19 ± 0.38^b	6.56 ± 0.34^c	6.56 ± 0.11^b
	B1	5.23 ± 0.24^b	3.92 ± 0.43^b	4.24 ± 0.31^c	6.35 ± 0.9^b	8.38 ± 0.08^b	6.60 ± 0.23^b
	B2	5.38 ± 0.65^b	3.98 ± 0.17^b	4.65 ± 0.44^c	6.49 ± 0.42^b	7.71 ± 0.57^b	6.48 ± 0.24^b
N1	B0	7.75 ± 0.17^a	8.23 ± 0.38^a	8.72 ± 0.07^a	8.67 ± 0.80^a	9.70 ± 0.34^a	9.61 ± 0.28^a
	B1	7.91 ± 0.20^a	8.18 ± 0.32^a	8.15 ± 0.28^b	9.29 ± 0.66^a	10.27 ± 0.70^a	9.67 ± 0.11^a
	B2	8.06 ± 0.39^a	8.39 ± 0.10^a	8.07 ± 0.19^b	9.57 ± 0.60^a	10.06 ± 0.81^a	9.50 ± 0.64^a
Analysis of variance	N	754.15**	2452.89**	915.74**	84.17**	94.88**	399.23**
	B	12.53**	4.63*	4.27*	1.28^{ns}	7.62**	0.32^{ns}
	N × B	2.82^{ns}	0.11^{ns}	2.89^{ns}	0.33^{ns}	2.05^{ns}	0.01^{ns}
Panicles per m^2							
N0	B0	261.4 ± 4.9^b	223.0 ± 14.5^b	239.0 ± 22.7^b	272.7 ± 2.6^b	273.1 ± 7.8^c	187.4 ± 12.5^b
	B1	259.3 ± 21.4^b	215.3 ± 4.3^b	225.4 ± 19.1^b	270.9 ± 4.2^b	294.4 ± 12.0^b	188.2 ± 10.1^b
	B2	259.3 ± 17.7^b	248.0 ± 7.5^b	221.3 ± 11.3^b	265.6 ± 23.9^b	279.4 ± 13.5^{bc}	184.3 ± 5.2^b
N1	B0	430.7 ± 3.2^a	382.7 ± 40.8^a	363.5 ± 1.5^a	322.9 ± 6.7^a	374.3 ± 3.6^a	272.3 ± 5.5^a
	B1	407.1 ± 33.0^a	360.6 ± 15.9^a	355.0 ± 10.2^a	313.5 ± 15.0^a	379.4 ± 8.2^a	259.3 ± 14.5^a
	B2	405.0 ± 14.7^a	372.2 ± 47.5^a	354.4 ± 13.4^a	309.0 ± 7.5^a	367.4 ± 3.8^a	273.0 ± 14.5^a
Analysis of variance	N	304.43**	123.99**	348.89**	60.26**	468.70**	243.65**
	B	1.02^{ns}	1.03^{ns}	1.43^{ns}	1.07^{ns}	4.45*	0.51^{ns}
	N × B	0.72^{ns}	0.65 ^{ns}	0.13^{ns}	0.17^{ns}	1.40^{ns}	1.05^{ns}
Spikelets per panicle							
N0	B0	86.9 ± 3.6^c	83.1 ± 5.4^b	83.2 ± 5.5^b	143.3 ± 11.4^d	124.7 ± 12.8^c	186.9 ± 2.6^{bc}
	B1	92.2 ± 6.4^c	80.1 ± 6.9^b	81.2 ± 7.0^b	150.2 ± 5.2^{cd}	131.9 ± 10.3^{bc}	190.7 ± 6.5^{abc}
	B2	93.8 ± 0.8^c	82.3 ± 7.3^b	87.9 ± 6.2^b	160.4 ± 7.2^{bc}	125.8 ± 12.2^c	180.3 ± 7.9^c
N1	B0	116.2 ± 6.4^b	96.4 ± 4.3^a	116.2 ± 4.1^a	174.7 ± 9.4^{ab}	153.0 ± 2.4^a	194.1 ± 8.0^{ab}
	B1	123.9 ± 9.0^{ab}	100.1 ± 6.1^a	117.3 ± 2.6^a	180.8 ± 5.7^a	147.3 ± 6.1^a	198.2 ± 1.9^{ab}
	B2	130.1 ± 7.1^a	106.4 ± 4.5^a	118.6 ± 9.1^a	188.5 ± 9.7^a	143.4 ± 0.5^{ab}	202.5 ± 4.0^a
Analysis of variance	N	124.86**	47.78**	134.05**	57.60**	24.56**	20.76**
	B	4.34*	1.14^{ns}	0.76^{ns}	5.10*	0.55^{ns}	0.78^{ns}
	N × B	0.49^{ns}	1.28^{ns}	0.30^{ns}	0.06^{ns}	0.94^{ns}	3.36^{ns}
Spikelets per m^2 (× 10^3)							
N0	B0	22.7 ± 1.4^b	18.6 ± 2.3^b	19.9 ± 2.9^b	39.1 ± 3.4^c	34.0 ± 2.6^d	35.0 ± 2.8^d
	B1	24.0 ± 3.6^b	17.3 ± 1.8^b	18.4 ± 2.9^b	40.7 ± 2^{bc}	38.9 ± 4.5^c	35.9 ± 3.1^d
	B2	24.3 ± 1.8^b	20.4 ± 2.3^b	19.5 ± 2.3^b	42.7 ± 5.4^b	35.3 ± 4.9^{cd}	33.2 ± 2.4^e
N1	B0	50.1 ± 3.1^a	36.9 ± 4.7^a	42.3 ± 1.6^a	56.5 ± 4.1^a	57.3 ± 0.4^a	52.9 ± 3.2^b
	B1	50.6 ± 7.4^a	36.2 ± 3.8^a	41.7 ± 2.1^a	56.7 ± 4.3^a	55.9 ± 3.4^{ab}	51.4 ± 3.4^c
	B2	52.7 ± 4.6^a	39.7 ± 6.5^a	42.1 ± 4.8^a	58.3 ± 4.3^a	52.7 ± 0.4^b	55.3 ± 4.0^a
Analysis of variance	N	195.11**	102.69**	266.24**	72.40**	157.97**	149.28**
	B	0.40^{ns}	1.16^{ns}	0.21^{ns}	0.70^{ns}	1.66	0.06^{ns}
	N × B	0.07^{ns}	0.02^{ns}	0.05^{ns}	0.07^{ns}	1.76^{ns}	1.64^{ns}

