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ORIGINAL PAPER

Rice Straw Biochar is More Beneficial to Soil Organic Carbon Accumulation and Stabilization than Rice Straw and Rice Straw Ash

Mengru He[1] · Ya Jiang[1] · Yuhang Han[1] · Wenhao Zhu[1] · Da Meng[1] · Cuilan Li[1] · Hongguang Cai[2] · Jinjing Zhang[1]

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Abstract

Purpose It is not well known how crop residue incorporation modes influence soil carbon (C) accumulation and stabilization. Here, an incubation experiment was performed to explore the influences of rice straw (RS) and its ash (RSA) and biochar (RSB) on the concentrations of soil organic carbon (SOC) and SOC fractions associated with bulk soil and its aggregate- and particle-size classes. Moreover, the variation in SOC chemical composition in bulk soil was examined.

Methods Soil samples with RS, RSA, and RSB incorporation and a control without incorporation (CK) were incubated under water logging conditions for 180 d. RSA and RSB were incorporated at a rate equivalent to an RS biomass of 50 g kg^{-1} soil. SOC chemical composition was determined by carbon-13 nuclear magnetic resonance spectroscopy.

Results The concentrations of SOC and SOC fractions generally increased following the order CK < RSA < RS < RSB. Compared with other treatments, RSB incorporation significantly decreased alkyl C and oxygen-alkyl C concentrations and aliphatic C/aromatic C ratio while significantly increased aromatic C concentration and alkyl C/oxygen-alkyl C and hydrophobic C/hydrophilic C ratios. The SOC and SOC fractions concentrations were positively correlated with each other as well as with aromatic carbon.

Conclusions RSB incorporation is more beneficial for SOC accumulation and stability. Physical protection in macroaggregates, chemical protection by clay, and biochemical protection through aromatic C are considered as the most important mechanisms for SOC preservation. This study provides a theoretical basis for rational utilization of RS in terms of improving soil carbon sequestration.

Keywords Soil organic carbon fractions · Soil aggregate-size classes · Soil particle-size classes · Rice straw biochar · ^{13}C nuclear magnetic resonance spectroscopy

1 Introduction

Soil organic carbon (SOC) plays a crucial role in soil fertility and the ecological environment because of its positive contribution to soil properties such as structuring and structural stability (Johannes et al. 2017), pH buffering (Murphy 2015), cation exchange capacity (Yost and Hartemink 2019), biological activity (Bhattacharyya et al. 2021), and carbon (C) sequestration (Han et al. 2016). Therefore, understanding SOC accumulation and stabilization mechanisms is of great importance for mitigating climate change and halting soil degradation.

SOC is a heterogeneous mixture of organic compounds that comprises various fractions with apparently different recalcitrance, decomposition degrees, and turnover rates (Ramesh et al. 2019). Relative to total SOC, SOC fractions are more sensitive to land use and management

✉ Jinjing Zhang
zhangjinjing@126.com

Cuilan Li
licuilan@126.com

Hongguang Cai
caihongguang1981@163.com

[1] Key Laboratory of Sustainable Utilization of Soil Resources in the Commodity Grain Bases of Jilin Province, College of Resource and Environmental Science, Jilin Agricultural University, Changchun, Jilin 130118, China

[2] Institute of Agricultural Resources and Environments, Jilin Academy of Agricultural Sciences, Changchun, Jilin 130033, China

changes (Plaza-Bonilla et al. 2014). Understanding the characteristics of SOC fractions is thus necessary to better reveal SOC accumulation and stabilization mechanisms. At present, physical and chemical fractionation has been widely used to isolate various SOC fractions. Based on physical fractionation methods, SOC fractions associated with aggregate- and particle-size classes can be separated (Stockmann et al. 2013). Meanwhile, it has been generally recognized that physical protection by encapsulation into stable aggregates and chemical protection by adsorption on silt and clay are major mechanisms of SOC stabilization (Christensen 2001; Wiesmeier et al. 2019). According to chemical fractionation methods, labile SOC fractions [e.g., water soluble organic C (WSOC), particulate organic C (POC), easily oxidized organic C (EOC)] and stable SOC fractions [e.g., fulvic acid C (FAC), humic acid C (HAC), humin C (HUC)] can be separated (von Lützow et al. 2007). Similar to SOC in bulk soil, SOC associated with aggregate- and particle-size classes was also a heterogeneous mixture consisting of a series of organic substances (Yu et al. 2022). The changes in labile and stable SOC fractions associated with aggregate- and particle-size classes will subsequently affect SOC accumulation and stabilization. A combination of physical and chemical fractionation methods is a promising method to clarify SOC accumulation and stabilization mechanisms in depth. Furthermore, SOC can be biochemically protected by forming recalcitrant compounds such as alkyl C and aromatic C (Six et al. 2002). As a consequence, information on the SOC chemical composition obtained by spectroscopy [e.g., nuclear magnetic resonance (NMR)] technologies is essential to reveal the biochemical mechanism of SOC accumulation and stabilization.

Rice (*Oryza sativa* L.) is the second largest cereal crop in the world (Chamara et al. 2018), and rice planting results in large amounts of rice straw (RS) being produced annually (Abaide et al. 2019). Burning RS on-field and returning RS directly to the field is two common management practices for RS (Gummert et al. 2020). In recent years, an optimized RS management practice, namely, incorporating biochar into the soil, has aroused widespread attention. Because of its particular properties including rich C content, large surface area, and high stability (Peng et al. 2011; Wu et al. 2012; Kaur et al. 2022), rice straw biochar (RSB) is considered promising for SOC accumulation and stability (Liu et al. 2021). However, most previous studies have mainly focused on the effects of RS and its derivatives (RSA and RSB) on SOC and its fractions in bulk soil (Zhao et al. 2019; Zheng et al. 2019; Bi et al. 2020; Dong et al. 2022; Zhang et al. 2022). There is relatively little information available regarding the impacts of RS and its derivatives on SOC fractions associated with soil aggregate- and particle-size classes, which limits our understanding of the potential mechanisms of SOC accumulation and stabilization after their incorporation into soil.

