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Insight in the characteristics of humic substances with cotton straw derived organic materials amendments

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Abstract

Carbon sequestration by application of organic materials and biochar in soil is an important strategy to increase soil organic carbon (SOC), but the stability of SOC, particularly humic substances (HS) vary with the types of organic material. In this study, cotton straw and its derived compost and biochar were added with equivalent carbon content to soil and incubated for 180 days. The structural characteristics of humic acid (HA), fulvic acid (FA) and humin (Hu) were investigated using solid-state ^{13}C nuclear magnetic resonance (NMR) and Fourier transform infrared (FTIR) spectroscopy. The results showed that biochar treatment increased the aryl C of HA, FA, and Hu by 1.38%, 1.68%, and 10.46% compared to straw treatment and increased the aryl C of HA, FA, and Hu by 1.46%, 1.99% and 2.01% compared to compost treatment. The O-alkyl C of HA was 10.59% and 10.65% in high biochar/straw and biochar/compost ratios respectively, while it was 9.81% and 9.61% in low biochar/straw and biochar/compost ratios. In addition, the O-alkyl C of FA was 62.83% and 58.48% in high ratios of biochar/straw and biochar/compost, respectively, while it was 55.85% and 55.94% in low ratios of biochar/straw and biochar/compost. These results suggest that biochar is advantageous for aryl C formation of FA and Hu due to its high aryl C content, whereas straw or compost is advantageous for alkyl C formation of HA. The stability of aryl C and O-alkyl C of HA, FA, and Hu can be improved in soils by incorporating biochar in combination with straw or compost.

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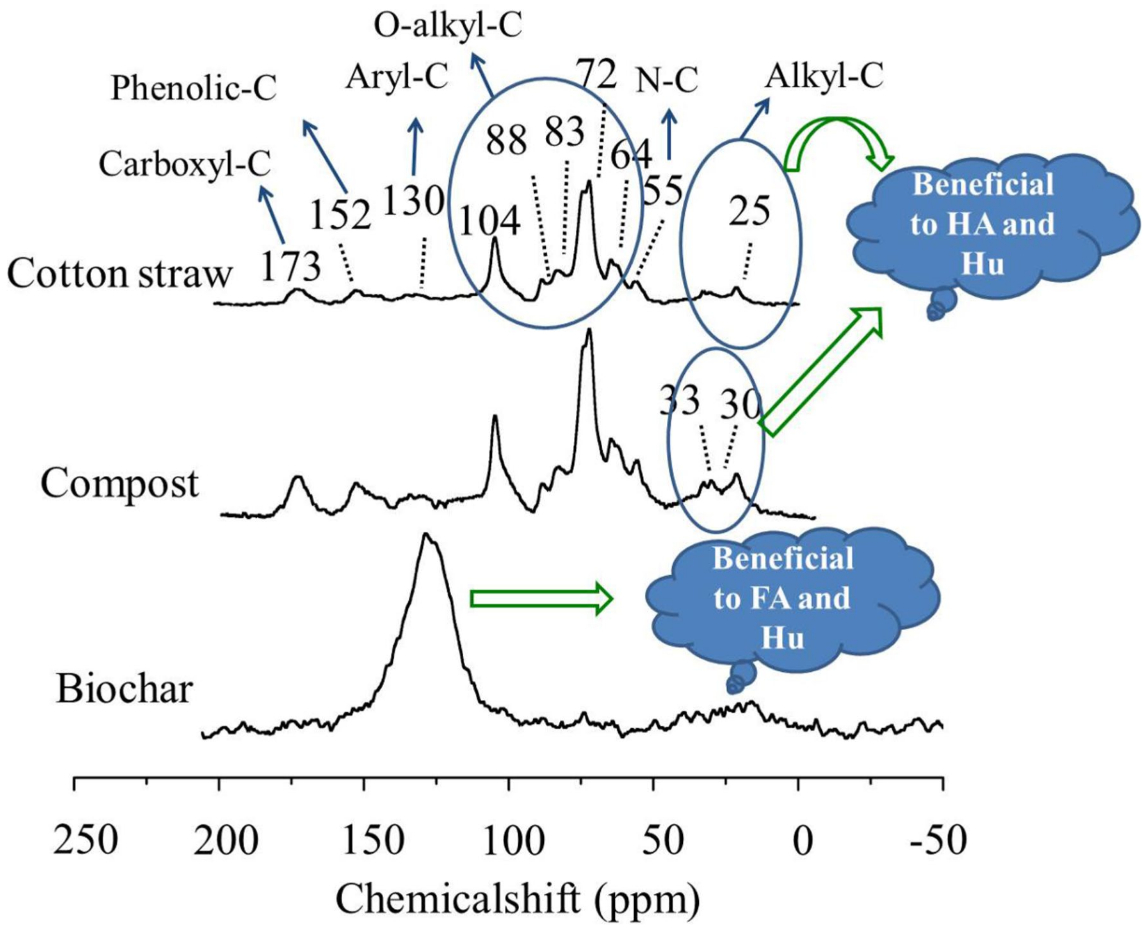
Graphical abstract



