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The effects of long-term rice straw and biochar return on soil humus composition and structure in paddy soil

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Abstract: The aim of this study was to evaluate the effects of continuous application of rice straw and biochar for 10 years on soil humus composition and structure in paddy soil. A 10-year field experiment was conducted in a paddy field and included three treatments: rice straw biochar (SC); rice straw (RS), no biochar or rice straw. The elemental analyser, Fourier transform infrared (FT-IR) spectrum, and three-dimensional excitation-emission matrix (3D EEM) fluorescence spectroscopy with fluorescence regional integration (FRI) analysis were used to study the soil humus composition and structure under different treatments. The results verified that the incorporation of rice straw and biochar significantly improved soil pH values and the soil organic carbon contents compared with the control. Rice straw significantly increased the contents of extractable humus, humic acid (HA) and fulvic acid in soil, while biochar only significantly affected HA and humic degree values. The molecular structure of HA affected by biochar is characterised by high humification and aromaticity, but rice straw increased the aliphaticity of the HA structure, as presented by elemental composition. Moreover, 3D EEM spectroscopy combined with FRI analysis showed that RS treatment formed soil humus had more aliphatic compounds, while SC treatment increased the aromatic components of humus. These results suggest that rice straw promotes the renewal of humus, and biochar enhances the humification degree of humus and the aromaticity of HA.

Keywords: ecosystem; FT-IR spectrum; carbon cycle; microorganism; structural properties

As the largest carbon pool within ecosystems, soil plays a crucial role in the global carbon cycle. Organic matter is a crucial component of soil carbon pool, and therefore, is essential for maintaining soil ecosystem functions and quality and closely related to terrestrial soil carbon cycling and stability (Smith et al. 2015). Soil organic matter (SOM) consists of a range of organic compounds that are produced through the breakdown of plant and animal remains, a process facilitated by microorganisms through bio-

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geochemical activities (Horáček et al. 2014). It is very difficult to study SOM because it is thoroughly mixed with and often adhere to minerals in soil. Before the advent of spectrometry, the chemical method was an important and widely used method for extracting soil organic carbon (SOC) (Zimmermann et al. 2007). The extraction efficiency of alkaline solution is very high. However, under strong alkaline conditions, the alkaline solution will exert an exaggerated chemical reactivity and extract portions of soil fractions (Lehmann and Kleber 2015). In order to avoid the adverse effects of alkaline conditions, although it is not as effective an extractant as an alkaline solution, neutral pyrophosphate is commonly used to extract SOC (Bremner and Lees 1949). In addition, organic solvents prefereed for humus extraction due to their unique dielectric constants and dipole moments (Hayes 2006). Therefore, the alkaline extraction method is still widely used in the fractionation of SOM due to its high efficiency and convenient reagent preparation.

Humic substances (HS) are supramolecular associations formed by the self-assembly of small, heterogeneous molecules derived from the degradation and decomposition of dead biological material, held together by multiple weak interactions such as hydrogen and van der Waals bonds (Piccolo 2002, Nebbioso and Piccolo 2012). HS in SOM has the highest stability, serving as a crucial indicator of carbon stability. It plays a crucial role in promoting soil aggregation, reducing nutrient losses, and stabilising organic carbon reservoirs (Gerke 2021). Humus can be divided into various constituents depending on its ability to dissolve in either acidic or alkaline solutions, including humic acid (HA), fulvic acid (FA), and humin (HM). The different components of humus, which have varying structures and roles, show different levels of resistance to biological decomposition. As a result, they play a key role in preserving the stability of soil carbon reservoirs. Generally, humic acids tend to have more aromatic composition and show higher resistance to decomposition by microbes, whereas fulvic acids are more susceptible to being leached from soil solutions and act as carbon sources for both microbes and plants. Changes in the structure and properties of fulvic acids can influence soil nutrient-holding and nutrient-supplying capacities, which are correlated with soil fertility status. However, the stability of humus components in soil varies due to anthropogenic activities (e.g., land management) and environmental factors (e.g., climate, soil types) (Ovchinnikova 2014). For example, the conversion of undisturbed original soil to farmland by tillage had a significant impact on surface soil humus, leading to a decrease in the level of topsoil humus to 92% of its initial value (Eremin 2016).

However, paddy soils have experienced a rapid decline in organic matter and nutrient due to the excessive cultivation and irrigation in recent years. Returning straw to the fields has become an effective means of increasing crop yields, as it can efficiently control soil erosion and enhance SOM content. Short-term research suggests that incorporating straw into the soil initially leads to a rapid increase in the content of dissolved organic carbon (Zhu et al. 2016), followed by a slow decrease, yet maintaining a higher level compared to the control treatment. Field studies have shown that adding straw to soil can boost organic matter levels and increase soil humus turnover rate (Fan et al. 2018). Zhang et al. (2021) showed that the short-term incorporation of corn straw into the soil is conducive to increasing SOC and aliphaticity in HA, which can potentially increase C sequestration. However, straw returning also weakened the stability of organic matter and significantly increased C mineralisation in the labile fraction. Furthermore, several studies have also indicated that long-term straw incorporation may result in a significant increase in soil diseases and exacerbating soil acidification in farmland (Yang et al. 2019). Straw biochar, pyrolysed from straw, has been recognised as an effective way to achieve agronomic benefits with decreasing bulk density (Dong et al. 2020), increasing water retention capacity and soil aggregate stability. Studies also have shown that biochar can promote the humification of HM compounds in soil and the humification of biochar itself (Orlova et al. 2019), supplementing HA to soil and thereby increasing the degree of soil humification. However, previous studies have mainly focused on the effects of straw and biochar on SOC and its composition in the short term (Fan et al. 2018, Zhang et al. 2019, 2021). Limited information is currently available regarding the long-term effects of straw and biochar on SOC fractions associated with soil humus, which hinders our comprehensive understanding of the underlying mechanisms.

