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The implication from six years of field experiment: the aging process induced lower rice production even with a high amount of biochar application

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Abstract

The single high-dose application of biochar to increase rice yield has been well reported. However, limited information is available about the long-term effects of increasing rice yield and soil fertility. This study was designed to perform a 6-year field experiment to unveil the rice yield with time due to various biochar application strategies. Moreover, an alternative strategy of the Annual Low dose biochar application (AL, $8 \times 35\% = 2.8 \text{ t ha}^{-1}$) was also conducted to make a comparison with the High Single dose (HS, 22.5 t ha^{-1}), and annual Rice Straw (RS, 8 t ha^{-1}) amendment to investigate the effects on annual rice yield attributes and soil nutrient concentrations. Results showed that the rice yield in AL with a lower biochar application exceeded that of HS significantly ($p < 0.05$) in the 6th experimental year. The rice yield increased by 14.3% in RS, 10.9% in AL, and 4.2% in HS. The unexpectedly higher rice yield in AL than HS resulted from enhanced soil total carbon (TC), pH, and available Ca. However, compared to AL, liable carbon fraction increased by 33.7% in HS, while refractory carbon fraction dropped by 22.3%. Likewise, biochar characterization showed that more oxygen functional groups existed in HS than in AL. Decreasing inert organic carbon pools due to the constant degradation of the aromatic part of biochar in HS led to a lower soil TC than AL, even with a higher amount of biochar application. Likewise, the annual depletion lowered the soil pH and available Ca declination in HS. Based on the obtained results, this study suggested AL as a promising strategy to enhance rice productivity, soil nutrient enrichment, and carbon sequestration in the paddy ecosystem.

Highlights

- Annual Low-rate biochar strategy showed higher rice yields than High Single in the 6th year.
- Higher total carbon, pH, and Ca^{2+} led to higher rice yields in Annual Low than High Single.
- Higher aromatic carbon loss in High Single contributed to lower inert organic carbon.

Keywords Biochar, Annual low rate, Single high dose, Rice yield, Carbon fractions, Soil quality

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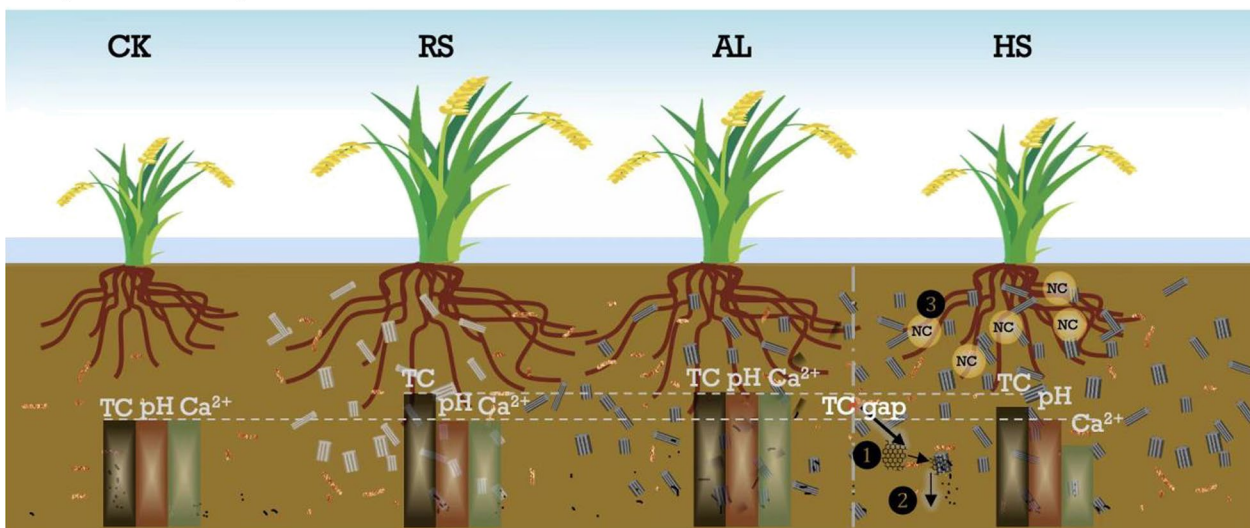
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Graphic abstract

Rice yield in the 6th year



CK: no biochar or straw RS: rice straw at 8 t ha⁻¹ AL: biochar at 2.8 t ha⁻¹ annually HS: biochar at 22.5 t ha⁻¹ only in 2015
 NC: soil native organic carbon ❶: biochar aromatic carbon degradation ❷: biochar migrates down
 ❸: soil native organic carbon utilization Rice straw Rice straw biochar

1 Introduction

Rice (*Oryza sativa* L.) is the primary dietary energy source and a major staple food for more than 3.5 billion people across the globe, particularly in Asia (Qin et al. 2023; Parashar et al. 2023). An increasing population leads to the increasing demand for food (Zhou et al. 2021; Mehmood et al. 2021), which results in significantly increased rice cultivation and production. To dispose of the accompanied massive amount of rice straw, incorporation into the paddy field is a sustainable management for superficial rice production (Nan et al. 2020b). It has been well reported that field incorporation of rice straw considerably improved the soil microbial biomass, soil organic carbon (SOC), total carbon (TC), and nitrogen (N) levels (Benbi et al. 2021; Zhou et al. 2020) and immobilization (Zhou et al. 2020; Chen et al. 2022). In addition, the mineralogical composition also depicts that the rice straw is rich in phosphorus (P), potassium (K) (Liu et al. 2019), magnesium (Mg) (Nan et al. 2020b), and other nutrients. However, rice straw amendment into the paddy soil will increase greenhouse gas emissions (GHGs) such as methane (CH₄), which gives negative feedback to the paddy ecosystem and is a poor strategy to achieve carbon neutrality (Jiang et al. 2019).

Considering the need for carbon sequestration and obtaining high rice yields through the use of agricultural waste (Ozturk et al. 2017; Kwoczyński and Čmelík 2021), rice straw was developed to be amended into the soil after pyrolysis to biochar (Thammasom et al. 2016; Si et al. 2018; Nan et al. 2020c; Zheng et al. 2020). Conversion of rice straw to biochar provides the dual benefits of managing the rice straw waste and offering additional environmental benefits, including soil amendment and carbon sequestration (Waqas et al. 2021). Biochar, a rich source of various inorganic minerals and organic matter contents, provides essential nutrients to plants (Qadeer et al. 2017). Likewise, owing to the carbon sequestration capabilities, the soil application of biochar has been recommended as a promising way for climate change mitigation.