Keywords ^{13}C NMR spectroscopy, Humic substances, FTIR spectroscopy, Biochar, Crop straw

Introduction

Carbon sequestration in soil is critical to the global carbon cycle. Soil contains about 2500 Pg C, which includes 1500 Pg soil organic carbon (SOC) and 950 Pg inorganic carbon [1]. Thus, C storage in soil depends on the formation and decomposition of SOC. The SOC is important for the carbon storage in soil, and manure or straw returning into the soil can enhance carbon sequestration [2]. The organic amendments can enhance the transformation of organic carbon into mineral-associate carbon [3]. The SOC contents are different in soil profile [4]. During the decomposition of organic materials, humic substances (HS) such as humic acid (HA), fulvic acid (FA) and humin (Hu) are formed, which are important in the environmental process as well as formation and transformation of soil organic matter (SOM) [5]. The HS has a strong relationship with the SOC [6]. In general, organic materials will be decomposed and form HS and HS contents in the large ration of SOC, especially Hu. Therefore, HS relates the content of SOC. The decomposition rate of SOC or HS depends on their accessibility to soil microbes or enzymes. In addition, organic materials such as straw or biochar can increase SOC and HS, as well as the SOC pool [7–9]. The HS is in a high stability and maybe in a fractal-cluster organization [10]. Soil organic carbon protection mechanisms, such as physical protection of soil aggregates and chemical protection via iron/aluminum oxides bond with SOC or HS, differ depending on the environmental conditions. The hydrophobic functional groups attached to HS also protect SOC which leads resistant to the accessibility of soil microbes or enzymes. The structural stability of HS is a critical component of the SOC protection mechanism. The effect of fertilization on HS structure was more visible in upland soil than in paddy soil [11]. In the long run, manure promoted alkyl C sequestration in HA [12]. Aside from physical protection of soil aggregates, HA has a hydrophobic protection in soil [13]. Hu is more stable among all humic fractions and makes up a large proportion of SOC rather than HA and FA [14, 15]. Long-term fertilization could change the heterogeneous and chemical structure of Hu in soil [16]. The ^{13}C isotope trace revealed a significant positive correlation between increased SOC and increased Hu, implying chemical stabilization of Hu in the long run [17]. The molecular analysis revealed that Hu primarily possessed aliphatic hydrocarbon functionalities [18, 19]. Long-term application of organic fertilizer increased the O-alkyl C of Hu [20]. The use of biochar can increase the relative content of aromatic C in Hu while decreasing the relative content of alkyl C and O-alkyl C [13]. In general, solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy and Fourier transform infrared (FTIR) spectroscopy could be used to investigate the chemical properties of SOC or HS [16, 21, 22]. ^{13}C NMR spectroscopy can be used to investigate the hydrophobic functional groups of HS, such as alkyl C and aromatic C, as well as the hydrophilic functional groups such as carboxyl C, O-alkyl C and methoxyl/N C [23–26]. FTIR spectroscopy can be used to investigate the modification of functional groups of HS functional groups [27]. Not only the contents of SOC and HS, but also the structure of them is very important for C sequestration and stability in soil and need further research.