The data are expressed as the average ± standard deviation of three replications (n = 3). The data after the "Analysis of variance" are F-values. N0 and N1 – with and without nitrogen fertiliser application; B0, B1 and B2 – biochar rates at three levels of 0, 20, and 40 t/ha; ^{ns}not significant; $^*P \le 0.05$; $^{**}P \le 0.01$; N × B – interaction between nitrogen fertiliser and biochar rates

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Table 2. Spikelet filling (%), grain weight (mg), total biomass (t/ha) and harvest index under different nitrogen fertiliser (N) and biochar rates (B) in the first and second seasons from 2017 to 2019

Nitrogen rate	Biochar rate	First season			Second season		
		2017	2018	2019	2017	2018	2019
Spikelet filling							
N0	B0	93.4 ± 0.1^{a}	89.7 ± 0.3^{ab}	92.6 ± 2.3^{a}	78.8 ± 3.3^{a}	84.7 ± 0.7^{a}	84.2 ± 2.1^{a}
	B1	92.9 ± 1.2^{a}	90.9 ± 0.4^{ab}	95.4 ± 0.5^{a}	82.7 ± 3.2^{a}	86.2 ± 1.2^{a}	85.3 ± 1.3^{a}
	B2	93.5 ± 2.1^{a}	91.2 ± 0.8^{a}	93.7 ± 1.3^{a}	82.1 ± 3.2^{a}	84.1 ± 0.8^{a}	83.2 ± 2.9^{a}
N1	B0	78.0 ± 2.9^{b}	88.9 ± 1.0^{b}	86.0 ± 2.5^{b}	80.1 ± 4.1^{a}	86.1 ± 1.8^{a}	82.2 ± 0.5^{a}
	B1	77.9 ± 1.0^{b}	90.0 ± 1.2^{ab}	86.1 ± 2.0^{b}	81.5 ± 2.0^{a}	85.2 ± 2.4^{a}	83.0 ± 2.7^{a}
	B2	81.0 ± 4.3^{b}	86.7 ± 0.9^{c}	86.1 ± 3.1^{b}	82.3 ± 7.0^{a}	86.6 ± 2.7^{a}	83.9 ± 2.0^{a}
Analysis of variance	N	158.93**	28.90**	60.70**	0.00^{ns}	1.18^{ns}	1.54^{ns}
	B	1.06^{ns}	5.46*	0.65^{ns}	0.88^{ns}	0.06^{ns}	0.28^{ns}
	N × B	0.61^{ns}	9.82**	0.60^{ns}	0.13^{ns}	1.54^{ns}	0.96^{ns}
Grain weight							
N0	B0	26.4 ± 0.3^{a}	25.7 ± 0.1^{a}	25.6 ± 0.4^{a}	24.1 ± 0.3^{ab}	24.2 ± 0.0^{b}	25.6 ± 0.3^{a}
	B1	27.2 ± 0.8^{a}	25.5 ± 0.5^{a}	25.6 ± 0.5^{a}	24.1 ± 0.7^{ab}	24.6 ± 0.2^{b}	25.8 ± 0.4^{a}
	B2	25.7 ± 1.6^{a}	25.6 ± 0.3^{a}	25.5 ± 0.3^{a}	23.8 ± 0.6^{b}	24.3 ± 0.5^{b}	25.7 ± 0.8^{a}
N1	B0	26.6 ± 1.3^{a}	25.5 ± 0.3^{a}	25.6 ± 0.1^{a}	25.0 ± 0.1^{a}	24.8 ± 0.6^{b}	26.5 ± 0.2^{a}