Furthermore, the straw-derived biochar is also enriched with various nutrients rice straw provides, ash content mitigating soil pH (Wu et al. 2022), recalcitrant carbon exerting a role in the carbon sequestration, and a small part of liable carbon contributing to SOC (Cross and Sohi 2011; Wang and Wang 2019). Moreover, biochar applications significantly improved the soil microbial communities and their enzymatic activities (Jaborova et al. 2021). It is well understood that soil is the home

to various microbes, including bacteria, algae, fungi, archaea, protozoa, and actinomycetes (Palansooriya et al. 2019). These soil-inhibited microorganisms are directly involved in various beneficial soil activities, including the decomposition of organic matter, disease and pest suppression, recycling of multiple nutrients, secretion of plant growth promoter hormones, soil structure formation, remediation of organic contaminants (Waqas et al. 2021; Farrell et al. 2013). However, it has been suggested that the effects of biochar on the soil microbial communities mainly depend on the application strategies of biochar, types of biochar, and soil (Palansooriya et al. 2019).

In addition, the high porosity and acid oxygen-functional groups on the surface make biochar an excellent candidate for N retention (Brennan et al. 2001; Nguyen et al. 2017) and provide habitat for microbial communities to colonize, promoting their growth in the soil environment (Waqas et al. 2018). Dong et al. (2015) reported that biochar application at 22.5 t ha⁻¹ increased the rice yield by 19.8%. Similarly, the findings of many researchers considerably proved that single high-rate biochar incorporation could improve the soil and enhance the crop (rice) yield in the subsequent years (Liu et al. 2014, 2021; Mehmood et al. 2020). However, how many years the crop production increased without supplementary addition of biochar is still under discussion. The exploration is of great importance for developing countermeasures to keep long-lasting rice yield.

Theoretically, the high rice production as a result of a high single biochar dose will vanish after a few years. Generally, TC increase under biochar application is a key factor for high rice production (Nan et al. 2020b). However, with the temporal aging process, biochar carbon experienced liable carbon mineralization, and aromatic carbon degradation after years of rice growth cycles could lead to lower TC content. Correspondingly, the nutrient concentration as a result of no biochar supplementary in the following years will also be gradually consumed and the liming effect would gradually disappear (Nan et al. 2021). Considering the economic aspects of biochar production and single high-dose application, the annual low-rate biochar amendment, incorporating low-rate biochar into the soil every single year, could be a promising way to achieve high rice production over a prolonged period (Awad et al. 2018). The reason behind this is that the annual biochar application at a lower rate could provide continuous and accumulative nutrient supply, soil quality improvement, and better rice production (Nan et al. 2020b).

To disclose the rice production promotion of declining points after years, a 6-year field experiment from 2015 to 2020 was conducted, with a promising alternative

strategy as a comparison. Soil properties and biochar characterization were analyzed to disclose the underlying mechanism that alters rice yield. It was hypothesized that an annual low-rate biochar application would increase the rice yield over a single high-rate biochar application after years of amendment.

2 Materials and methods

2.1 Collection of feedstock and biochar preparation

Rice (*Oryza sativa* L. *Japonica* rice *Xiushui* 134) straw was used as the feedstock for biochar production. Detailed information about biochar production can be found in the Additional file 1. Briefly, biochar was produced under 500 °C in oxygen-deficient conditions for 2 h in a self-made auto-carbonizing furnace. Biochar yield produced from rice straw was 35%. Attributes of biochar and rice straw are listed in the Additional file 1: Table S1. Carbon content in produced biochar was 47.2%. Likewise, the pH of the produced biochar was 10.58.

2.2 Field experiments

The field situation was described in the previously published article (Nan et al. 2020a). Briefly, the field was located in Jingshan town in Hangzhou. The paddy field soil was classified as Ultisol with a clay loam texture. Soil properties are given in Additional file 1: Table S1. The field was conventional paddy before the experiment.

The experimental design was a Randomized Complete Block Design (RCBD) with three replications. Each plot size was kept at 4 × 5 m. Plastic film and quartzite were covered on the ridges to separate the plots and to facilitate the researcher's walking for data collection. Fields were used continuously from 2015 to 2020. The single high-dose biochar amendment at 22.5 t ha⁻¹ (Liou et al. 2003) was applied only in 2015. Correspondingly, rice straw at 8 t ha⁻¹ (RS) and biochar at 2.8 t ha⁻¹ (8 × 35% = 2.8 t ha⁻¹, AL, of which 35% is the biochar yield when pyrolyzed with rice straw) were applied during each experimental year before the addition of fertilizer. An unamendment treatment was kept as a control to compare the effect of each treatment. Biochar and rice straw were incorporated to a depth of 20 cm using a rake one day before fertilization and transplanting. Then, fertilizer of 270 kg nitrogen (N, Urea) ha⁻¹, 32.75 kg phosphorus (P, superphosphate) ha⁻¹, and 74.5 kg potassium (K, potassium chloride) ha⁻¹ was added to each plot and kept constant during the following years. Rice grew from late June and was harvested in November without a rotation crop. The paddy field was maintained by intermittent irrigation from the grain-filling stage to the maturing stage.

2.3 Determination of soil nutrients

Rice yields were determined each year of the experimental duration (2015 to 2020). Soil samples were collected by diagonal sampling method after rice was harvested. Five soil samples were randomly collected from each plot and composed together as one soil sample. After collection, the soil samples were sealed in plastic bags and transported to the laboratory to be air-dried, sieved through a 2 mm sieve, and analyzed for pH, TC contents of available P (Melich III-P), K, Ca, Mg, zinc (Zn), iron (Fe), aluminum (Al) and manganese (Mn). The detailed measuring method can be found in the Additional file 1.