According to our previous research, the chemical structure of SOC is affected by the type of organic materials added to the soil, and biochar is more effective at increasing aryl C of SOC than cotton straw or cotton straw derived compost [28]. Moreover, organic materials derived C is mainly sequestrated in Hu, and straw or compost are more likely to contribute to the formation of HA and FA in soil, whereas biochar promotes the formation of Hu [29]. Han et al. [30] review the literature and propose that humification in soils is related to changes in soil conditions induced by biochar. However, the effects of various organic materials on HS stability are still unclear. Despite the fact that Hu accounts for more than 50% of SOC [25], the limited knowledge is available related to changes in structural characteristics of Hu with different organic material amendments. The differences in chemical structure of HS amended with crop straw, compost and biochar are also not clear, but they are critical for understanding the resistance of microbial attack and oxidation of SOC, as well as mechanism of C sequestration in soils. The structure characteristics of HS amended with organic materials need to be investigated by multi-technique.

Our previous research has demonstrated the C sequestration of straw, compost and biochar in SOC and HS using the ^{13}C isotope tracing method [28, 29]. However, the effect of cotton straw and its derived compost and biochar on the structural stability of HA, FA and Hu, as well as their chemical protection mechanism, remains unknown. In this study, ^{13}C NMR and FTIR spectroscopy were used to investigate the structural characteristics of HA, FA, and Hu with cotton straw (straw), cotton straw derived compost (compost), and cotton straw derived biochar (biochar) after 180 days of incubation.

Materials and methods

Experimental site

This research was based on our previous work, and the materials and methods were described by Song et al. [28]. Topsoil (0–20 cm) was collected in 2015 in Jilin Province (43°48′53″ N, 125°19′1″ E), which classified as Typic Hapludoll [31]. Before incubation, the naturally dried soil was sieved through a 2 mm sieve. The soil contained 1.35 g kg^{-1} and 16.10 g kg^{-1} total nitrogen (TN) and SOC, respectively.

Organic materials amendment

Cotton straw was collected after cotton harvest, and parts of it were pyrolyzed at 500 °C for 4 h before being converted to biochar. A portion of cotton straw was composted for 6 months in the field to form compost. The straw, compost, and biochar were air dried at room temperature and then passed through a 1 mm sieve. The contents and chemical structure of SOC of organic materials were analyzed by wet oxidation method, dry combustion method, and ^{13}C solid state NMR spectra [28].

Laboratory incubation

The soil samples were pre-incubated for one week at 20 °C. The soil samples were then mixed with biochar (CB), compost (CC), and straw (CS) on 2% (w: w) carbon equivalent basis. In addition, straw or compost mixed with CB in the following ratios: 1:2 (CSB1/CCB1), 1:1 (CSB2/CCB2) and 2:1 (CSB3/CCB3) were incubated. Ammonium sulfate was used to adjust the C/N ratio to that of the soil before incubation. The moisture content of these samples was adjusted and kept at 60% of field capacity (FC).