In this study, a 10-year field experiment with rice straw and biochar incorporation was conducted from 2013 to 2022 to investigate the impact of long-term rice straw and biochar amendments on the humifica-

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tion of SOM in paddy field by analysing the chemical composition and structure of the humus with three-dimensional excitation-emission matrix (3D EEM) fluorescence spectroscopy coupled with Fourier transform infrared (FT-IR) spectrum and element analyser. Our hypothesis was that the long-term application of biochar would have a greater effect on decomposition and composition of organic matter and HA structure than rice straw amendment.

MATERIAL AND METHODS

Site description and experimental design. The experimental plot was in Yuhang District, northwest of Hangzhou City, Zhejiang Province, China (119.90°E, 30.37°N). The climate in this area is the North subtropical southern edge monsoon with seasonal changes. The soil within the experimental plot is identified as a typically hydromorphic paddy soil, comprising 73% sand, 23% silt and 4% clay. The soil texture is sandy loam according to USDA-NRCS soil classification. The properties of the topsoil, rice straw and rice straw biochar were described in detail by Dong et al. (2013).

For ten consecutive years (2013–2022), we planted single-season rice in paddy fields in Jingshan Town and conducted field experimental management. The entire plot was divided into 9 experimental blocks of 4 m × 5 m each by hardening the ridges between the blocks, covering them with plastic sheeting and laying cement stone slabs. The trials were conducted in 9 experimental blocks, including three types of experimental management with triplicate: SC treatment (add rice straw biochar); RS treatment (add dried rice straw), and CK treatment (no rice straw and biochar). Rice straw was applied annually at a rate of 8 t/ha, aligning with the regional standard practice for returning straw to the paddy field. The carbon conversion rate of rice straw during the carbonisation process was 35%. Rice straw biochar was therefore incorporated into the soil at a rate of 2.8 t/ha, which, in quantities, was equivalent to the biomass of rice straw used in the RS treatment. Before transplanting rice seedlings in the RS and SC treatments, biochar and straw were thoroughly mixed with the soil layer at a depth of approximately 0–15 cm using a hoe. The control was also evenly blended but without adding straw and biochar. Among all the treatments, the urea was applied at a rate of 270 kg N/ha. Urea was applied in three splits, 40% of the total fertiliser was applied as base fertiliser before transplantation, 30% was applied during the tillering stage, and the remaining 30% was applied during the panicle stage. Calcium superphosphate and potassium chloride were applied at the rate of 33 kg P/ha and 74.7 kg K/ha, respectively, and both fertilisers were applied simultaneously as base fertilisers before transplanting seedlings. The paddy field was managed with a typical water management system (flooding-drainage-reflooding-moist) for the area.

Soil sample collection and physicochemical property analysis. Soil samples (0–15 cm) were collected from each block using the five-point method after rice harvest. Soil samples were air-dried and sieved, and then individual soil samples were divided into two parts: one part was used to measure soil physical and chemical properties, and the other was used to extract the soil humus. Soil pH was determined with a pH meter (Leichi PHS-3E, Shanghai, China) after suspending soil in water at a 1:2.5 *w*/*v* ratio. After removing roots, rice stems, and leaves, soil samples were ground to pass through a 0.15 mm sieve to analyse soil total C, total N, and SOC. The total C and N of the paddy soil were determined using an Element analyser (Elementar vario EL cube, Frankfurt, Germany). The SOC content was determined by wet oxidation with $K_2Cr_2O_7$ and titration with $FeSO_4$.

Extraction of humic compounds and purification of humic acid. The various humic compounds extraction procedure was performed followed Wang et al. (2014) and Gaffney et al. (1996). Soil samples were extracted with 0.1 mol/L NaOH at a ratio of 1:20 (*w*/*v*) in an oscillator at 180 rpm for 10 h. After extraction, the mixture was centrifuged at 3 000 *g* for 10 min, and the supernatant was collected. The residue underwent three more extractions, and the supernatants collected from all four extractions together represented the extractable humic substances. Subsequently, the residual substances after extracting all the extractable humic substances were cleaned and dried at low temperature to obtain HM. We used 6 mol/L HCl to adjust the pH of the extractable humic substances to 1.0–2.0 until a red-brown flocculent precipitate was visible. The solution was then heated at 80 °C for 30 min and left overnight at 4 °C. The solution containing precipitates was separated *via* centrifugation (6 000 *g*, 15 min). The precipitates were humic acid (HA), and the supernatants was fulvic acid (FA). The precipitated HA was purified by dissolving it in 0.1 mol/L NaOH and dialysing through a Spectra Por membrane (1000 Dalton MWCO, Hunan, China) to remove excessive salt and finally

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freeze-dried (72 h). The elemental composition of HM and purified HA were analysed by elemental analyser. The TOC/TN Analyser (multi N/C 2100, Analytik Jena AG multi N/C 2100, Jena, Germany) was employed to quantify two important carbon fractions: extractable humus carbon (EXC) and fulvic acid carbon (FAC). The humic acid carbon content (HAC) was calculated as the difference between FAC and EXC. The PQ value represents the degree of soil humification, which was computed as HAC/(HAC + FAC).