2.4 Determination of carbon fractions

Soil total organic carbon was determined by the Walkley–Black method (Li et al. 2016). The dissolved organic carbon was extracted by 1 M KCl solution and measured by dichromate oxidation. Microbial biomass carbon (MBC) was determined using the CHCl_3 fumigation-extraction method (Vance et al. 1987). The liable organic carbon of the bulk soil was measured according to the process of Weil et al. (2003). The light fraction organic carbon (LFOC) was determined according to Roscoe and Burman (Roscoe and Buurman 2003). Particular organic carbon (POC) and mineral-associated organic carbon fractions were determined according to Lagomarsino et al. (2011). Heavy fraction organic carbon (HFOC) was determined according to Falloon and Smith (2000). Soil DOC, MBC, and LOC were classified as active organic carbon pools (AC) (Song et al. 2012). Soil POC and LFOC were classified as chronic organic carbon pools (Cambardella and Elliott 1992). Soil HFOC and MOC were classified as inert organic carbon pools (IOC) (Falloon and Smith 2000). The detailed detection method is listed in the Additional file 1. Soil inorganic carbon (IC) was obtained by TC with TOC deduction.

2.5 Biochar characterization

For the biochar collection, surface soil samples (0–20 cm) were collected through a 5 cm diameter sampling auger during the rice tillering stage in 2020. For each plot, five soil samples were collected on the diagonal and composed of one sample. The collected soil sample was mixed evenly and transported into the laboratory for biochar particle sampling. Biochar particles of 150 μm to 1 mm diameter were hand-picked from the soil samples using tweezers under an optical microscope (45 \times , SZ61, Olympus) until no visible biochar particles were observed. Then, to get the clean biochar particles, they were washed with deionized water and then oven-dried at 60 °C (Yi et al. 2020). Elemental analysis (EA, Flash EA1112, Thermo Finnigan, Italy), Fourier-transform infrared spectroscopy (FTIR, Nicolet, USA), ^{13}C nuclear

magnetic resonance (NMR, Bruker BioSpin AG, Switzerland), and X-ray photoelectron spectroscopy (XPS, VG Escalab-Mark II, England) were conducted to explore the surface chemistry of the biochar.

2.6 Quantification of Gram-positive bacteria and Gram-negative bacteria by qPCR

The microbial community composition was also assessed by the ratios of gram-negative bacteria/gram-positive bacteria (G^-/G^+) in the soil at the mature stage in 2020 to analyze the biochar degradation potential better. The specific sequences of primers (5-AGAGTTTGATCCTGG CTCAG-3) and (5-ACGGCTACCTTGTTACGACTT-3) were used for G^+ . Primers of (5-CCAGCAGCCGCGGTA ATAC-3) and (5-TAACCCAACATYTCACRACACGAG -3) were used for G^- . The detailed protocol is supplied in Additional file 1.

2.7 Data analysis

All the collected data were subjected to R 3.6.1 and SPSS 24.0 statistical software by testing the significance among various treatments at a 5% probability level. One-way ANOVA and the least significant difference (LSD) method were employed to calculate the difference between treatments. Moreover, regression analysis was done to reveal the relationship between treatments and crop parameters. The function of gvlma was used to testify and assure all the linear assumption assessments were acceptable. The importance of soil nutrients on rice yield was calculated by the real weight function after data was standardized by scale function.

3 Results

3.1 Rice yield

The results in the given Fig. 1 depict that all the amendment strategies (biochar and rice straw treatments) significantly ($p < 0.05$) increased rice production over the duration of six years of field experiments (2015 to 2020) (Fig. 1). The results revealed that in comparison to the control treatment (CK), the rice yield in the 6th year increased by 14.3% in RS, 10.9% in AL, and 4.2% in HS respectively. A significant ($p < 0.05$) higher rice yield for AL was observed in 2020. Furthermore, no significant difference was observed from 2016 to 2019 in AL compared to HS. The result is in line with the proposed hypothesis that the annual low-rate application of biochar will considerably increase the rice yield over a single high-dose biochar application.

3.2 Soil nutrients

To investigate the key indicators contributing to higher rice yields, soil TC, TN, and available nutrient elements were detected. Most of the nutrient increase was

observed for RS treatment. In comparison with CK, soil TC, TN, NH₄⁺-N, available Mg, Zn, and Mn in RS were significantly (*p* < 0.05) enhanced by 25.5%, 13.9%, 25.3%, 26%, 42.3%, and 53.6%, respectively (Fig. 2, Additional file 1: Fig. S1). Likewise, in comparison to CK, AL significantly (*p* < 0.05) increased soil TC, TN, NH₄⁺-N, available K, Ca, and soil pH by 29%, 11.4%, 23.9%, 53.3%, and 6.4%, respectively. HS significantly (*p* < 0.05) enhanced the soil TN, NH₄⁺-N, available Mg, Zn, and Mn by 16.7%, 29.6%, 31%, 43.8%, and 51.8%, respectively, as compared to CK. It is worth noting that, compared to HS, the soil pH and available Ca in AL were considerably increased to or by ?) 2.8% (*p* = 0.0497) and 13.2%, respectively (*p* = 0.0414) (Fig. 2). Moreover,

the high soil TC was recorded in AL, whereas as compared to CK no significant difference was observed for HS.

3.3 Mechanism of higher rice yield in AL relative to HS

Without considering the loss, biochar was applied at 22.5 t ha⁻¹ for HS, whereas AL contained an annual application of 16.8 t ha⁻¹. The results depicted that a higher rice yield than HS was observed for AL in 2020, with no significant difference observed from 2016 to 2019. To explore the increasing effect of AL for higher rice production than HS in 2020, a stepwise regression (*n* = 52, *R*² = 0.847) among rice yield and soil nutrients was conducted. The results in the given Table 1 showed that soil TC (*p* = 0.0008), pH (*p* = 0.0021), available Ca (*p* < 0.0001), Fe (*p* = 0.0019), and Mg (*p* = 0.0124) showed a positive relation to the rice yield. However, soil available AL showed a considerable (*p* < 0.0001) negative interaction with the rice yield. The result was similar to the correlation PCA analysis (Additional file 1: Fig. S4). The relative importance analysis for the soil nutrients to the rice yield showed the contribution order of soil nutrients to rice yield: available Ca > Al > TC > Fe > TN (*p* = 0.0779) > pH > Mg (Fig. 3). Soil TC, TN available Ca and pH were significantly increased (*p* < 0.05) in AL treatments in 2020, while in comparison to CK, HS only increased the soil available Mg (*p* < 0.05) content. Hence, the lower rice yield in HS could be due to the lower contribution to soil TC, pH, and available Ca compared with AL.