	B1	25.9 ± 2.0^{a}	25.8 ± 0.6^{a}	25.6 ± 0.3^{a}	24.7 ± 0.2^{ab}	24.7 ± 0.2^{ab}	26.7 ± 0.3^{a}
	B2	25.1 ± 1.2^{a}	25.5 ± 0.7^{a}	25.5 ± 0.3^{a}	24.7 ± 0.6^{ab}	25.5 ± 0.3^{a}	26.5 ± 0.5^{a}
Analysis of variance	N	0.85^{ns}	0.00^{ns}	0.02^{ns}	12.86**	10.70**	16.69**
	B	1.53^{ns}	0.14^{ns}	0.15^{ns}	0.64^{ns}	2.42^{ns}	0.31^{ns}
	N × B	0.54^{ns}	0.59^{ns}	0.00^{ns}	0.35^{ns}	3.37^{ns}	0.01^{ns}
Total biomass							
N0	B0	9.4 ± 0.5^{e}	6.3 ± 0.4^{d}	7.9 ± 0.4^{c}	9.5 ± 0.3^{d}	10.8 ± 0.7^{c}	11.1 ± 0.6^{c}
	B1	10.1 ± 0.9^{d}	6.5 ± 0.2^{d}	7.6 ± 0.3^{d}	9.8 ± 0.4^{d}	10.9 ± 0.6^{c}	11.4 ± 0.3^{c}
	B2	10.8 ± 0.8^{c}	7.4 ± 0.7^{c}	7.5 ± 0.4^{d}	10.5 ± 0.4^{c}	9.9 ± 0.3^{d}	10.6 ± 0.6^{c}
N1	B0	12.3 ± 0.7^{b}	12.4 ± 0.8^{b}	13.1 ± 0.3^{b}	16.0 ± 0.0^{b}	15.8 ± 0.1^{b}	16.4 ± 0.4^{b}
	B1	12.5 ± 0.5^{ab}	13.0 ± 1.3^{ab}	13.3 ± 0.2^{b}	17.0 ± 1.1^{a}	17.0 ± 0.3^{a}	16.2 ± 0.9^{b}
	B2	12.9 ± 0.4^{a}	13.4 ± 1.2^{a}	13.7 ± 0.2^{a}	17.2 ± 0.5^{a}	16.8 ± 0.5^{a}	17.2 ± 0.7^{a}
Analysis of variance	N	61.02**	238.86**	1807.89**	720.83**	712.02**	354.85**
	B	3.44^{ns}	2.30^{ns}	0.34^{ns}	6.14*	3.60*	0.12^{ns}
	N × B	0.40^{ns}	0.18^{ns}	4.35*	0.53^{ns}	5.90*	3.30^{ns}
Harvest index							
N0	B0	0.54 ± 0.06^{abc}	0.62 ± 0.12^{a}	0.63 ± 0.02^{c}	0.65 ± 0.04^{a}	0.65 ± 0.02^{a}	0.63 ± 0.01^{ab}
	B1	0.52 ± 0.06^{bc}	0.55 ± 0.02^{a}	0.65 ± 0.03^{bc}	0.65 ± 0.09^{a}	0.66 ± 0.03^{a}	0.64 ± 0.00^{ab}
	B2	0.50 ± 0.10^{c}	0.61 ± 0.08^{a}	0.65 ± 0.01^{abc}	0.62 ± 0.02^{a}	0.71 ± 0.13^{a}	0.62 ± 0.02^{ab}
N1	B0	0.63 ± 0.04^{a}	0.63 ± 0.01^{a}	0.68 ± 0.01^{a}	0.54 ± 0.05^{a}	0.67 ± 0.02^{a}	0.61 ± 0.06^{b}
	B1	0.63 ± 0.04^{a}	0.63 ± 0.01^{a}	0.67 ± 0.01^{ab}	0.55 ± 0.04^{a}	0.63 ± 0.06^{a}	0.65 ± 0.01^{a}
	B2	0.62 ± 0.03^{ab}	0.63 ± 0.03^{a}	0.67 ± 0.00^{ab}	0.56 ± 0.05^{a}	0.67 ± 0.02^{a}	0.64 ± 0.01^{ab}
Analysis of variance	N	12.50**	0.00^{ns}	18.00**	12.80**	0.07^{ns}	1.33^{ns}
	B	0.13^{ns}	0.70^{ns}	1.50^{ns}	0.20^{ns}	0.07^{ns}	2.33^{ns}
	N × B	0.13^{ns}	0.30^{ns}	1.50^{ns}	0.20^{ns}	0.07^{ns}	2.33^{ns}