FT-IR and 3D EEM fluorescence spectroscopy. The Fourier transform infrared spectrometer (Shimadzu IRPrestige-21, Kyoto, Japan) at wave numbers of 400–4 000/cm was used to acquire the infrared spectral characteristics of HA. 2 mg HA sample was mixed evenly with 200 mg KBr, grinded thoroughly, pressed into thin slices using a tablet press and then analysed. The spectra were recorded 32 times, with a resolution of 4/cm. Besides, the background of pure KBr was analysed to correct the baseline of the spectra. To quantify the relative changes in the FT-IR spectra and facilitate spectral comparison, the relative absorbance was calculated by dividing the corrected height of a specific peak (e.g., 3 409, 2 925, 2 851, 1 650, 1 412 or 1 035/cm) by the total height of all peaks at these wavenumbers, and then multiplying the result by 100 (Gerzabek et al. 2006). Corrected peak heights were obtained using the OMNIC software (Thermo Fisher Scientific, Waltham, USA).

The extractable humus sample was passed through a 0.45 μm filter membrane prior to dilution (organic carbon concentration is approximately 10 mg/L). We scanned extractable humus samples with a fluorescence spectrophotometer (Hitachi F-7000, Tokyo, Japan) to obtain spectral data of the 3D EEM. The scanning parameters were the following: the excitation fluorescence wavelength (Ex) scanned from 200 to 500 nm with an increment of 4 nm, and the emission fluorescence wavelength (Em) scanned from 250 to 600 nm with an increment of 3 nm. The recorded EEM spectra were obtained using a scanning speed of 12 000 nm/min. We analysed the fluorescence intensity data extracted by the FL Solutions software with MATLAB R2022a data processing software (MathWorks, Natick, USA). The EEM spectral data of ultrapure water was subtracted from the EEM spectral data of each sample to eliminate Raman scattering. After removing the first- and second-order Rayleigh scattering regions, the EEM data was quantitatively analysed using the fluorescence regional integration (FRI) technique through interpolation on the MATLAB R2022a data processing software platform. The EEM data could be divided into five categories of substances, i.e., tyrosine-like organic compounds (region I); tryptophan-like organic compounds (region II); fulvic acid-like substances (region III); soluble microbial by product-like substances (region IV), and humic acid-like substances (region V) (Chen et al. 2003). We used $\Phi_{i,n}$ to define the integral volume of each region. $\Phi_{i,n}$ can be determined by normalising the volume derived from multiplying the area of the specified region with its fluorescence intensity. The integral volume ($\Phi_{i,n}$) was calculated by Eq. 1:

$$\Phi_{i,n} = \mathrm{MF}_i \Phi_i = MF_i \sum_{ex} \sum_{em} I(\lambda_{ex}\lambda_{em})\, \Delta\lambda_{ex} \Delta\lambda_{em} \quad (1)$$

where: MF_i – fixed multiplication factor for each region; $I(\lambda_{ex}\lambda_{em})$ – fluorescence intensity at each excitation (ex) and emission (em) wavelength pair; $\Delta\lambda_{ex}$ – excitation wavelength gradient (4 nm); $\Delta\lambda_{em}$ – emission wavelength gradient (3 nm).

Statistical analyses. All data were expressed as means and standard error of the means. The statistical significance of the biochar and rice straw amendment on soil properties and humus composition was determined using analysis of variance (ANOVA) and least significant difference (*LSD*) tests by the SPSS 24.0 statistical software package (IBM, Armonk, USA). Any differences with a *P*-value less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Effects of rice straw and biochar amendment on soil properties and humus composition. According to Table 1, the long-term continuous application of rice straw (RS) and biochar (SC) in paddy fields can significantly impact the physicochemical properties of the soil, including TC, TN, SOC, and pH. The addition of straw significantly increased the TC and TN content. Although adding biochar can increase TC content, it did not have a significant impact on TN. Additionally, the long-term addition of biochar or straw significantly increased the C/N ratio of the soil. The addition of biochar and straw significantly increased soil SOC content by 47% and 26%, respectively, compared to the CK treatment. We demonstrated that incorporating rice straw or biochar into the soil can notably enhance SOC content. This finding was consistent with existing studies (Cui et al. 2017, Zheng et al. 2019). The increase in SOC is mainly due to the microbial decomposition

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Table 1. Effects of rice straw and biochar amendment on soil properties in paddy fields