Table 1 Regression information of rice yield and soil nutrients by stepwise method

	Estimate	Standard error	t value	Pr(> t)	Significance label
(Intercept)	-33.3200	11.6900	-2.8510	0.0067	**
pH	7.8930	2.4080	3.2770	0.0021	**
TC	1.5160	0.4198	3.6130	0.0008	***
TN	0.8471	0.4688	1.8070	0.0779	.
Mg	0.0040	0.0015	2.6140	0.0124	*
Ca	0.0032	0.0006	5.6850	0.0000	***
Al	-0.0043	0.0006	-6.6710	0.0000	***
Fe	0.0014	0.0004	3.3080	0.0019	**
pH*TC	-0.2925	0.0832	-3.5150	0.0011	**

*R*² = 0.847

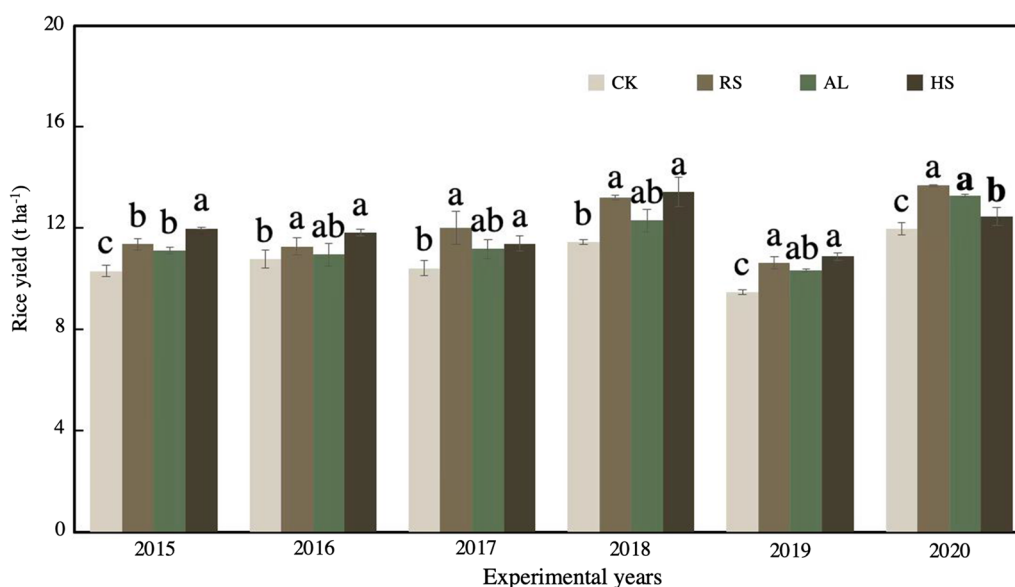


Fig. 1 Rice yield from 2015 to 2020. CK represents control treatment. RS represents the annual rice straw treatment. AL represents biochar collected from annual low biochar strategy treatment and HS represents biochar collected from high single biochar strategy

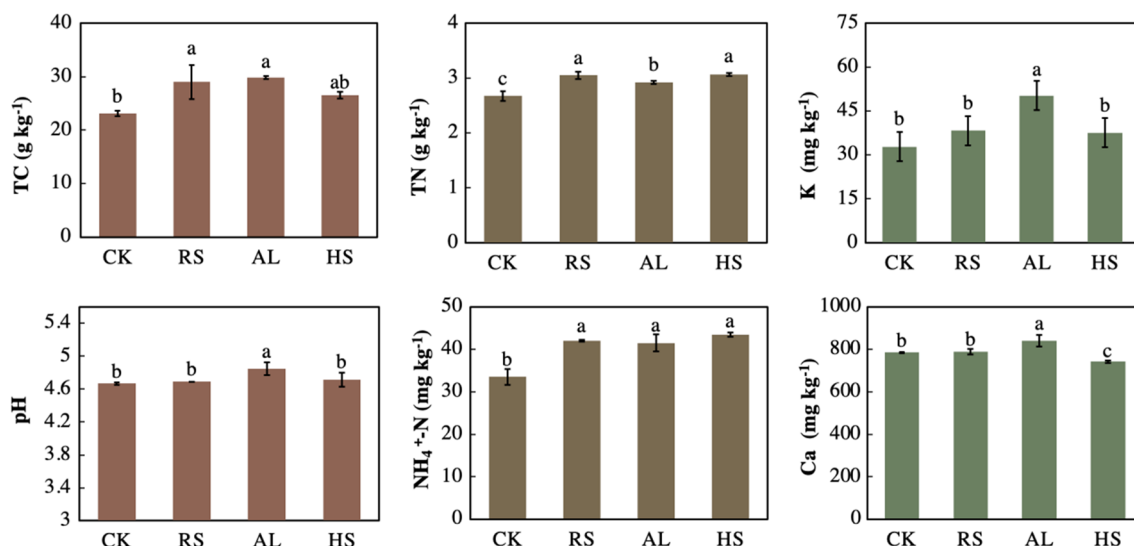


Fig. 2 Soil properties after rice was harvested in 2020. CK represents control treatment. RS represents the annual rice straw treatment. AL represents biochar collected from annual low biochar strategy treatment and HS represents biochar collected from high single biochar strategy

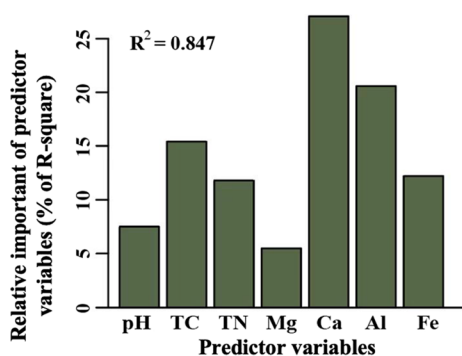


Fig. 3 Relative importance of key soil properties on rice yield. R3.6.0 was used for stepwise regression analysis to make sure all assumptions were acceptable. Then weight function was used to get the relative importance of soil properties on rice yield

3.4 Mechanism of higher soil TC in AL than HS

The significantly lower pH and Ca content in HS seem reasonable compared to AL. However, the biochar application amount in AL would be equal to that in HS in the eighth year ($2.8 \times 8 \approx 22.5 \text{ t ha}^{-1}$). Considering the recalcitrant nature, biochar significantly increased ($p=0.008$) TC in AL, whereas no significant difference ($p=0.099$) was observed for HS compared with CK, indicating fast biochar degradation. In this regard, different soil carbon fractions and biochar characterization were carried out to demonstrate the various possible phenomenon (Fig. 4).