The data are expressed as the average ± standard deviation of three replications (n = 3). The data after the "Analysis of variance" are F-values. N0 and N1 – with and without nitrogen fertiliser application; B0, B1 and B2 – biochar rates at three levels of 0, 20, and 40 t/ha; ^{ns}not significant; $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; N × B – interaction between nitrogen fertiliser and biochar rates

https://doi.org/10.17221/147/2022-PSE

In the first four seasons, total biomass was higher in B1 compared to B0, and changes comparing B1 to B0 (from 2.22% to 3.34%) were significant (Figure 2C – B1). Similarly, total biomass in B2 was greater than that of B0 in the first three seasons, and changes comparing B2 to B1 (from 2.23% to 6.63%) were significant (Figure 2C – B2). There was no significant change comparing B1 to B0 and B2 to B0 for harvest index in all six seasons (Figure 2D).

Pearson correlations showed that relationships between grain yield and yield attributes varied depending on parameters (Table 3). Panicles per m^2, spikelets per m^2, spikelets per panicle and total biomass were significantly positively correlated with yield. However, the yield was negatively correlated with the spikelet filling percentage. In addition, there was no significant correlation between grain weight and yield.

DISCUSSION

Our results showed that the effects of biochar on rice yield varied over time. In the first four seasons, rice yields were significantly increased with biochar application for B2. Chen et al. (2021) showed that applying biochar could increase rice yields. However, there was no significant change with biochar application in the sixth season. This indicated that the positive effects of biochar application on rice yield depended on the duration of biochar application. According to Huang et al. (2019), however, it was found that there was no significant change in yield with biochar application in the first three seasons, while it increased in the fourth to sixth seasons. The inconsistent results may be due to the way of biochar application and the differences in physical and chemical properties of soil. Biochar was applied only before the first year of transplanting in our experiment, while it was applied in each season in the research of Huang et al. (2019).