	TC (g/kg)	TN (g/kg)	C/N	SOC (g/kg)	pH
CK	25.07 ± 1.33^{c}	3.19 ± 0.17^{b}	7.87 ± 0.13^{c}	23.50 ± 1.10^{b}	4.98 ± 0.02^{c}
RS	31.81 ± 1.14^{b}	3.76 ± 0.08^{a}	8.46 ± 0.14^{b}	29.68 ± 0.57^{a}	5.06 ± 0.04^{b}
SC	36.94 ± 3.76^{a}	3.67 ± 0.44^{ab}	10.07 ± 0.23^{a}	34.50 ± 4.18^{a}	5.12 ± 0.01^{a}

Means ± standard error; different letters within the same column indicate significant differences ($P < 0.05$) among the treatments. TC – total carbon; TN – total nitrogen; SOC – soil organic carbon; CK – control; RS – rice straw; SC – rice straw biochar

of rice straw in paddy soil, which produce organic acids, and thus increase organic matter (Cui et al. 2017, Zhang et al. 2020). Additionally, long-term straw returning could enhance soil organic matter-minerals interactions, decrease the mineralisation of pre-existing SOM, and promote soil aggregation and aggregate stability (Zhang et al. 2020). Relative to the RS treatment, the SC treatment exerts a more pronounced positive impact on the content of SOC and the C:N ratio in paddy soil. Biochar is highly porous and has large surface area, which could effectively adsorb organic carbon in the soil and prevent it from microbial decomposition. Adding biochar to paddy soil also inhibited the mineralisation of SOC by reducing the activity of enzymes (e.g., sucrase, β-glucosidase) that hydrolyse soil carbon (Demisie et al. 2014, Zheng et al. 2019). Besides, the carbon in biochar is indeed a kind of hard-degradable carbon, which is different from the easily degradable carbon provided by rice straw. The addition of straw and biochar to soil impacted SOC stability through changing soil cation mobility. In acidic soils, organic acids caused metal minerals to dissolve, resulting in the release of SOC that was bound to metals. Incorporating straw or biochar into the soil can increase pH, which reduce the effectiveness and mobility of metal cations and thus microorganisms' activity and respiration (Gondek et al. 2020). This contributed to SOC stability in the paddy soil.

Soil humus is an important component of SOC, and its content is governed by the combined effects of humification and respiratory processes. Changes in the content of humic composition in response to biochar and rice straw application are shown in Figure 1. Compared to the CK treatment, the RS treatment significantly increased the C content of EXC, HA, FA, and HM in the soil, which was mainly caused by the humification process that converted returned straw into humic substances. A significant increase in the content of FAC in the RS treatment has been widely reported. Our results are in consistent with existing studies that the relatively labile FA was initially formed and then continuously increased during the decomposition of straw into humus (Zhang et al. 2019). Additionally, incorporating biochar significantly increased the content of HAC and humin carbon, but had negligible impact on FAC. On the one hand, this might be due to the abundance of stable aromatic components in biochar, which makes greater stability of biochar C (Tan et al. 2017), leading to the accumulation of larger molecular structures of HA and HM. On the other hand, the excitatory effect caused by biochar in the soil might result in easier decomposition of smaller molecular structures of FA (Maestrini et al. 2015). Further calculations revealed that the SC treatment had a higher PQ value than other treatments, indicating a higher degree of humification under the SC treatment (Zhang et al. 2019). The release of humic-like compounds from biochar and increased pH value might contribute to the decrease in the proportion of low molecular weight humus bonds in the soil (Sadej and Zolnowski 2019, Sun et al. 2022). This also can be attributed to the fact that FA was more easily decomposed by microbes compared to HA in the SC treatment. Therefore, adding rice straw or biochar to the fields contributes to improving soil maturity and fertility (Zhang et al. 2021).

Elemental composition of soil humic acid and humin. The changes in the elemental composition (C, N, H, S) of HA and HM after the long-term continuous application of rice straw and biochar in paddy fields are presented in Table 2. In comparison to the elemental composition of HM, HA had higher contents of C, N, H and S. The RS treatment significantly increased the contents of C, N, H and S in HA compared to the CK treatment. However, the SC treatment only had a significant impact on the C and N contents in HA. Both the RS and SC treatments substantially increased the C content in