Separation and purification of HS

The HS were extracted and purified using a dilute alkaline solution [12, 15]. The water dissolved substances (WSS) in soil were first extracted using distilled water (1:10, w/v). The humic extractable substances (HE) were then extracted using 50:50 (v/v) solution of 0.1 mol/L NaOH and 0.1 M $Na_4P_2O_7$. After bringing the pH of HE to 1 with 0.5 mol/L H_2SO_4, then the HA and FA were separated as precipitate and solution, respectively. They were then dialyzed and freeze-dried. The soil residue was Hu, which was washed with distilled water to bring it to pH 7. Hu was treated with a mix solution of 30% HF + 30% HCl for 24 h at room temperature. The mixture was then centrifuged at 3552 g/min for 10 min before discarding the supernatant. This acidic solution treatment and centrifugation procedure was repeated six times. The Hu was then washed with distilled water and the pH was adjusted to 6–7 before being air-dried and sieved (0.25 mm) for solid-state ^{13}C NMR and FTIR spectroscopy analysis.

Analysis of ^{13}C NMR spectra

The technique of ^{13}C NMR spectra is an essential way to investigate the structure of HA, FA and Hu. It can't destroy the samples and get accurately structure information of samples. The procedure about the characterization of HA, FA and Hu by using the solid-state ^{13}C NMR spectroscopy was described by Song et al. [12]. The ^{13}C NMR spectra were analyzed using a Bruker AVANCE III 400 WB spectrometer equipped with a 4 mm standard bore CPMAS probehead. The experiments were carried out with a contact time of 2 ms and 10,000 scans with a recycle delay of 6 s for each sample.

The chemical shift range of ^{13}C CPMAS NMR spectra of HS was divided as follows: 0–45 ppm (alkyl C), 45–60 ppm (methoxyl C or N-C), 60–110 ppm (O-alkyl-C), 110–160 ppm (aromatic-C), including 110–145 ppm (aryl C) and 145–160 ppm (phenolic C), carboxyl- and carbonyl-C (160–190 ppm). The chemical structure of straw, compost, and biochar were described by Song et al. [28].

FTIR spectroscopy

The FTIR spectroscopy is a traditional technique to investigate HS structure. There will be more information to be revealed associated FTIR spectroscopy and ^{13}C NMR spectroscopy. The detail about functional groups of HA, FA and Hu using FTIR spectroscopy is provided in a study by Song et al. [32]. A Themo FTIR spectrometer was used to characterize HA, FA, and Hu in the mid IR range of 4000 to 400 cm^{-1}. Spectra with a resolution of 4 cm^{-1} and 16 scans per sample were recorded. The spectrum was obtained on pellet containing 1 mg of HS sample and 200 mg of KBr. The software OMNIC version 8.2 for Windows (Thermo Nicolet Instrument Corp. Madison, WI) was used to analyze the data. The absorbance peak area was calculated by integrating the tangents of peak's two trough points.

Statistical analysis

Excel 2010 software was used for data analysis including the correlation analysis and two-way ANOVA.

Results

Characteristics of HS determined by ^{13}C NMR spectroscopy

Song et al. [12] described the assignment details of HS functional groups, and their ^{13}C NMR spectra are shown in Fig. 1. Furthermore, the chemical structure of organic materials investigated by ^{13}C NMR spectra was demonstrated in a previous study [28]. The signal at 25 ppm was assigned to short-chain polymethylene and linked to aryl C [33, 34]. The peaks at 30 ppm and 33 ppm corresponded to amorphous – (CH_2) – and crystalline – (CH_2) – groups in aliphatic compounds [34–39]. The peak at 56 ppm was attributed to methoxyl-to-α-amino [40] and overlapped with N-alkyl intensity [41]. The peak at around 73 ppm corresponded to the overlapping resonances of C2, C3 and C5 carbons in the pyranoside structure of cellulose and hemicellulose [26]. The signal at 103 ppm was assigned to the anomeric C1 carbon [42]. Moreover, a broadband around 130 ppm was assigned to lignin, suberin biopolymers or condensed aromatic olefinic carbons [43]. The peaks at about 152 ppm and 174 ppm indicated a low content of O-substituted ring carbons and carboxyl groups, respectively [26, 42].