The main aim of this study was to compare the influences of different RS incorporation modes, i.e., RS, rice straw ash (RSA), and RSB incorporation, and a control without incorporation, on SOC and its fractions concentrations from bulk soil and its aggregate- and particle-size classes by laboratory an incubation experiment. Moreover, the variation in SOC chemical composition in bulk soil was also examined by ^{13}C NMR spectroscopy. We attempted to answer the following two questions: 1) Is conversion of RS into biochar more effective in increasing SOC quantity and stability? 2) What are the underlying mechanisms controlling SOC accumulation and stabilization under RSB? As reported in previous studies, RS and its derivatives are different in their physical, chemical, thermal, and surface properties (Gummert et al. 2020; Kaur et al. 2022; Halder et al. 2023). Thus, we hypothesize that the incorporations of RS and its derivatives into soil will have distinct effects on the accumulation and stabilization of SOC. This study tried to provide a theoretical basis for improving C sequestration capacity in saline-alkali soil through rational utilization of RS.

2 Materials and Methods

2.1 Incubation Experiment

The soil samples (0–20 cm) were collected from an uncultivated saline-alkali land located in Zhenlai County, Jilin Province, Northeast China (46°11′39.8"N, 123°26′55.7"E). The RSA with an output ratio of 18% was prepared by burning RS using a DL-1 electric furnace (Zhongxingweiye Instrument, Beijing, China) under ambient laboratory conditions. RSB with an output ratio of 45% was produced through pyrolysis of RS in a QSXL-1002 muffle furnace (Bozhen Instrument, Shanghai, China) at 350 °C for 2 h. The basic properties of the soil, RS, RSA, and RSB used were tested using the recommended procedures (Lu 2000; Singh et al. 2017), and the results are listed in Table 1 and Fig. S1.

Approximately 200 g of air-dried soil samples (< 2 mm) were rewetted to 60% of the field water holding capacity and pre-incubated aerobically for 7 days at 25 °C in a DHP120 incubator (Shanghai Laboratory Instrument, Shanghai, China). RS, RSA, and RSB, to which $(NH_4)_2SO_4$ was added to give a carbon to nitrogen ratio of 25, were mixed with soil samples at 50, 9, and 22.5 g kg^{-1} dry soil (50 g of RS biomass can produce 9 g of RSA and 22.5 g of RSB in the present study), respectively. To reduce the negative effects of salt and alkali on microbial activity, aluminum sulfate that has been proven to be a promising chemical amendment for the amelioration of saline-sodic soil (Zhao et al. 2019) was added to the soil samples at a rate of 4 g kg^{-1}. The

Table 1 Basic characteristics of soil, rice straw, rice straw ash, and rice straw biochar used in this experiment

	Soil	Rice straw	Rice straw ash	Rice straw biochar
pH	9.81	6.79	11.1	10.4
Specific surface area (m^2/g)	ND	26.8	26.8	32.1
Total pore volume (cm^3/g)	ND	0.044	0.063	0.067
Soil organic carbon (g/kg)	4.70	ND	ND	ND
Total carbon (g/kg)	16.8	368.9	54.3	479.5
Total nitrogen (g/kg)	0.57	5.88	1.86	8.52
Total phosphorus (g/kg)	0.31	3.88	1.81	4.88
Total potassium (g/kg)	19.4	6.00	31.8	24.1
Available nitrogen (mg/kg)	20.9	ND	ND	ND
Available phosphorus (mg/kg)	9.26	ND	ND	ND
Available potassium (mg/kg)	9.73	ND	ND	ND
Water soluble organic carbon (g/kg)	0.17	10.2	6.56	2.93
Humic acid carbon (g/kg)	1.61	14.3	2.12	2.95
Fulvic acid carbon (g/kg)	0.69	9.08	2.58	5.70
Humin carbon (g/kg)	2.23	335.4	43.0	467.9
Alkyl carbon (%)	24.2	5.50	7.45	1.66
Oxygen-alkyl carbon (%)	30.8	83.3	0.12	1.31
Aromatic carbon (%)	32.5	8.08	90.9	98.7
Carboxyl carbon (%)	12.6	3.34	2.02	ND
A/O-A	0.79	0.07	63.2	1.27
Ali/Aro	1.72	11.0	0.08	0.03
HB/HI	1.31	0.16	46.0	76.6

A/O-A, alkyl carbon to oxygen-alkyl carbon ratio; *Ali/Aro*, aliphatic carbon to aromatic carbon ratio; *HB/HI*, hydrophobic carbon to hydrophilic carbon ratio; *ND*, not determined

composites were filled into plastic beakers and incubated at 30 °C under the condition of water logging (approximately 1 cm water layer on the soil surface) in the incubator.

After 180 days of incubation, three replicates of each treatment were removed, sieved to 10 mm, and subsequently divided into three equal parts: 1) the first part was used for soil aggregate fractionation, 2) the second part was used for soil particle-size fractionation, and 3) the third part was used for determining the SOC concentration and chemical composition in bulk soil.