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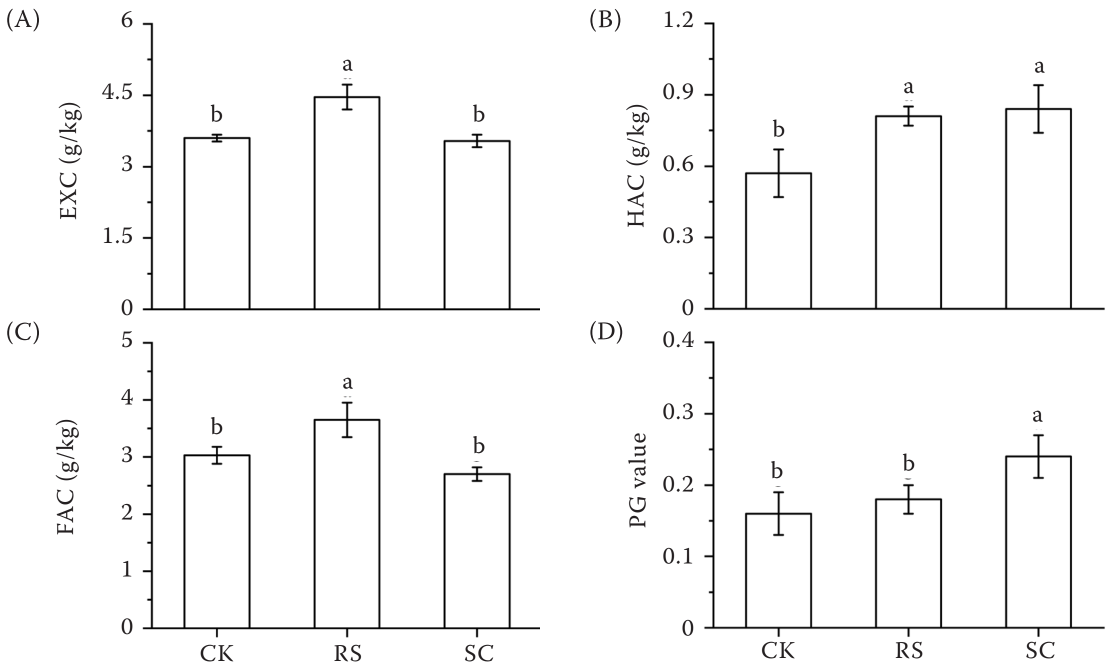

Figure 1. Effects of rice straw and biochar amendment on humus composition. (A) Carbon content of extractable humus (EXC); (B) carbon content of humic acid (HAC); (C) carbon content of fulvic acid (FAC), and (D) PQ value computed as HAC/ (HAC + FAC). Different letters above the error bars indicate significant differences (P < 0.05) among the treatments. CK – control; RS – rice straw; SC – rice straw biochar

HM compared to the CK treatment. The RS treatment also had a significant effect on the H content in HM. In all treatments, the C/N ratios in HA and HM were the highest in the SC treatment, reaching a significant level compared to those in the RS and CK treatments. The ratio of hydrogen to carbon (H/C) could serve as an indicator of the degree of aromatic condensation in the molecular structure of HA. A higher H/C ratio indicates a stronger aliphatic property of HA, while a lower H/C ratio represents a higher degree of aromatic condensation in HA. The decreasing H/C ratio in the SC treatment illustrated that applying biochar to paddy soil could enhance the aromaticity of HA molecules, increase C aromaticity, promote the aggregation of HA, and make the molecular structure more complex (Zhang et al. 2019). Compared with the CK treatment, straw and biochar significantly decreased the H/C ratio of HA in paddy soil. This was mainly attributed to the fact that both straw and biochar contain aromatic C compounds. In the SC treatment, incorporating biochar not only promotes soil humification process

Table 2. Effects of rice straw and biochar amendment on the elemental composition of humic acid (HA) and humin (HM) in paddy fields

	Treatment	C	N	H	S	C/N	H/C
		(g/kg)					
HA	CK	172.44 ± 3.38^{c}	24.63 ± 0.54^{b}	36.04 ± 3.19^{b}	2.92 ± 0.49^{b}	7.00 ± 0.05^{c}	2.51 ± 0.24^{a}
	RS	234.27 ± 1.52^{a}	28.92 ± 0.77^{a}	41.22 ± 0.46^{a}	3.91 ± 0.57^{a}	8.10 ± 0.17^{b}	2.11 ± 0.03^{b}
	SC	197.92 ± 1.56^{b}	22.14 ± 0.54^{c}	31.63 ± 2.33^{b}	2.79 ± 0.31^{b}	8.94 ± 0.29^{a}	1.92 ± 0.16^{b}
HM	CK	14.03 ± 0.55^{c}	2.11 ± 0.21^{a}	5.71 ± 0.29^{b}	0.41 ± 0.38^{a}	6.67 ± 0.45^{c}	0.22 ± 0.00^{a}
	RS	19.21 ± 1.49^{b}	2.26 ± 0.26^{a}	6.63 ± 0.32^{a}	0.13 ± 0.05^{a}	8.21 ± 0.33^{b}	0.37 ± 0.01^{b}
	SC	28.20 ± 3.88^{a}	2.50 ± 0.25^{a}	6.26 ± 0.33^{ab}	0.61 ± 0.39^{a}	11.24 ± 0.52^{a}	0.41 ± 0.02^{c}

Means ± standard error; different letters within the same column indicate significant differences (P < 0.05) among the treatments. CK – control; RS – rice straw; SC – rice straw biochar