Soil active and inert organic carbon pools were detected. Even though no significant difference in TOC between AL and HS was observed ($p=0.133$),

TOC constituted the main difference in the soil TC between AL and HS, as IC showed a similar value (Additional file 1: Fig. S2). Hence, soil organic carbon fractions were further explored. Both the two biochar treatments decreased soil AC significantly ($p < 0.05$) while increased CC and IOC significantly ($p < 0.05$) (Additional file 1: Fig. S3), as compared to CK. The significantly decreased AC in biochar treatments mainly resulted from the reduced MBC (Fig. 4), not DOC (Additional file 1: Fig. S2). While HS increased LOC particularly ($p=0.021$) in comparison to CK. POC and LFOC were significantly increased compared to CK in AL ($p=0.002, 0.001$) and HS ($p=0.029, 0.002$). In contrast to CK, the significantly increased IOC in AL resulted from HFOC and MOC ($p=0.027, 0.016$, respectively). However, only MOC contributed to a significant increase ($p=0.025$) of IOC in HS compared to CK. RS only increased AC significantly ($p < 0.05$) compared with CK. The result showed that IOC loss mainly led to decreased TC in HS compared to AL.

Furthermore, EA, XPS, and FTIR analyses were also conducted to explore the changes in biochar characteristics to sort out the decreased IOC content in HS compared with AL (Fig. 5). For FTIR, the bands at 647, 699, and 700–900 cm^{-1} represented aromatic O–H, mono polycyclic and branched aromatic groups and aromatic C–H, respectively (Liu et al. 2020). The bands at 1110, 1031, 1160, 1600, and 1700 cm^{-1} represented aliphatic C–O, aliphatic C–O–C, aromatic CO– stretching, aromatic C=C, and aromatic C=O stretching, respectively (Guang-Cai Chen et al. 2008). Likewise, the bands at 2845, 2925, and 2977 were assigned to aliphatic C–H (Yi

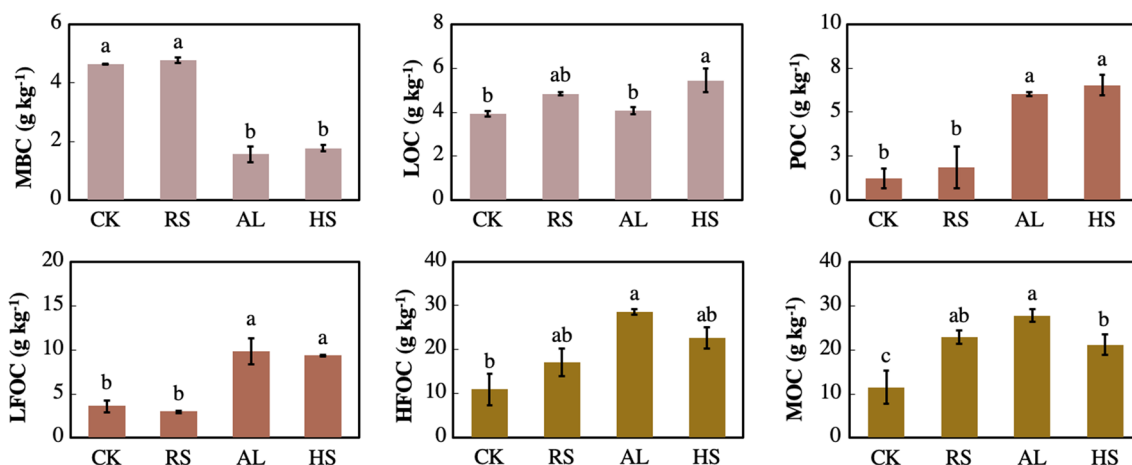


Fig. 4 Soil carbon fractions in CK, RS, AL, and HS. CK represents control treatment. RS represents the annual rice straw treatment. AL represents biochar collected from annual low biochar strategy treatment and HS represents biochar collected from high single biochar strategy

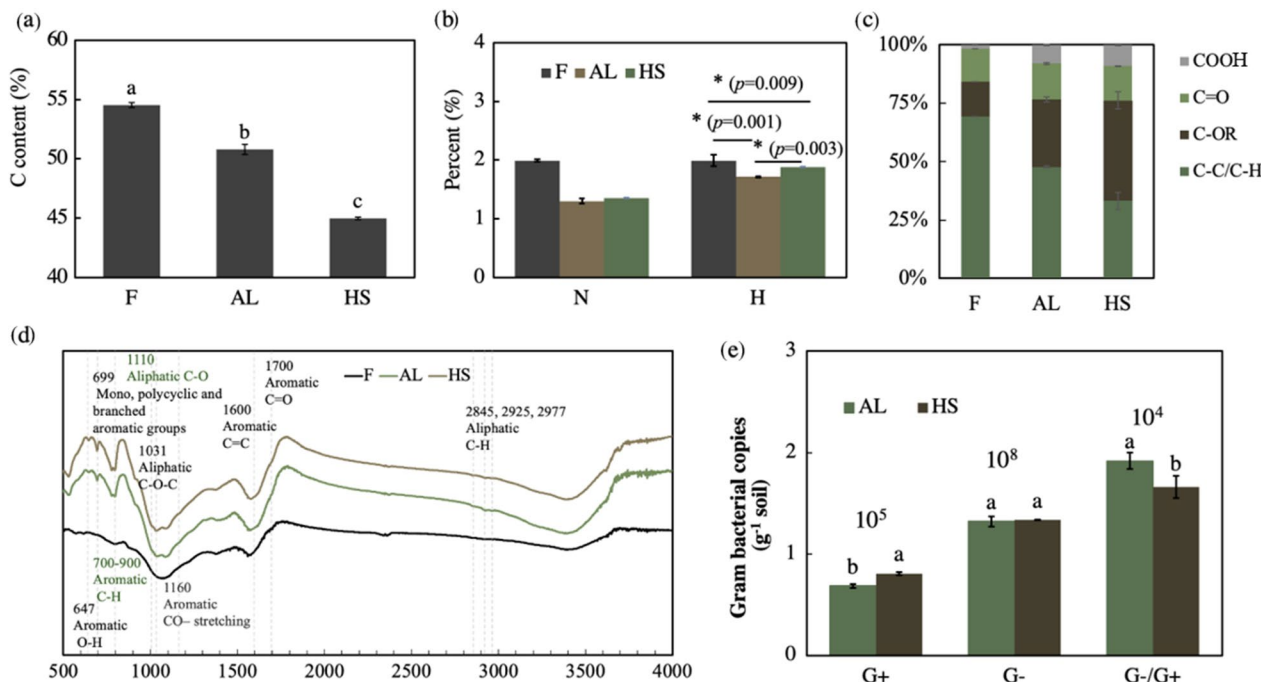


Fig. 5 Element analysis of carbon (a), nitrogen, and hydrogen (b), XPS result (c), FTIR result (d) of biochar characteristic, and Gram bacterial abundance (e) in AL and HS. F represents fresh biochar; AL represents biochar collected from annual low biochar strategy treatment and HS represents biochar collected from high single biochar strategy