The importance of grain weight in improving rice yield has been recognised in some studies (Yang et al. 2002, Huang et al. 2019). Although the decreased grain weight was observed in the first season, grain yield was increased with biochar application in this study. Correlation analysis indicated that the trend of increased grain yield in rice with biochar application during the first four seasons was mainly attributable to an increase in sink size (spikelets per m^2) (Table 3). It is generally believed that increasing spikelets per panicle is the most promising way to increase sink size (Peng et al. 2008). Indeed, our study showed that the larger sink size in rice applied with biochar was attributed to increased panicle size (spikelets per panicle). Nonetheless, it was reported that there was a compensatory relationship between panicle size and panicle number (Ying et al. 1998, Huang et al. 2011). However, the synergistic increase of panicles per m^2 and spikelets per panicle showed that the contradiction between them can be effectively improved by the application of biochar. In this regard, increasing biomass production is a feasible way to decouple the compensation relationship between the two yield components, including rice (Ying et al. 1998, Huang et al. 2019). This may also be the reason for the compatible relationship between panicles per m^2 and spikelets per panicle of rice applied with biochar in this study. The positive effects of biochar application on yield components in rice varied over time. From the fourth season on, there was no significant change in sink size and panicle size. As a result, grain yield tended to decrease with biochar application in the last two seasons. The significant impact of biochar application on yield components of rice varied over time, which may be due to its dynamic effect on soil properties.

On the other hand, the trend of rice grain yield with biochar application could be explained by the change in total biomass and harvest index for six seasons. The harvest index is determined by the remobilisation of stored reserves into growing grains and transient photosynthesis during grain formation (Blum 1993). Huang et al. (2019) showed that the trend of rice grain yield with biochar application could be explained by the dynamic of the harvest index. In this study, nonetheless, there was no significant change in harvest index with biochar application for six seasons. Obviously, correlation analysis showed that there was a significant positive correlation between biomass and yield (the correlation coefficient was 0.939, Table 3), which was consistent with the results of Pal et al. (2017). The effect of biomass by biochar application may be one of the main reasons leading to the change in yield in this study. In addition, biochar affects N cycling through different mechanisms, including sorption of NO_3^-, NH_3, NH_4^+ and organic-N, as well as through changes in microbial processes and activities (Bai et al. 2015). The biomass of B1 in the first four seasons and B2 in the first three seasons were higher than that of B0 and the changes of them were significant; it was consistent with the results of previous studies