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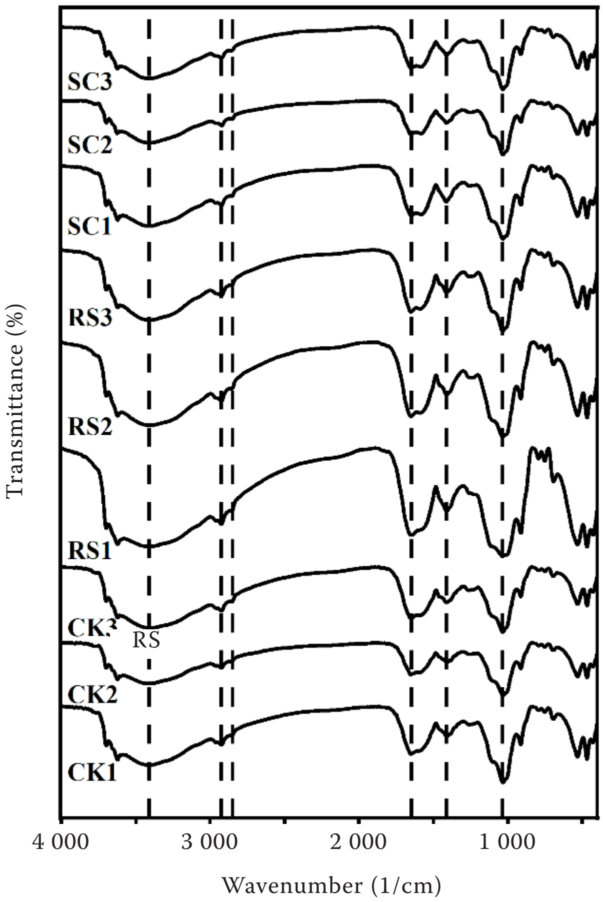

Figure 2. Fourier transforms infrared spectra of soil humic acid using different treatments. CK – control; RS – rice straw; SC – rice straw biochar

but also concurrently decarboxylates and diminishes carbohydrate and amino acid fragments in its structure (Lodygin and Beznosikov 2010).

FT-IR analysis of soil humic acid. Fourier transform infrared spectra of soil humic acid and the relative absorption of selected peaks under different treatments are shown in Figure 2 and Table 3. The absorption peaks in the infrared spectra of humic acid showed intense bands near 3 409/cm, 1 650/cm, 1 412/cm, and 1 035/cm, generally attributed to the stretching vibration of O-H, the aromatic structure C=C stretching, COO-, polysaccharide-like structure bands and Si-O of silicate impurities, respectively (Fan et al. 2018, Liu et al. 2021, Zhang et al. 2021). In addition, the peaks at 2 925/cm and 2 851/cm were related to symmetric and asymmetric C-H stretching in the CH_3 and CH_2 of the aliphatic chains. However, there were no significant differences in the relative intensity of those absorption peaks among the treatments.

3D EEM of soil extractable humus. The 3D EEM fluorescence spectrum was conducted to evaluate the level of humification and chemical composition of extractable humus (Figure 3). The 3D EEM fluorescence spectra of extractable humus from different treatments exhibited similar peak features, but significant differences were observed in the fluorescence intensity of peak A between RS and SC treatments (Table 4). Those changes directly reflected the impacts of biochar and rice straw amendment on soil humus. As shown in Figure 3, the EEM spectrum from soil samples revealed two distinctive peaks at excitation/emission (EX/EM) wavelengths of 250/390–460 nm (peak A), 252/427–442 nm (peak B) and 310/380–410 nm (peak C). According to Guo et al. (2021), peak A is attributable to fulvic-like substances, while peak B and peak C are attributable to humic-like substances. Interestingly, the fluorescence intensities of peaks A and B were decreased in the SC treatment compared to the CK treatment, while the RS treatment showed the opposite trend. Stronger fluorescence intensity was linked to the presence of hydroxyl, alkoxy, methoxy, and amino groups within the humus (Senesi et al. 2003). Therefore, long-term application of straw can increase soil extractable humus with a higher content of aliphatic C compounds. Zhang et al. (2021) also found that following the applica-

Table 3. Mean relative absorbance in percentage of the sum of all selected peak heights of the FT-IR spectra of humic acid (HA) from different treatments

	Relative absorbance as a percentage of the sum of selected peaks					
	3 409/cm	2 925/cm	2 851/cm	1 650/cm	1 412/cm	1 035/cm
CK	22.18[a]	12.52[a]	8.89[a]	16.49[a]	10.12[a]	29.80[a]
RS	22.31[a]	12.83[a]	9.31[a]	16.04[a]	9.55[a]	29.96[a]
SC	21.81[a]	11.78[a]	8.38[a]	16.93[a]	9.85[a]	31.25[a]

Different letters within the same column indicate significant differences ($P < 0.05$) among the treatments. CK – control; RS – rice straw; SC – rice straw biochar