et al. 2020). For XPS, Peak energy for C1s was conducted at 284.6 eV for C–C, C=C, and C–H, at 286.2 eV for C–O, 286.8 eV for C=O, and 287.6 eV for COOR (Singh et al. 2014).

EA analysis (Fig. 5a) showed that biochar carbon (BC) content in AL (50.79%) was significantly higher ($p=0.039$) than that in HS (44.98%) and decreased significantly compared with fresh biochar. Accompanied

by lower BC in HS, a significantly higher H content ($p=0.04$) was observed for HS as compared to AL (Fig. 5b). XPS results (Fig. 5c, Additional file 1: Table S2) showed that, after 6 years of the aging process, the relative content of oxygen functional groups (COOR, C-OR) and mainly C-OR increase in HS biochar resulted in the lower BC content. The increased C-OR consisted of aromatic CO– stretching and aliphatic C–O functional

groups according to the FTIR result (Fig. 5d). The increased oxygen functional group of aromatic O–H (647 cm^{-1}) also confirmed the increased H content in HS related to AL. These results showed that more aromatic biochar carbon in HS was oxidized than in AL. G^+ is responsible for enhanced biochar degradation and co-metabolism of soil TOC, and G^-/G^+ is negatively related to the priming effect (Sheng et al. 2016). Significantly ($p=0.046$) increased G^+ in AL than in HS was observed (Fig. 5e). The significantly higher ($p=0.034$) ratio of G^-/G^+ in AL was observed as compared to HS. The results showed that biochar in HS was more fragile to degrade than AL.

4 Discussion

Keeping under consideration the higher rice yield effect, RS showed good performance in promoting rice yield. Even though this effect was comparable with those of HS, however, for RS, the rice straw cost was lower in 6 experimental years ($8 \times 6\text{ t ha}^{-1} < 22.5/0.35\text{ t ha}^{-1}$) compared to HS. However, in 2020 HS showed a decreasing trend in rice yield. Similar results were reported by previous studies (Nan et al. 2020a; Dong et al. 2013). The 6 years' field experiments demonstrated high rice growth and production attributes for rice straw application strategy.

The overwhelming rice yield increasing effects of RS over biochar treatments might result from the higher carbon input than that of AL (considering only 47.2% carbon content remained when rice straw was converted to biochar) on an annual basis. Whereas, soil TC in RS was lower than AL in the third year. This was mainly because of the recalcitrant carbon accumulation in AL, as RS put a large amount of labile organic carbon into the soil (Yin et al. 2014) while AL contained mainly the introduced recalcitrant carbon (Mia et al. 2017a). Labile organic carbon can be easily metabolized by microbes compared to the recalcitrant carbon (Farrell et al. 2013; Gorovtsov et al. 2020; Calvelo Pereira et al. 2011). This was also confirmed by higher soil AC and lower CC and IOC content in RS than in AL and HS (Additional file 1: Fig. S2). Hence, biochar amendment plays another vital role in carbon sequestration (Lehmann et al. 2006; Spokas et al. 2012). On the other hand, the annual rice straw amendment gave nutrient supplement once a year which contributed to yearly nutrient replenishment like soil TN and available Mg (Fig. 2; Additional file 1: Fig. S1), benefiting rice production promotion insistently. The higher soil TN in RS than in AL resulted from the higher TN content in rice straw than in biomass equivalent biochar. In addition to the higher rice production for the rice straw amendment strategy, the promotion of substantial CH_4 emission induced by this strategy could not be ignored

(Yang et al. 2021; Woolf et al. 2010). Hence, the application of biochar is encouraging to fulfill the need for high yield and CH_4 emission reduction (Wang et al. 2012; Zhang et al. 2010).

AL is expected to achieve continuous yield-increasing effects as an alternative strategy in the long run. When in comparison to CK, AL significantly ($p < 0.05$) increased rice production in the last two experimental years, and the yield-promoting effects showed an increasing trend (Fig. 1). The growing promotion of rice yield in AL probably resulted from the cumulative nutrient effect (Nan et al. 2020b). Moreover, the continuous ash content (Al-Wabel et al. 2013; Smider and Singh 2014; Yao et al. 2010) was supplemented by AL, and the nutritive element can be preserved mainly due to the unique surface functionality of biochar (Ippolito et al. 2012) and higher availability than RS due to its liming effect. This is likely the reason for higher rice production in AL than in HS in 2020. The 6-year field experiment also tested our hypothesis that, in the 6th year, AL surpassed HS in rice yield increasing effect. Further, the higher soil CC in AL than in CK indicated that AL had a strong soil carbon supply capacity, as CC is a temporary storage reservoir for soil organic matter turnover and crop-effective nutrients (Jandl and Sollins 1997). This indicates that AL was conceived of great potential to maintain and increase soil fertility, thus achieving a stable or better rice yield stimulation effect in the following long term.