2.2 Soil Analysis

The SOC concentration was measured using the H_2SO_4 and $K_2Cr_2O_7$ oxidation method (Lu 2000). Particulate organic matter (POM) was separated by passing soil samples through a 0.053 mm sieve after dispersing them using $(NaPO_3)_6$ solution, and the POM maintained on the sieve was dried and weighed (Cambardella and Elliott 1992). The EOC concentration was tested with the $KMnO_4$ oxidation method (Blair et al. 1995). Distilled water and 0.1 M NaOH and 0.1 M $Na_4P_2O_7$ mixture were used to extract water soluble substance (WSS) and alkali soluble humic fractions, respectively, the alkaline supernatants were further acidified to isolate fulvic acid (FA) from humic acid (HA) fractions, and the insoluble humic fraction was the humin (HU) fraction (Wang et al. 2016). The C concentrations in POM, WSS, HA, FA, and HU were tested with the same method as that for SOC.

Soil particle-size fractions, i.e., sand- (2–0.05 mm), silt- (0.05–0.002 mm), and clay-size fractions (< 0.002 mm), were obtained by a combination of ultrasonic dispersion at 500 W for 8 min on a SCIENTZ-IID Ultrasonic Homogenizer (Xinzhi Biotechnology, Ningbo, China), wet sieving, and centrifugation in water (Anderson et al. 1981; Tiessen and Stewart 1983). Soil water-stable aggregates, i.e., large macroaggregates (LMac, > 2 mm), small macroaggregates (SMac, 2–0.25 mm), microaggregates (Mic, 0.25–0.053 mm), and silt + clay (SC) fractions (< 0.053 mm), were separated according to the wet sieving procedure (Cambardella and Elliott 1993). The concentrations of SOC, WSOC, HAC, FAC, and HUC associated with aggregate- and particle-size-size classes were measured with the same method as that of SOC. Mean weight diameter (MWD) was calculated to characterize the water stability of aggregates (Zhang et al. 2017).

Solid-state ^{13}C NMR analysis was performed with an AVANCE III 400 WB spectrometer (Bruker BioSpin,

Fällanden, Switzerland) after treating bulk soil samples using HF-HCl solution (10%, v/v) (Schmidt et al. 1997). The following NMR parameters were set: contact time, 2 ms; acquisition time, 20 ms; and recycle time, 3 s. Chemical shifts were referenced to the methylene resonance from adamantane standard. Semi-quantification was carried out with MestReNova software (version 5.3.1, Mestrelab Research, Santiago de Compostela, Spain).

2.3 Statistical Analyses

To evaluate whether straw incorporation modes had statistically significant influences on SOC concentration, SOC fractions concentrations, SOC chemical composition, and soil aggregate- and particle-size distributions, one-way ANOVA followed by LSD test was employed when data were normally distributed (Shapiro−Wilks tests, $P > 0.05$) and variances complied with homogeneity (Levene's tests, $P > 0.05$). If necessary, data were log- or square root-transformed and retested. Pearson correlation was utilized to assess relationships between SOC concentration, SOC fractions concentrations, and SOC chemical composition at $P < 0.05$ and $P < 0.01$. SPSS software (version 16.0, SPSS, Chicago, USA) was utilized for statistical analysis.

3 Results

3.1 SOC and SOC Fractions Concentrations in Bulk Soil

The SOC concentration followed the order of CK (4.78 g kg^{-1}) < RSA (4.83 g kg^{-1}) < RS (7.54 g kg^{-1}) < RSB (10.5 g kg^{-1}), but no significant difference was found between the CK and RSA treatments. The concentrations of WSOC, EOC, POC, HAC, FAC, and HUC, which accounted for 3.45% − 4.29%, 23.6% − 39.0%, 18.0% − 31.2%, 34.5% − 35.3%, 8.86% − 13.5%, and 47.8% − 52.3% of SOC, respectively, also generally displayed a similar pattern to the SOC concentration. However, there were no significant differences for WSOC, EOC, HAC, FAC, and HUC between the CK and RSA treatments, for WSOC between the RSA and RS treatments, and for FAC between the RS and RSB treatments (Table 2).

3.2 SOC and SOC Fractions Concentrations in Soil Aggregate-Size Classes

Across all treatments, LMac displayed a lower proportion (3.93%–7.79%) than SMac (14.9%–33.2%), Mic (27.4%–44.5%), and SC fractions (28.8%–47.6%). Compared with other treatments, the RS treatment had a significantly greater proportion of LMac and a smaller proportion of Mic, while the RSB treatment had a significantly greater proportion of SMac and a smaller proportion of SC fractions (Fig. 1a). The MWD was 44.0% ($P < 0.05$) and 40.0% ($P < 0.05$) higher in the RS and RSB treatments than in the RSA treatment, respectively (Fig. 1b).

Across all treatments, the concentration of SOC associated with SC fractions was highest (3.62 − 15.7 g kg^{-1}), followed by SOC associated with Mac (2.59 − 14.8 g kg^{-1}), and the concentration of SOC associated with Mic was the lowest (2.06 − 5.33 g kg^{-1}). Among the different treatments, the concentrations of SOC associated with all aggregate-size classes increased significantly in the order of CK < RSA < RS < RSB. Similarly, the concentrations of WSOC, HAC, FAC, and HUC associated with all aggregate-size classes generally increased in the order of CK < RSA < RS < RSB. However, there were no significant differences for WSOC associated with Mac between the CK and RSA as well as between the RS and RSB treatments, for FAC associated with Mac between the RS and RSB treatments, and for HAC, FAC, and HUC associated with Mic between the RSA and RS treatments (Table 3).