Hydrophilic and hydrophobic functional groups of HS determined by ^{13}C NMR spectroscopy

The relative percentage of functional groups and hydrophobic or hydrophilic indexes of HA, FA and Hu were shown in Tables 1 and 2. Compared to control treatment, carboxyl C of HA increased by 1.26% in CS treatment, while it decreased by 0.30% and 0.99% for FA and Hu, respectively (Fig. 2a). In addition, carboxyl C of HA increased by 1.26% and 0.52% in CSB1 treatment and CSB2 treatment, while decreased by 3.49% and 2.13% for FA in CSB1 treatment and CSB2 treatment. The results showed that straw contributed to the transformation to hydrophilic C of HA, FA and Hu, especially the HA. The O-alkyl C of HA and Hu increased by 1.17% and 6.24% in CC treatment, while it decreased by 1% for FA (Fig. 2b). However, the methoxyl C of FA increased by 2.02% in CC treatment (Fig. 2c). The results showed that compost preferred to form O-alkyl C of HA and Hu, but it was good for methoxyl C formation of FA. Correspondingly, the hydrophilic C of HA and Hu increased by 1.06% and 16.42% in CS treatment, respectively (Fig. 3a). The results showed that straw was good for hydrophilic C formation of HA and Hu. In addition, phenolic C of HA and Hu increased by 0.46% and 0.68% in CB treatment, respectively (Fig. 2d). But aryl C of FA and Hu increased by 1.83% and 14.50% in CB treatment, respectively (Fig. 2e). The results showed that biochar could transform to hydrophobic C of FA too. The aromaticity of HA, FA and Hu increased by 2.47%, 1.91% and 17.65%, respectively (Fig. 3b). The alkyl C of HA increased for all treatments, but decreased for Hu in all treatments (Fig. 2f). The aliphaticity of HA and FA increased by 1.28% and 3.04% in CS treatment, while increased by 3.03% for Hu in CB treatment (Fig. 3c). However, the hydrophobic C of HA and Hu increased by 2.24% and 11.26% in CB treatment (Fig. 3d). Similarly, the hydrophobicity of HA and Hu increased by 0.17% and 1.44% in CB treatment,