https://doi.org/10.17221/147/2022-PSE

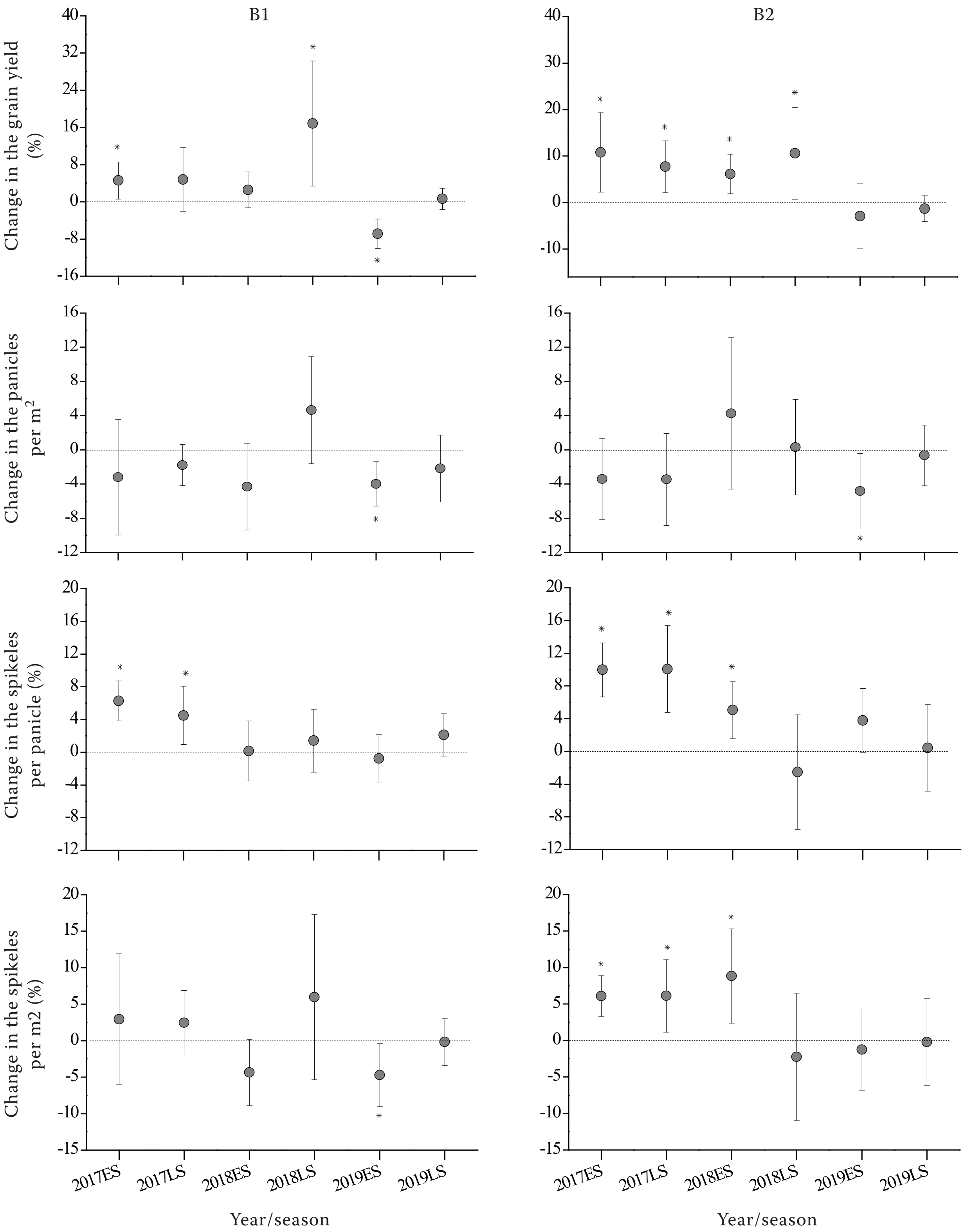

Figure 1. Change in (A) grain yield; (B) panicles per m^2; (C) spikelets per panicle and (D) spikelets per m^2 comparing B1 to B0 (B1) and B2 to B0 (B2) in rice grown in the first and second seasons from 2017 to 2019. B0, B1 and B2 – biochar rates at three levels of 0, 20 and 40 t/ha. Error bars are 95% confidence intervals, and dashed lines are the zero reference lines. Significant changes are denoted by * (where error bars do not overlap zero). ES – first season; LS – second season