3.3 SOC and SOC Fractions Concentrations in Soil Particle-Size Classes

Across all treatments, the proportion of sand-size fraction was the largest (44.0%–54.8%), followed by coarse

Table 2 Soil organic carbon and its fractions concentrations in bulk soil under treatments receiving rice straw ash (RSA), rice straw (RS), rice straw biochar (RSB), and control (CK)

Treatment	SOC (g kg^{-1})	WSOC (g kg^{-1})	EOC (g kg^{-1})	POC (g kg^{-1})	HAC (g kg^{-1})	FAC (g kg^{-1})	HUC (g kg^{-1})
CK	4.78 ± 0.04 c	0.17 ± 0.01 c	1.21 ± 0.12 c	0.86 ± 0.03 d	1.65 ± 0.01 c	0.63 ± 0.02 b	2.33 ± 0.02 c
RSA	4.83 ± 0.02 c	0.20 ± 0.02 bc	1.14 ± 0.05 c	1.40 ± 0.07 c	1.68 ± 0.02 c	0.65 ± 0.02 b	2.31 ± 0.03 c
RS	7.54 ± 0.06 b	0.26 ± 0.05 b	2.81 ± 0.11 b	2.35 ± 0.03 b	2.66 ± 0.05 b	0.90 ± 0.02 a	3.72 ± 0.10 b
RSB	10.5 ± 0.04 a	0.45 ± 0.08 a	4.10 ± 0.03 a	3.20 ± 0.08 a	3.67 ± 0.02 a	0.93 ± 0.03 a	5.49 ± 0.12 a

Different lowercase letters within the same column indicate significant differences among treatments at $P < 0.05$ (mean ± standard deviation, $n = 3$). *SOC*, soil organic carbon; *WSOC*, water soluble organic carbon; *EOC*, easily oxidized organic carbon; *POC*, particulate organic carbon; *HAC*, humic acid carbon; *FAC*, fulvic acid carbon; *HUC*, humin carbon

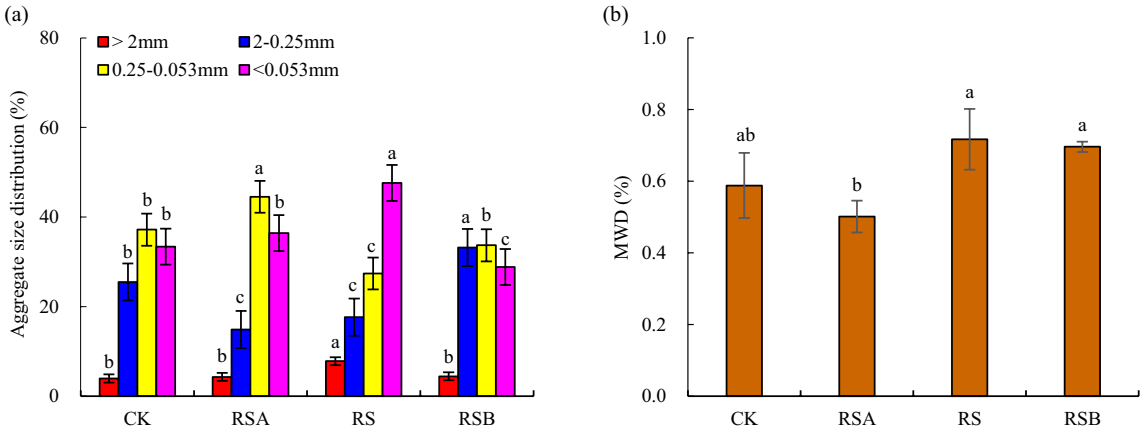

Fig. 1 Size distributions (**a**) and mean weight diameter (MWD) (**b**) of soil water-stable aggregates under treatments receiving rice straw ash (RSA), rice straw (RS), rice straw biochar (RSB), and control (CK). The bars having same letters among different treatments are not significantly different ($P < 0.05$)

silt- (25.0%–32.7%) and clay-size fractions (12.0%–20.1%), and the proportion of fine silt-size fractions was the lowest (2.62%–11.3%). Among the different treatments, the RS treatment showed a significantly greater proportion of sand-size fraction than the RSA and RSB treatments, the RSA treatment showed a significantly greater proportion of fine silt-size fraction than the other treatments, and the CK and RSB treatments had a significantly greater proportion of clay-size fraction than the RSA treatment (Fig. 2).

Across all treatments, the concentration of SOC associated with clay-size fraction was the highest (11.5–23.8 g kg^{-1}), followed by SOC associated with silt- (6.19–15.7 g kg^{-1}) and sand-size fractions (3.73–9.13 g kg^{-1}). Among the different treatments, the concentrations of SOC associated with all particle-size fractions significantly increased in the order of CK < RSA < RS < RSB. Likewise, the concentrations of WSOC, HAC, FAC, and HUC associated with all particle-size fractions also generally increased in the order of CK < RSA < RS < RSB. However, there were no observable differences for HAC and HUC associated with sand-size fraction between the RS and RSB treatments, for HAC associated with sand-size fraction between the RSA and RS treatments, for FAC associated with sand-size fraction between the CK and RSA treatments, and for HUC associated with clay-size fraction between the RAS and RS treatments (Table 4).