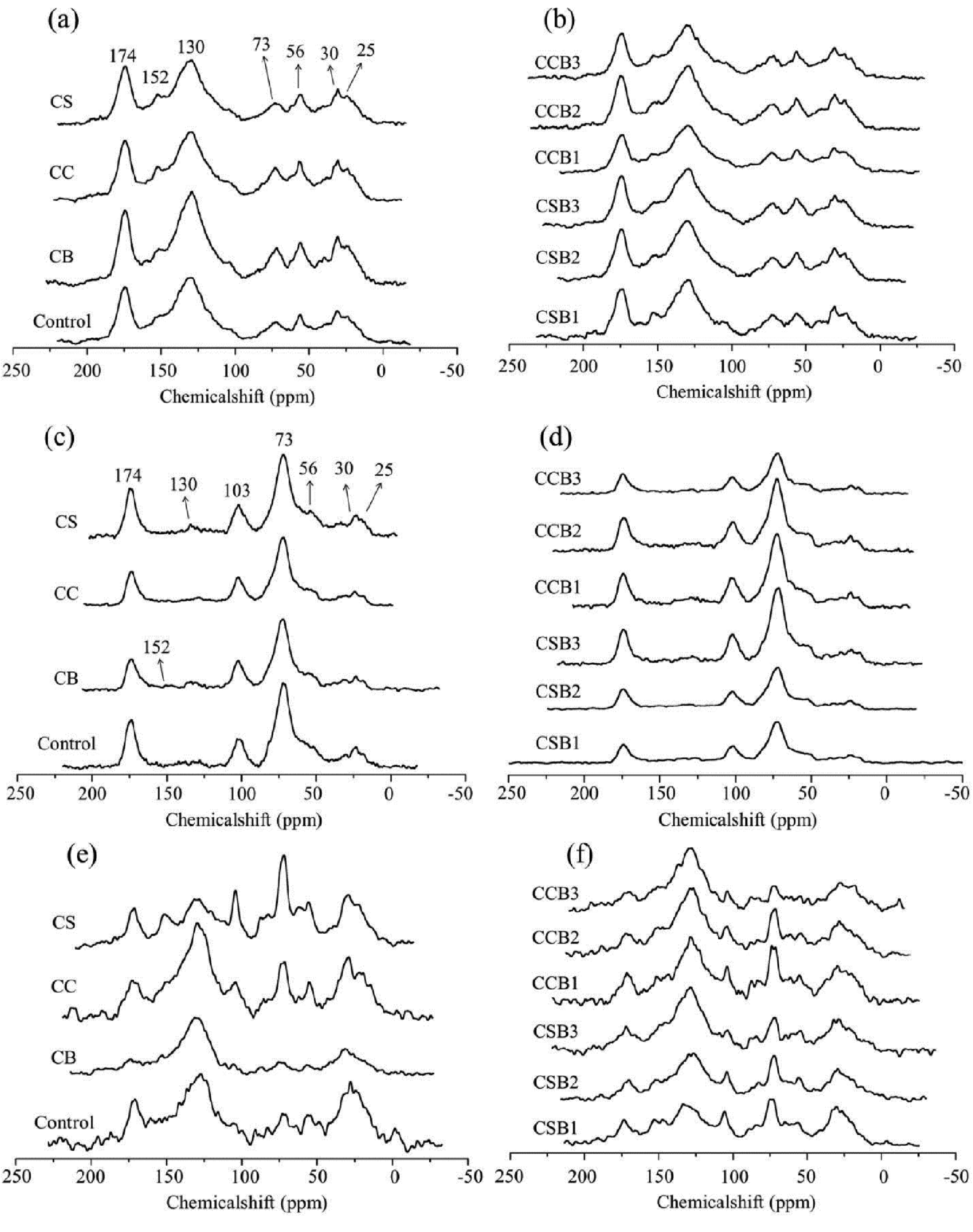


Fig. 1 ^{13}C NMR spectra of HA (**a** and **b**), FA (**c** and **d**) and Hu (**e** and **f**) incubated for 180 days

respectively (Fig. 3e). The results showed that the hydrophobic C and hydrophobicity of HA and FA were mainly affected by aromatic C. The correlation between aryl C and HA hydrophobicity was found to be significantly positive ($R^2 = 0.42$, $P = 0.04$, $n = 10$) (Fig. 4a).

Table 1 Relative distribution (%) of signal area over chemical shift regions (ppm) in CPMAS ^{13}C NMR spectra of HS incubated for 180 days

Treatment	160-190			145-160			110-145			60-110			45-60			0-45		
	HA	FA	Hu	HA	FA	Hu	HA	FA	Hu	HA	FA	Hu	HA	FA	Hu	HA	FA	Hu
Control	16.69	17.51	11.43	7.35	1.40	6.74	41.57	1.40	33.03	10.02	62.52	14.97	7.51	5.78	6.97	16.86	11.38	26.86
CS	17.86	17.21	10.44	8.39	0.00	8.25	38.04	3.61	20.67	9.46	56.45	33.09	8.57	8.78	6.26	17.68	13.94	21.29
CC	16.21	16.95	11.22	6.65	0.00	5.27	38.74	2.71	33.45	11.18	61.53	21.21	9.56	7.80	5.05	17.67	11.02	23.79
CB	15.92	14.71	10.31	7.80	1.18	7.42	39.81	3.24	47.53	10.19	64.85	10.52	8.60	6.76	3.51	17.68	9.26	20.72
CSB1	17.95	14.03	11.25	6.10	2.66	8.89	39.50	5.33	36.56	10.59	62.83	19.24	8.44	4.21	4.39	17.41	10.94	19.69
CSB2	16.39	14.39	8.40	7.70	2.45	6.21	39.67	5.47	33.67	10.33	62.30	25.36	8.20	4.32	5.21	17.70	11.08	21.16
CSB3	17.21	15.38	10.62	6.88	2.46	10.08	39.07	5.08	23.14	9.81	55.85	28.98	9.12	7.69	6.69	17.90	13.54	20.49
CCB1	15.43	15.15	10.10	6.17	1.82	9.39	41.05	4.85	41.72	10.65	58.48	16.26	9.26	8.48	2.12	17.44	11.21	20.40
CCB2	16.42	15.53	10.07	7.39	3.11	8.56	38.75	5.12	34.14	10.34	61.96	23.36	8.54	3.88	5.24	18.56	10.40	18.63
CCB3	15.75	15.85	7.59	6.14	3.33	10.02	40.79	4.12	30.52	9.61	55.94	26.27	8.82	9.98	6.00	18.90	10.78	19.59