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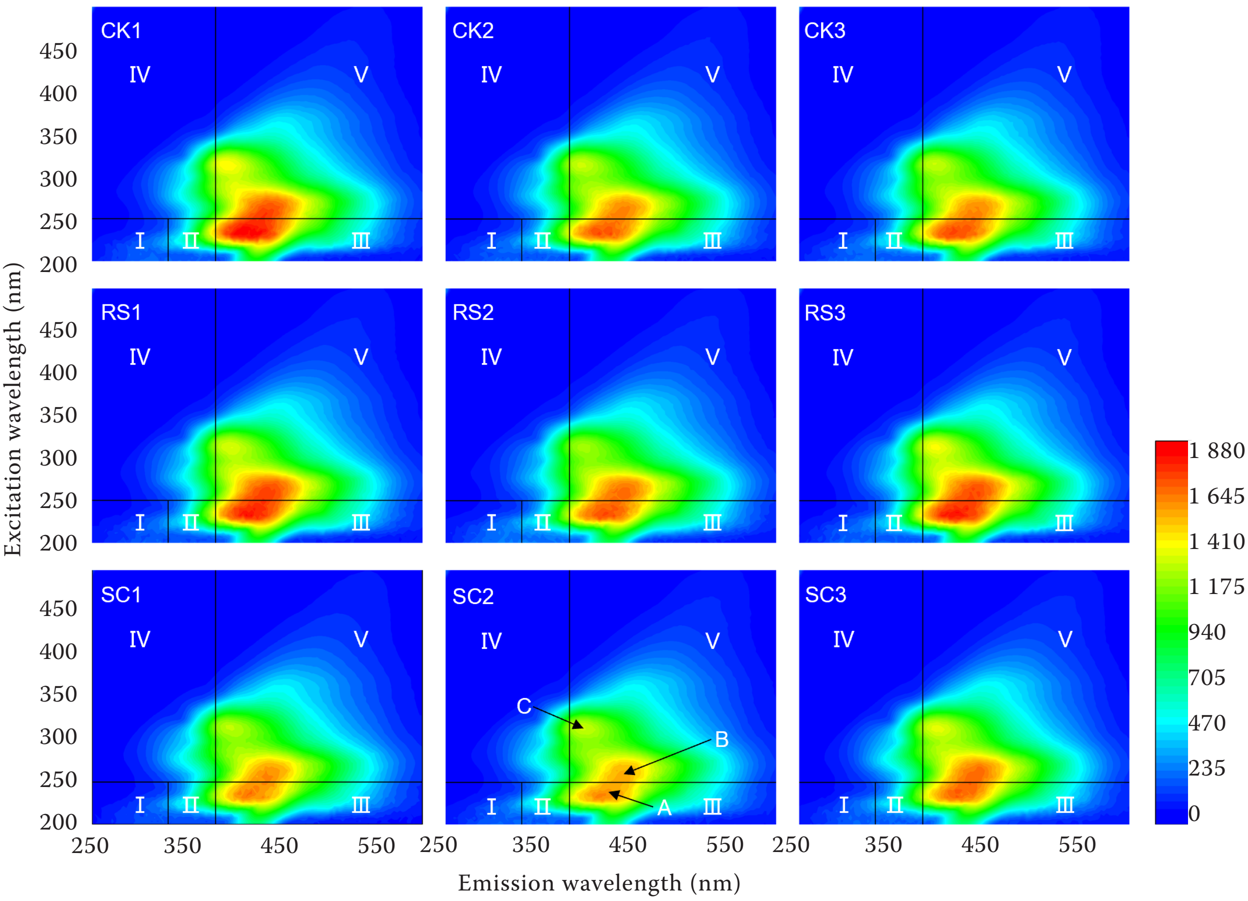

Figure 3. 3D EEM fluorescence spectra of extractable humus from samples of different treatments. The colour variation represents the fluorescence intensity in arbitrary units. CK – control; RS – rice straw; SC – rice straw biochar; A – fulvic acid-like substances; B – humic acid-like substances; C – humic acid-like substances; Region I – tyrosine-like organic compounds; Region II – tryptophan-like organic compounds; Region III – fulvic acid-like substances; Region IV – soluble microbial by-products; Region V – humic acid-like substances

tion of straw to the soil, there was an increase in the unstable aliphatic components of HS, whereas the stable aromatic carbon and amide components showed a decrease after 180 days. The presence of high molecular-weight compounds, which are rich in linearly-condensed aromatic ring systems, might

Table 4. Fluorescence spectra parameters of extractable humic substances from different treatments

Peak	Composition	Treatment	Intensity
		CK	1 724.7 ± 28.7^{ab}
A	fulvic acid-like	RS	1 802.7 ± 67.6^{a}
		SC	1 665.0 ± 45.6^{b}
		CK	1 639.0 ± 22.9^{a}
B	humic acid-like	RS	1 705.7 ± 52.6^{a}
		SC	1 618.3 ± 67.1^{a}
		CK	1 293.0 ± 19.7^{a}
C	humic acid-like	RS	1 319.7 ± 76.8^{a}
		SC	1 311.7 ± 34.0^{a}

Different letters within the same column indicate significant differences ($P < 0.05$) among the treatments. CK – control; RS – rice straw; SC – rice straw biochar

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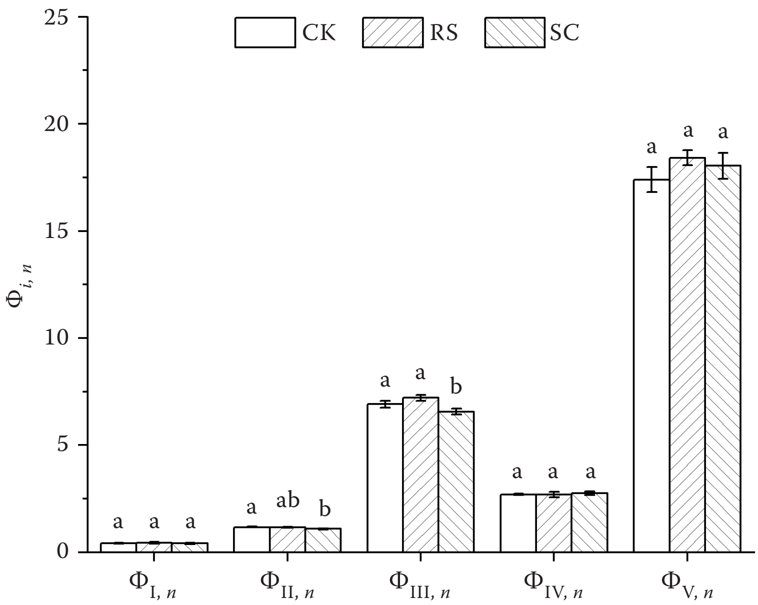