Soil TC, available Ca, and pH were the most significant factors contributing to the increasing rice yield of AL over HS in 2020. It is reasonable that (1) soil pH in HS showed no significant difference with CK and was significantly lower ($p < 0.05$) than AL, and (2) soil available Ca in HS was significantly lower ($p < 0.05$) than that in CK and AL. The fading liming effect of biochar in HS was mainly due to the loss of ash content induced by years of plant utilization and leaching process; meanwhile, and the H^+ released by increased acid oxygen-containing functional groups with biochar aging process (Li et al. 2019). As biochar was only applied in 2015 with no supplementary in the following years, soil available Ca was taken up by plants and probably was deficient in the early experimental years with the abundant of other nutrients like soil TN, available Mg, and Mn (Fig. 2; Additional file 1: Fig. S1). This was also consistent with the higher rice yield in HS in the early experiment years. Therefore, soil available Ca in HS was significantly lower ($p < 0.05$) than that in CK. In contrast, with the annual biochar application and nutrient supplement, soil available Ca in AL was significantly higher than ($p < 0.05$) that in CK and HS. Even so, it was intricate that soil TC in AL was higher ($p = 0.1$) than that in HS, with 16.8 t ha^{-1} (2.8×6) in AL

while 22.5 t ha^{-1} biochar was applied in HS in total till 2020.

Higher IOC in AL led to higher soil TC than HS. Both AL and HS decreased AC pools while increasing IOC pools. The difference was that the higher IOC content (HFOC and MOC) and lower AC (mainly LOC) were observed in AL than in HS, indicating a transformation of IOC into AC in HS. IOC, with members of HFOC mainly composed of aromatic compounds, and MOC, whose carbon is often associated with mineral elements, plays significant roles in carbon sequestration (Georgiou et al. 2022). With no extra carbon supplementation except for biochar, the increased HFOC in AL probably suggested a higher biochar aromatic carbon than HS. A significantly higher ($p = 0.035$) MOC in AL indicated higher aromatic carbon than in HS, considering higher mineral content in HS (Additional file 1: Fig. S1) except for available Ca. These results showed that biochar in HS probably experienced constant and prominent degradation of an aromatic carbon during 6 years of rice growth cycles.

Biochar aromatic carbon oxidation induced a lower IOC content in HS than in AL. Though more biochar (also more recalcitrant carbon) was added in HS than in AL in the 6 years, the inert carbon in HS was lower than in AL. Stronger aromatic carbon oxidation of biochar in HS was observed than that in AL, which was confirmed by FTIR, XPS, and G^+ abundance results. The oxidized organic aromatic carbon was converted to relatively labile carbon, resulting in higher LOC content and lower HFOC in HS. After biochar was applied to soil, labile carbon and volatile organic compounds (15.3%) were first mineralized to CO_2 (Wang et al. 2020) and then left the hard to degraded and stabilized recalcitrant carbon (Quilliam et al. 2013). Usually, biochar-labile carbon will be consumed after 2 years of field incubation (Yi et al. 2020). With low labile carbon of biochar presence in HS treatment, recalcitrant carbon contributed to the main carbon content of biochar and suffered oxidation, thus increasing the oxygen functional groups (Fig. 5d). A study by Yi et al. (Yi et al. 2020) explored long years of moiety changes of biochar after its application into the soil, and reported that biochar recalcitrant carbon decreased by 8.7% after nine years. With a large amount of input, all biochar experienced the oxidation process synchronously, resulting in more LOC and less inert carbon. The result indicated that after 6 years of aging process, the recalcitrant composition of biochar also underwent an oxidation process, which contributed to lower TOC in HS than in AL. In the other research, Nan et al. (2020c) reported that annual low-rate biochar application decreases CH_4 emission stably. Combined with tardiness biochar oxidation in AL, the result is of great climate

combination importance for it reduced carbon emission and also increased carbon sequestration.

Biochar aromatic carbon loss is not the single reason for lower soil TOC in HS than in AL. Rough biochar aromatic C (BAC) content calculation suggested that there should be higher BAC in HS than in AL without consideration of BAC oxidation: there was still 19.06 t ha^{-1} (22.5×0.847) of biochar in HS treatment after deducting the labile carbon and 14.23 t ha^{-1} ($2.8 \times 6 \times 0.847$) of biochar should have been applied in AL. The higher IOC content in AL than in HS meant that at least 25% of BAC was oxidized, which is unrealistic. There must be extra reasons for the lower IOC in HS relative to AL. First, biochar migrated down. Rice roots grow actively in the soil 0–20 cm. With agricultural activity like plowing and gravity function on small pieces of biochar degraded or broken from big ones (Wang et al. 2020, Mia et al. 2017b), part of the biochar carbon would migrate down to deeper depth (50 cm) in soil (Singh et al. 2015) leading to lower soil IOC detection in HS. Moreover, the abundant nutrients provided by biochar in HS might cause native AC first and then inert carbon (humus) consumption combined with biochar oxidation. HS still had the effect of increasing soil available content of Mg, Zn, and TN (Fig. 2; Additional file 1: Fig. S1) to promote rice yield; accordingly more organic carbon was needed to support it. Whereas no significant difference in soil TC was observed between CK and HS, with much recalcitrant carbon difficult to be used by microbes, soil native organic carbon (AC and IOC) might have to be replenishment. The conceptual figure of the supposed carbon loss mechanisms in HS is displayed in Fig. 6.

Annual low-rate biochar strategy has an enormous potential to be conducted globally worldwide. Here are three main reasons behind this claim. First, the biomass needed for the annual low-rate biochar strategy is easily reachable and thus applicable for every square paddy. Moreover, as time flies, the increasing rice effect accumulates with the soil's total carbon content. Further, it's pretty easy to operate by incorporating it in the field before applying fertilizer. However, the biggest obstacle is the cost of the biochar production process. Lowering the production cost is the key to pushing the biochar application from theory to practical application.

5 Conclusion

The 6 years of field experiments demonstrated a declined rice production promotion effect for HS and an economically promising biochar application strategy for rice yield promoting products in AL. RS showed promising results in enhancing the rice yield due to its annual nutrients and active carbon supplementation. However, the CH_4 stimulation factor under this