Table 3 Soil organic carbon and its fractions concentrations in soil aggregate-size classes under treatments receiving rice straw ash (RSA), rice straw (RS), rice straw biochar (RSB), and control (CK)

Treatment	SOC (g kg^{-1})	WSOC (g kg^{-1})	HAC (g kg^{-1})	FAC (g kg^{-1})	HUC (g kg^{-1})
Macroaggregates (> 0.25 mm)					
CK	2.59 ± 0.03 d	0.09 ± 0.01 b	0.73 ± 0.03 d	0.47 ± 0.08 c	1.30 ± 0.03 d
RSA	6.32 ± 0.10 c	0.12 ± 0.00 ab	1.45 ± 0.04 c	1.28 ± 0.03 b	3.47 ± 0.08 c
RS	9.52 ± 0.06 b	0.15 ± 0.03 a	1.79 ± 0.04 b	1.40 ± 0.05 a	5.78 ± 0.11 b
RSB	14.8 ± 0.06 a	0.16 ± 0.03 a	1.88 ± 0.06 a	1.58 ± 0.06 a	10.3 ± 0.02 a
Microaggregates (0.25 – 0.053 mm)					
CK	2.06 ± 0.07 d	0.13 ± 0.00 d	0.46 ± 0.04 c	0.37 ± 0.08 c	1.10 ± 0.11 c
RSA	3.96 ± 0.09 c	0.15 ± 0.00 c	0.99 ± 0.01 b	0.89 ± 0.09 b	1.94 ± 0.09 b
RS	4.23 ± 0.06 b	0.19 ± 0.00 b	1.04 ± 0.05 b	0.86 ± 0.08 b	2.14 ± 0.10 b
RSB	5.33 ± 0.10 a	0.21 ± 0.01 a	1.32 ± 0.02 a	1.12 ± 0.03 a	2.68 ± 0.12 a
Silt + clay fractions (< 0.053 mm)					
CK	3.62 ± 0.20 d	0.10 ± 0.00 d	0.65 ± 0.04 d	0.71 ± 0.02 d	2.16 ± 0.25 d
RSA	7.23 ± 0.10 c	0.16 ± 0.00 c	1.55 ± 0.03 c	1.56 ± 0.05 c	3.95 ± 0.09 c
RS	10.6 ± 0.19 b	0.20 ± 0.03 b	2.32 ± 0.01 b	1.93 ± 0.06 b	5.42 ± 0.19 b
RSB	15.7 ± 0.26 a	0.22 ± 0.01 a	3.44 ± 0.10 a	3.00 ± 0.05 a	8.28 ± 0.31 a

Different lowercase letters within the same column indicate significant differences among treatments in the same aggregate-size class at $P < 0.05$ (mean ± standard deviation, $n = 3$). *SOC*, soil organic carbon; *WSOC*, water soluble organic carbon; *HAC*, humic acid carbon; *FAC*, fulvic acid carbon; *HUC*, humin carbon

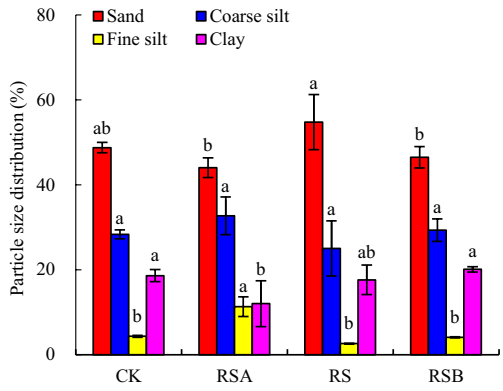

Fig. 2 Size distributions of soil particle-size classes under treatments receiving rice straw ash (RSA), rice straw (RS), rice straw biochar (RSB), and control (CK). The bars having same letters among different treatments are not significantly different ($P < 0.05$)

3.4 SOC Chemical Composition

The NMR spectra included carboxyl C (190–160 ppm), aromatic C (160–110 ppm), O-alkyl C (110–50 ppm), and alkyl C (50–0 ppm). O-alkyl C predominated in the CK and RS treatments, while aromatic C predominated in the RSA and RSB treatments (Fig. 3).

No significant differences were found for the proportions and concentrations of all SOC functional groups among the CK, RSA, and RS treatments. The exceptions were for the proportion of carboxyl C that was significantly greater in the CK than in the RSA and RS treatments, for the concentration of O-alkyl C that was significantly greater in the RS than in the CK and RSA treatments, and for the concentration of carboxyl C that was significantly greater in the RS than in the RSA treatment. In contrast, the proportions and concentrations of carboxyl C, O-alkyl C, and alkyl C were significantly lower, while those of aromatic C were significantly higher for the RSB than for the other treatments. The ratios of alkyl C/O-alkyl C, aliphatic C/aromatic C, and hydrophobic C/hydrophilic C among the CK, RSA, and RS treatments were not significantly different. However, RSB had significantly higher alkyl C/O-alkyl C and hydrophobic C/hydrophilic C ratios but significantly lower aliphatic C/aromatic C ratio than the other treatments (Table 5).

3.5 Relationships Between SOC Concentration, SOC Fractions Concentrations, and SOC Chemical Composition in Bulk Soil and its Aggregate- and Particle-Size Classes

Significant and positive correlations were observed between SOC, WSOC, EOC, POC, HAC, FAC, and HUC concentrations in bulk soil with SOC, WSOC, HAC, FAC, and HUC concentrations in soil aggregate-size classes. There were also significant and positive correlations between SOC, WSOC, EOC, POC, HAC, FAC, and HUC concentrations in bulk soil with SOC, WSOC, HAC, FAC, and HUC in soil particle-size classes. Moreover, SOC and SOC fractions concentrations in bulk soil and its aggregate- and particle-size classes were significantly and positively correlated with aromatic C concentration (Fig. S2).