Figure 4. Distribution of the integral volume ($\Phi_{i, n}$) (× 10^6, aun m^2/[mg/L]) of extractable humus sample from regional integrity analysis. Different letters above the error bars indicate significant difference. CK – control; RS – rice straw; SC – rice straw biochar

lower fluorescence intensity in the extractable humus of the SC treatment (Rodríguez et al. 2014). The biochar amendment promoted the humification process in the soil, increasing the aromatic structures in the extractable humus.

In order to better understand the differences in the EEM fluorescence region intensities between the SC and RS treatments, we further performed a quantitative analysis of the EEM spectrum with the FRI technique. The volumes ($\Phi_{I, n}$) representing the cumulative fluorescence response of each region in the EEM spectra were calculated based on the FRI technique (Figure 4). The fluorescence intensities for the extractable humus extracted from the CK, RS, and SC treatments were in the order of: region V > region III > region IV > region II > region I. Thus, humic-like acids belonging to region V were the leading fluorescent component in the extractable humus. When compared with the CK treatment, the RS treatment increased the content of $\Phi_{I, n}$, $\Phi_{III, n}$ and $\Phi_{V, n}$, but slightly reduced the content of $\Phi_{II, n}$. In compared with the CK treatment, the SC treatment significantly reduced the content of $\Phi_{II, n}$ and $\Phi_{III, n}$, but increased the content of $\Phi_{IV, n}$ and $\Phi_{V, n}$. The SC treatment increased the content of humic-like substances (region III and V), which confirmed our previous conclusions. Biochar can cause changes in the fluorescence properties of soil humus composition through several mechanisms, primarily through the adsorption of soil native dissolved organic matter onto biochar, the release of dissolved organic matter components from biochar, and the biochar's effect on the microbial decomposition and transformation of SOM (Feng et al. 2021).

We also analysed up the humus-like/protein-like components ratio (H/P). Region (III + V) represents a relativity stable humic-like substances (H), while region (I + II) indicates a protein-like components (P) that are easily decomposed by microorganisms. The ratio of H/P reveals the stability of extractable humus through evaluating whether the humic acid structure is more abundant (Huang et al. 2023). The H/P for three treatments were in the order of: SC > RS > CK (Figure 5). Previous research has shown that applying biochar to soil leads to an increase in

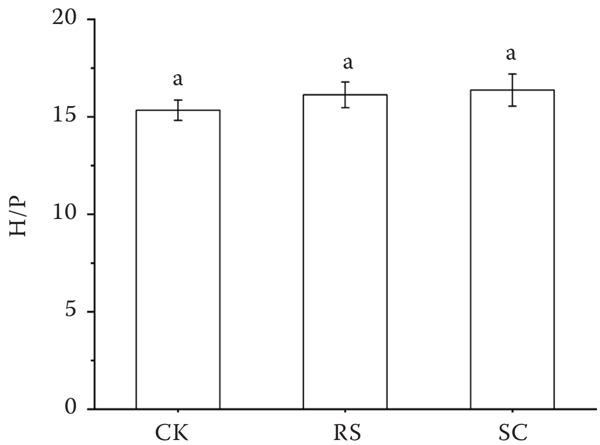

Figure 5. The ratio of humic-like to protein-like (H/P) in different treatments; H/P = Σ ($\Phi_{\text{ш}, n}$ + $\Phi_{V, n}$)/Σ($\Phi_{I, n}$ + $\Phi_{II, n}$). Different letters above the error bars indicate significant differences (P < 0.05) among the treatments. CK – control; RS – rice straw; SC – rice straw biochar

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aromatic components and humus composition (Huang et al. 2022), enhancing the degree of soil humification. This agreed with the effect of biochar amendment in terms of FRI and the H/P in the SC treatment. Compared with the SC treatment, RS treatment increased the content of protein-like components (region I and II), and decreased H/P. This could be explained by the fact that straw contains easily broken-down materials, such as polysaccharides and aliphatic chemicals, which boost the protein-like components of soil humus.

In this ten-year study, our findings showed that both the extended application of biochar and rice straw significantly influenced the organic matter status as well as HA structure. The addition of straw induced the formation of soil fulvic acids and increased the aliphaticity of the HA molecular structure, which made it simpler and promoted the activity of humus. Biochar increased the HA structure's aromaticity, making the structure more complex and enhancing soil carbon pool stability. However, the long-term effects and microbiological mechanisms associated with carbon cycling functional genes on biochar and rice straw amendment need to be further investigated.

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