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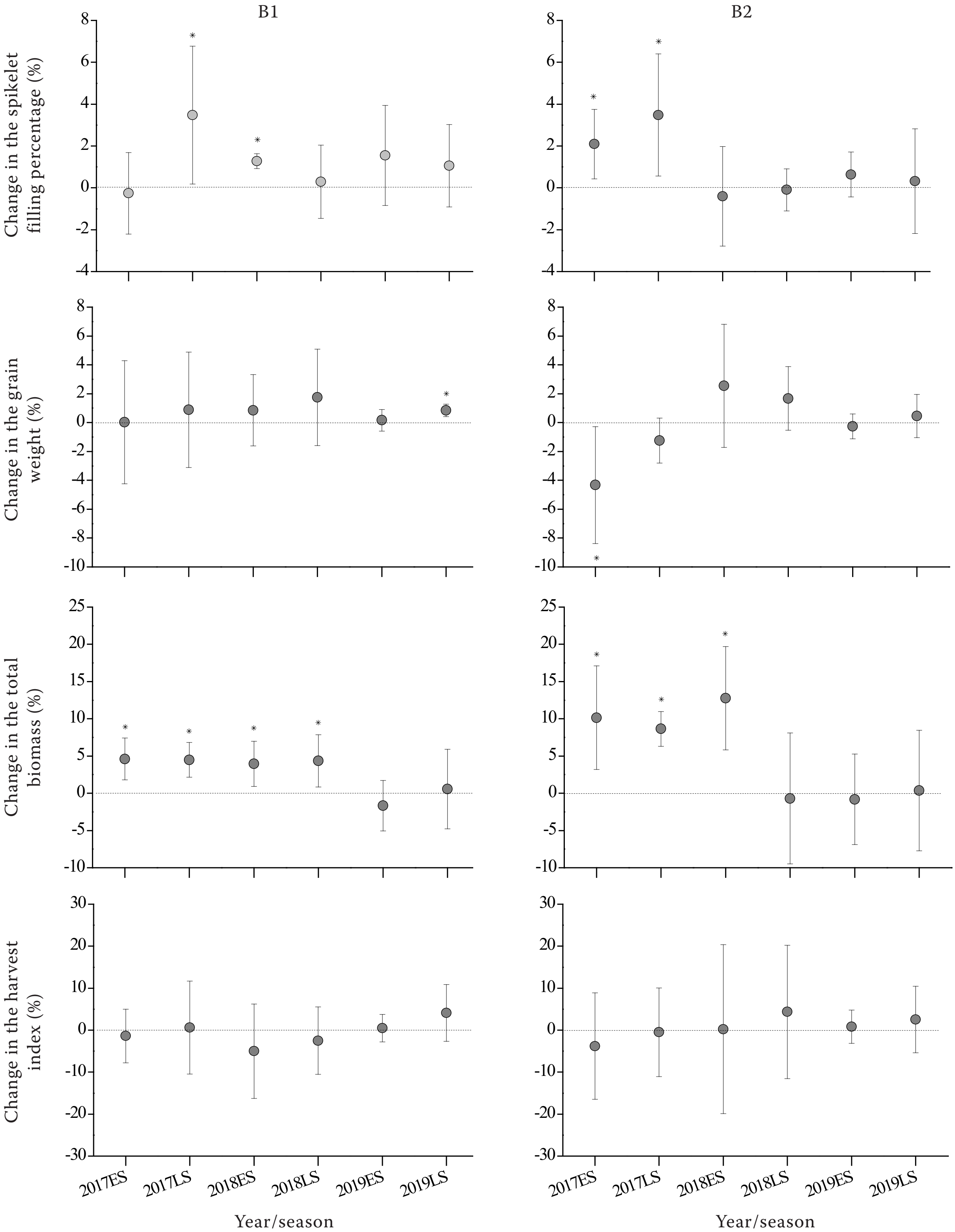

Figure 2. Change in (A) spikelet filling percentage; (B) grain weight; (C) total biomass and (D) harvest index comparing B1 to B0 (B1) and B2 to B0 (B2) in rice grown in the first and second seasons from 2017 to 2019. B0, B1 and B2 – biochar rates at three levels of 0, 20 and 40 t/ha. Error bars are 95% confidence intervals, and dashed lines are the zero reference lines. Significant changes are denoted by * (where error bars do not overlap zero). ES – first season; LS – second season

https://doi.org/10.17221/147/2022-PSE

Table 3. The correlation coefficient among grain yield and yield attributes in rice grown in the first and second seasons from 2017 to 2019

Parameter	Grain yield	Panicles per m^2	Spikelets per panicle	Spikelets per m^2	Spikelet filling percentage	Grain weight	Total biomass	Harvest index
Grain yield	1	0.624**	0.637**	0.912**	–0.489**	–0.023	0.939**	0.184
Panicles per m^2		1	–0.055	0.624**	–0.306**	0.026	0.550**	0.231*
Spikelets per panicle			1	0.730**	–0.593**	–0.056	0.677**	–0.031
Spikelets per m^2				1	–0.641**	–0.054	0.906**	0.114
Spikelet filling percentage					1	0.370**	–0.443**	–0.042
Grain weight						1	0.079	–0.056
Total biomass							1	0.016
Harvest index								1

*$P \le 0.05$; **$P \le 0.01$

(Cui et al. 2021, Liu et al. 2021b). It was likely to be related to the increase of soil available nutrients, such as N, K, Ca and Mg, by biochar addition during rice production (Rajkovich et al. 2012, Zhang et al. 2015). Therefore, the increase of biomass by biochar addition may be because biochar promoted the uptake of nutrients for the plant from the soil. However, further investigation was needed to confirm this conjecture. It would be interesting to research what effect changes in the soil's physical, chemical and biological properties after biochar application and how these changes affect the morphology and physiology of rice plants.

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Received: April 27, 2022
Accepted: July 1, 2022
Published online: July 25, 2022