Table 4 Soil organic carbon and its fractions concentrations in soil particle-size classes under treatments receiving rice straw ash (RSA), rice straw (RS), rice straw biochar (RSB), and control (CK)

Treatment	SOC (g kg^{-1})	WSOC (g kg^{-1})	HAC (g kg^{-1})	FAC (g kg^{-1})	HUC (g kg^{-1})
Sand-size fractions					
CK	3.73 ± 0.07 d	0.14 ± 0.01 d	1.15 ± 0.03 c	0.17 ± 0.03 c	2.28 ± 0.07 c
RSA	5.72 ± 0.06 c	0.18 ± 0.00 c	1.52 ± 0.01 b	0.49 ± 0.03 c	3.53 ± 0.06 b
RS	8.25 ± 0.22 b	0.22 ± 0.00 b	1.70 ± 0.03 ab	1.41 ± 0.02 b	4.91 ± 0.18 a
RSB	9.13 ± 0.29 a	0.25 ± 0.00 a	1.98 ± 0.35 a	1.77 ± 0.37 a	5.13 ± 0.28 a
Silt-size fractions					
CK	6.19 ± 0.05 d	0.14 ± 0.00 d	0.95 ± 0.03 d	0.95 ± 0.02 d	4.15 ± 0.02 d
RSA	9.99 ± 0.07 c	0.16 ± 0.00 c	1.27 ± 0.02 c	1.22 ± 0.04 c	7.34 ± 0.07 c
RS	12.2 ± 0.13 b	0.18 ± 0.00 b	1.78 ± 0.07 b	1.38 ± 0.14 b	8.82 ± 0.11 b
RSB	15.7 ± 0.12 a	0.26 ± 0.00 a	3.06 ± 0.05 a	1.83 ± 0.06 a	10.6 ± 0.17 a
Clay-size fractions					
CK	11.5 ± 0.04 d	0.15 ± 0.00 d	2.99 ± 0.00 d	1.75 ± 0.06 d	6.58 ± 0.10 c
RSA	14.9 ± 0.14 c	0.18 ± 0.00 c	3.46 ± 0.02 c	3.96 ± 0.06 b	7.27 ± 0.13 b
RS	15.1 ± 0.12 b	0.21 ± 0.01 b	4.60 ± 0.04 b	3.13 ± 0.05 c	7.18 ± 0.14 b
RSB	23.8 ± 0.17 a	0.26 ± 0.01 a	6.54 ± 0.05 a	4.36 ± 0.16 a	12.6 ± 0.11 a

Different lowercase letters within the same column indicate significant differences among treatments in the same aggregate-size class at $P < 0.05$ (mean ± standard deviation, $n = 3$). *SOC*, soil organic carbon; *WSOC*, water soluble organic carbon; *HAC*, humic acid carbon; *FAC*, fulvic acid carbon; *HUC*, humin carbon

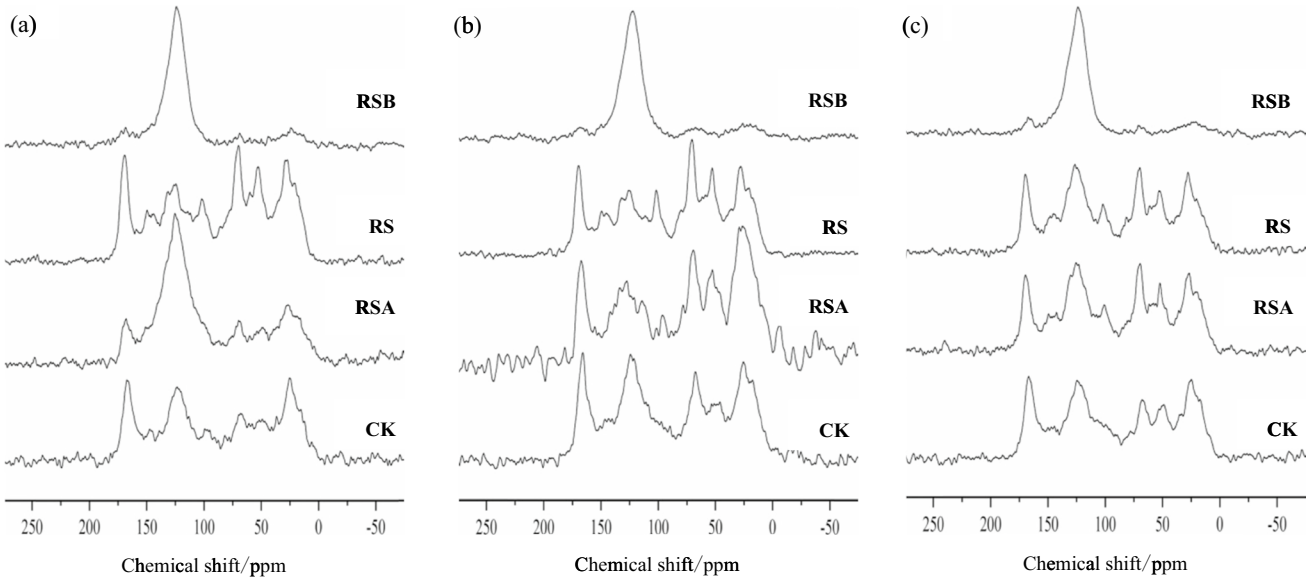

Fig. 3 Solid-state ^{13}C NMR spectra of soil samples under treatments receiving rice straw ash (RSA), rice straw (RS), rice straw biochar (RSB), and control (CK). The subfigures (**a**), (**b**), and (**c**) represent three replicates from each treatment