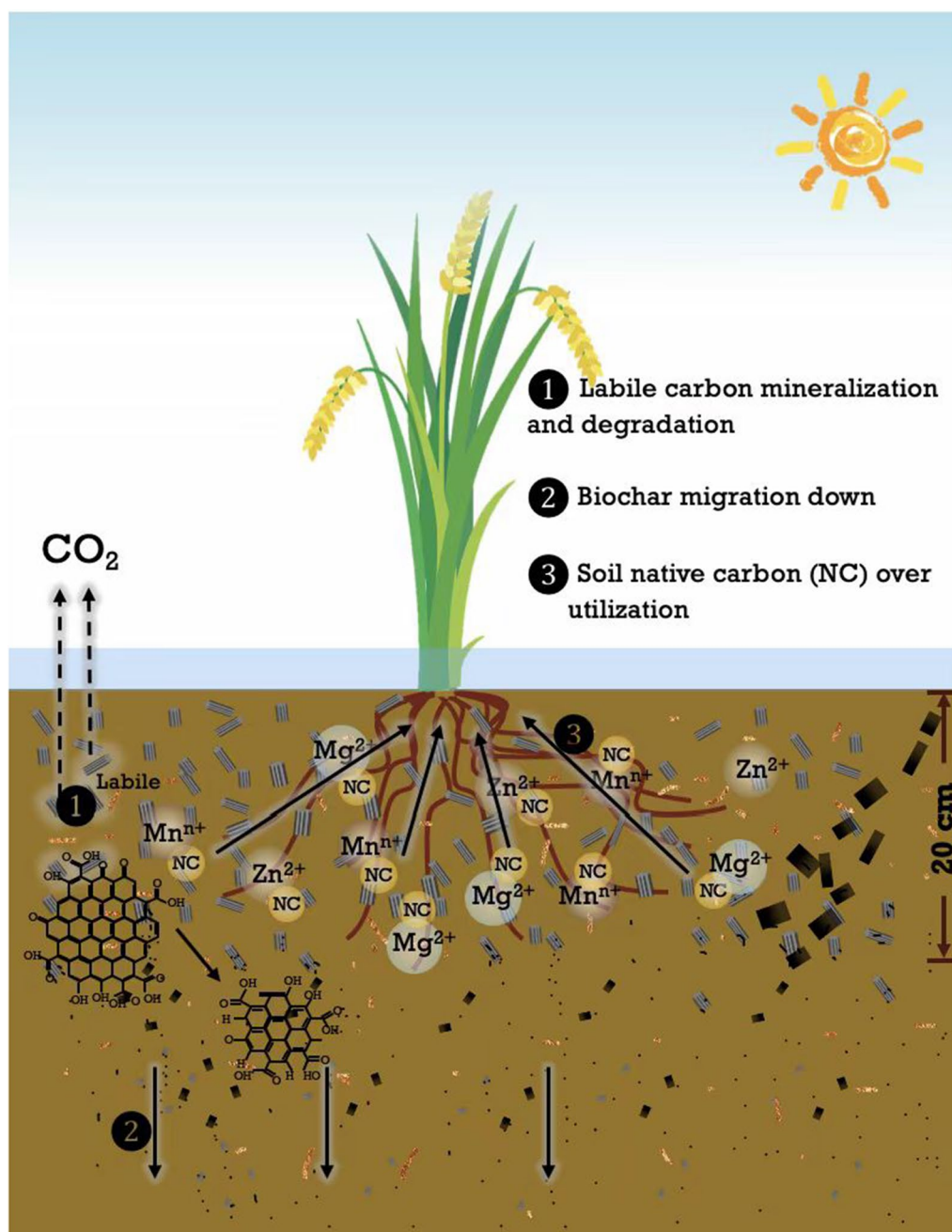


Fig. 6 The conceptual figure of the supposed carbon loss mechanisms in HS.

scenario should be seriously considered, especially considering the significant demand for pursuing carbon neutrality to combat climate change. HS also increased rice yield over 6 years. However, the rice-increasing effect of HS seems to be impaired in the 6th year compared with AL. The sustainable AL model accumulated soil TC, guaranteed available soil nutrients, and increased soil pH, which resulted in higher rice

productivity than HS in 2020., Moreover, a higher rice yield in AL during the following year is expected. The results highlighted the great environmental potential benefits of this sustainable amendment strategy.

A particularly intriguing consequence of our finding is the higher soil TC in AL than in HS during the 6th year, even with a lower biochar application rate. Further exploration disclosed a fast inert biochar carbon

degradation in paddy, which resulted in lower soil TC in HS than in AL. The evidence can be combined with the insight that biochar stability in paddy fields under rice growth has been overestimated. Of particular interest, the results remind researchers of the biochar stability variation in the paddy soils. This phenomenon enlightens us with the significance of attention to the long-term soil quality improvement with biochar incorporation and elevation in the soil pH due to the acid nature.

Abbreviations

CK	Control treatment
RS	8 T rice straw ha ⁻¹ incorporation into paddy field annually
AL	2.8 T biochar ha ⁻¹ incorporation into paddy field annually
HS	22.5 T biochar ha ⁻¹ incorporation into paddy field only in the first year
GHGs	Greenhouse gases emission
CH ₄	Methane
DOC	Dissolved soil organic carbon
NO ₃ ⁻ -N	Soil nitrate
NH ₄ ⁺ -N	Soil ammonia
TC	Soil total carbon
TN	Soil total nitrogen
TOC	Soil total organic carbon
MBC	Microbial biomass carbon
LOC	Labile organic carbon
LFOC	Light fraction organic carbon
POC	Particular organic carbon
MOC	Mineral associate organic carbon
HFOC	Heavy fraction organic carbon
CC	Chronic organic carbon pool
AC	Active organic carbon pool
IOC	Inert organic carbon pool
IC	Soil inorganic carbon
SI	Supplementary Information file
EA	Elemental analysis
FTIR	Fourier-transform infrared spectroscopy
NMR	¹³ C nuclear magnetic resonance
G ⁻ /G ⁺	Gram-negative bacteria/gram-positive bacteria
BAC	Biochar aromatic C

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1007/s42773-023-00218-w>.

Additional file 1. Table S1. Properties of experimental field soil and rice straw and rice straw biochar. **Table S2.** Relative abundance of functional groups obtained from XPS result. **Figure S1.** Soil properties in 2020 of different treatments at a mature stage. **Figure S2.** Soil TC, TOC, IOC, and DOC content at a mature stage in 2020. **Figure S3.** Soil active carbon, slow carbon, and recalcitrant carbon content at a mature stage in 2020. **Figure S4.** PCA analysis for rice yield and soil properties. PCA analysis was conducted by the data in 2015 ~ 2020. **Figure S5.** Soil total carbon (TC) content at the mature stage during 2015 ~ 2020.

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Author contribution

Qiong Nan: Conceptualization, formal analysis, investigation, data curation, writing-original draft, visualization. Lepeng Tang and Wenchen Chi: Investigation. Muhammad Waqas: revising, language editing. Weixiang Wu:

Data curation, funding acquiring, revising, experiment design. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary fields.

Declarations

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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