4 Discussion

In the present study, RS and its derivatives exhibited different effects on the concentrations of SOC and its fractions. The distinct physical and chemical properties of these amendments were considered responsible for the differences in the accumulation of SOC and its fractions after their application to soil. After RS burning, RSA still contained a certain amount of C. The incorporation of RSA into soil can thus increase SOC and SOC fractions concentrations. As expected, RS incorporation further increased SOC and SOC fractions concentrations compared with RSA incorporation due to the higher C concentration in RS. In the study of Shu et al. (2016), they observed that POC was larger in RS than in RSA, which could explain the higher POC concentration in RS- than in RSA-amended soil. Our results were identical to the findings from previous studies that showed that the concentrations of SOC and SOC fractions were generally higher in soils with than without RS (Yin et al. 2014; Zhao et al. 2019; Dutta et al. 2022) and with RS than with RSA incorporation (Dutta et al. 2022). The present study proved that residue retention rather than burning was more beneficial for enhancing SOC sequestration. Consistent with the results from previous studies (Liu et al. 2016; Zheng et al. 2019), we observed that SOC and SOC fractions

Table 5 Soil organic carbon chemical composition from solid-state ^{13}C NMR spectra of soil samples under treatments receiving rice straw ash (RSA), rice straw (RS), rice straw biochar (RSB), and control (CK)

Treatment	Alkyl C	O-alkyl C	Aromatic C	Carboxyl C	A/O-A	Ali/Aro	HB/HI
Proportion (%)							
CK	24.5 ± 1.73 a	31.3 ± 2.35 a	29.0 ± 1.09 b	15.2 ± 0.92 a	0.79 ± 0.12 b	1.93 ± 0.09 a	1.15 ± 0.10 b
RSA	24.4 ± 7.28 a	30.2 ± 8.29 a	37.1 ± 16.7 b	8.34 ± 3.61 b	0.83 ± 0.20 b	1.78 ± 1.04 a	1.81 ± 1.03 b
RS	22.7 ± 0.66 a	38.4 ± 3.56 a	28.3 ± 4.02 b	10.7 ± 0.31 b	0.59 ± 0.05 b	2.19 ± 0.42 a	1.05 ± 0.15 b
RSB	6.87 ± 0.74 b	1.70 ± 0.68 b	87.8 ± 2.38 a	3.61 ± 1.39 c	4.54 ± 2.12 a	0.10 ± 0.01 b	19.2 ± 6.37 a
Concentration (g kg^{-1})							
CK	1.17 ± 0.08 a	1.50 ± 0.12 b	1.39 ± 0.06 b	0.73 ± 0.04 a			
RSA	1.21 ± 0.36 a	1.50 ± 0.41 b	1.84 ± 0.83 b	0.41 ± 0.18 b			
RS	1.58 ± 0.06 a	2.68 ± 0.26 a	1.98 ± 0.27 b	0.75 ± 0.03 a			
RSB	0.60 ± 0.06 b	0.15 ± 0.06 c	7.71 ± 0.24 a	0.32 ± 0.12 b			

Different lowercase letters within the same column indicate significant differences among treatments at $P < 0.05$ (mean ± standard deviation, $n = 3$). *A/O-A*, alkyl carbon to oxygen-alkyl carbon ratio; *Ali/Aro*, aliphatic carbon to aromatic carbon ratio; *HB/HI*, hydrophobic carbon to hydrophilic carbon ratio

concentrations were higher after RSB incorporation than after RS incorporation. Our results further proved that RSB was more efficient than its source material in promoting SOC accumulation. The reasons may be summarized as follows. First, RSB itself contained more C, especially more resistant humin C and condensed aromatic C structures relative to RS. Thus, the incorporation of RSB can significantly increase SOC concentrations although the rate of RSB incorporation was lower than that of RS incorporation in the present study. In addition, we observed that WSOC, HAC, and FAC concentrations from the RSB-amended soil were higher than those from the RS-amended soil, although RS contained more WSOC, HAC, and FAC. This implied that RSB incorporation was more beneficial to the formation and accumulation of WSOC, HAC, and FAC. A significant increase in WSOC concentration after the application of biochar to soil was also observed in previous studies (Yang et al. 2020; Dong et al. 2022). It was reported that biochar-derived dissolved organic matter can be stabilized in soil via adsorption onto soil components or by biotic and abiotic oxidation processes after soil application (Sun et al. 2021). In recent studies, Zhang et al. (2022) observed that maize straw biochar could partly transform into extractable humic substances after soil addition. Dong et al. (2022) reported that the application of wheat straw biochar increased the concentrations of HA- and FA-like compounds in WSOC. The increasing humic fractions could be partly responsible for the increases in HAC and FAC in the biochar-amended soil. Second, RSB has a larger surface area than RS (Table 1), which may promote the accumulation of some SOC fractions (e.g., WSOC) in soil by their adsorption onto the biochar surface (Chen et al. 2021; Feng et al. 2021). Third, RSB possessed highly porous structure that could also protect some SOC fractions against microbial degradation. In an incubation experiment, Zheng et al. (2021) reported that the mineralization of hydrophilic dissolved organic matter was slower than that of hydrophobic dissolved organic matter due to the accessibility of the former to the pore space of biochar. Zhu et al. (2019) observed that the mineralization rate was higher for RS than for RSB after they were applied to soil. Fourth, RSB could induce a positive or negative priming effect on native SOC (Zhu et al. 2019; Chen et al. 2021). In this study, the inducement of the negative priming effect was possibly partly responsible for the increase in SOC concentration following the incorporation of RSB. However, some previous studies showed that RSB incorporation did not influence SOC concentration (Liu et al. 2016) or significantly decreased SOC fractions (e.g., dissolved organic C, microbial C, mineralizable C) concentrations compared with RS incorporation (Li et al. 2013; Yin et al. 2014). The discrepancies might be ascribed to the influences of many factors, for example biochar diversities and soil types used in experiments. A meta-analysis of 169 studies found that soils with low initial C concentrations showed higher percent increases after biochar application than soils with high initial C concentrations. The influence of the initial soil C concentration on SOC accumulation was even greater than that of soil pH (Chagas et al. 2022). In this study, the saline-alkali soil used contained a low initial SOC concentration (< 10 g kg^{-1}), which could cause significant increases in SOC fractions after soil incorporation. Overall, our present study suggested that converting RS into RSB followed by its incorporation into soil should contribute to the dual increases in labile and stable SOC fractions.

In the present study, RS and its derivatives also exhibited different effects on the chemical composition of SOC. Although RSA was predominated by aromatic C compounds, the incorporation of RSA only slightly increased the concentration of aromatic C in soil. Meanwhile, RSA addition also did not influence the concentrations of other SOC functional groups. Our results suggested that the C-containing functional groups from RSA hardly took part in the formation of SOC molecular structures after they were incorporated into the soil. In contrast, the incorporation of RS significantly increased the O-alkyl C concentration. The high O-alkyl C of RS was responsible for the significant increase in the O-alkyl C concentration following RS incorporation into the soil. Relative to RS and RSA, RSB presented greater proportion of aromatic C and lower proportions of alkyl C, O-alkyl C, and carboxyl C. In a previous study, Li et al. (2013) also showed that O-alkyl C in RS tended to eliminate and aromatic C became the predominant component when the charring temperature was over 300 °C. The incorporation of RSB can thus significantly increase aromatic C concentration but decrease alkyl C, O-alkyl C, and carboxyl C concentrations. In addition, it was previously reported that recalcitrant C functional groups (e.g., aromatic C) could accumulate by consuming or converting labile and degradable C compounds (e.g., O-alkyl C) due to the enhancement of soil microbial activity under biochar amendment (Situ et al. 2022; Zheng et al. 2022). The changes in the alkyl C/O-alkyl C, aliphatic C/aromatic C, and hydrophobic C/hydrophilic C ratios implied that the humification, aromaticity, and hydrophobicity degrees of soil organic matter increased significantly after RSB addition, which contributed to the stabilization of SOC. Our results suggested that RSB amendment had a profound influence on the SOC chemical composition. Bi et al. (2020) also reported that aromatic structures in soil increased after the incorporation of RSB. In addition, the predominance of aromatic structures was responsible for the large specific surface area, porous structure (Kaur et al. 2022), high pH, and electrical conductivity of RSB (Li et al. 2013).

It was previously reported that SOC could be protected physically through aggregate formation, chemically via silt and clay, and biochemically by forming refractory compounds (Six et al. 2002). In the present study, a positive

correlation between SOC and SOC fractions concentrations from bulk soil with those from Mac and Mic were observed, which demonstrated that SOC was physically protected in aggregate classes. In the RSB-amended soil, SOC and SOC fractions concentrations were generally higher in Mac than in Mic, suggesting that physical protection via Mac was a more important mechanism of SOC accumulation and stabilization. Some previous researchers indicated that SOC decomposition was more susceptible to increasing temperatures in Mic than in Mac (Tao and Song 2013; Sandeep and Manjaiah 2014). The predominance of Mac-associated SOC over Mic-associated SOC in RSB-amended soil would be beneficial to combat future climate change. On the other hand, a positive correlation between SOC and SOC fractions concentrations in bulk soil with those in SC fractions was observed, which confirmed that chemical protection by silt and clay was another mechanism for SOC accumulation and stabilization. The higher concentrations of SOC and SOC fractions in SC fractions than in Mac and Mic suggested that chemical protection was a more important mechanism for SOC preservation than physical protection in RSB-amended soil. The clay minerals have been proven to protect SOC against microbial degradation by forming organic-mineral complexes and promoting aggregate establishment (Sarkar et al. 2018). Our present findings also showed that the concentrations of SOC and SOC fractions in clay-size fraction were larger than those associated with silt-size fraction in RSB-amended soil. In addition, we found that there was a positive correlation between SOC and SOC fractions concentrations with aromatic C concentration, which implied that SOC was biochemically protected by forming refractory aromatic C compounds in RSB-amended soil.

5 Conclusions

It is important to study soil organic carbon accumulation and stabilization under different rice straw incorporation modes to obtain valuable information on how to improve soil carbon sequestration capacity through rational utilization of rice straw. In the present study, we demonstrated that the incorporation of rice straw and its derivatives (i.e., rice straw ash and rice straw biochar) into soil had distinct effects on the accumulation and stabilization of soil organic carbon. Compared with rice straw and rice straw ash, the incorporation of rice straw biochar was more beneficial for soil organic carbon accumulation and stabilization due to the higher concentrations of soil organic carbon and its fractions and the greater degrees of humification, aromaticity, and hydrophobicity of soil organic carbon in rice straw biochar-amended soil. The physical protection in macroaggregates, chemical protection by clay, and biochemical protection through aromatic carbon are considered as the most important mechanisms for soil organic carbon accumulation and stabilization in rice straw biochar-amended soil. Based on the findings obtained from the present study, turning rice straw into rice straw biochar followed by its incorporation into soil is beneficial for climate change mitigation, soil quality improvement, and crop residue management. This is the first attempt to systematically compare the effects of rice straw, rice straw ash, and rice straw biochar on soil organic carbon accumulation and stabilization from the perspective of both bulk soil and its physically and chemically separated fractions. However, the findings from our present study need to be further confirmed by using in situ field experiments.

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Declarations

Conflict of Interest The authors declare no competing interests.

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