Table 2 Relative distribution (%) of signal area over chemical shift regions (ppm) in CPMAS ^{13}C NMR spectra of Hu incubated for 180 days

Treatment	Hydrophilic C (%) [a]			Hydrophobic C (%) [b]			Aromaticity (%) [c]			Aliphaticity (%) [d]			Hydrophobicity [e]		
	HA	FA	Hu	HA	FA	Hu	HA	FA	Hu	HA	FA	Hu	HA	FA	Hu
Control	34.22	85.81	33.37	65.78	14.19	66.63	58.72	3.40	44.90	20.24	13.80	30.32	1.92	0.17	2.00
CS	35.89	82.44	49.79	64.11	17.56	50.21	56.52	4.37	32.28	21.52	16.84	23.78	1.79	0.21	1.01
CC	36.95	86.27	37.49	63.05	13.73	62.51	54.16	3.27	43.62	21.08	13.27	26.80	1.71	0.16	1.67
CB	34.71	86.32	24.33	65.29	13.68	75.67	56.63	5.17	61.26	21.02	10.86	23.10	1.88	0.16	3.11
CSB1	36.98	81.07	34.87	63.02	18.93	65.13	55.58	9.30	51.20	21.23	12.72	22.18	1.70	0.23	1.87
CSB2	34.92	81.01	38.96	65.08	18.99	61.04	56.67	9.24	43.54	21.18	12.94	23.10	1.86	0.23	1.57
CSB3	36.14	78.92	46.28	63.86	21.08	53.72	55.51	8.91	37.17	21.62	16.00	22.92	1.77	0.27	1.16
CCB1	35.34	82.12	28.48	64.66	17.88	71.52	55.84	7.86	56.85	20.62	13.21	22.70	1.83	0.22	2.51
CCB2	35.30	81.37	38.67	64.70	18.63	61.33	55.21	9.74	47.48	22.20	12.32	20.72	1.83	0.23	1.59
CCB3	34.17	81.77	39.86	65.83	18.23	60.14	55.70	8.85	43.88	22.43	12.81	21.20	1.93	0.22	1.51

Thecalculationwereasfollows:[a] $\text{Hydrophilic} = \text{carboxyl C} + \text{O-alkyl C} + \text{methoxyl C}$,[b] $\text{Hydrophobic} = \text{aromatic C} + \text{alkyl C}$,[c] $\text{Aromaticity} = \text{aromatic C}/\ (\text{aromatic C} + \text{O-alkyl C} + \text{alkyl C}) \times 100$,[d] $\text{Aliphaticity} = \text{alkyl C}/(\text{aromatic C}+\text{O-alkyl C}+\text{alkyl C}) \times 100$,[e] $\text{Hydrophobicity} = (\text{aromatic C}+\text{alkyl C})/(\text{carboxyl C}+\text{O-alkyl C}+\text{methoxyl C})$

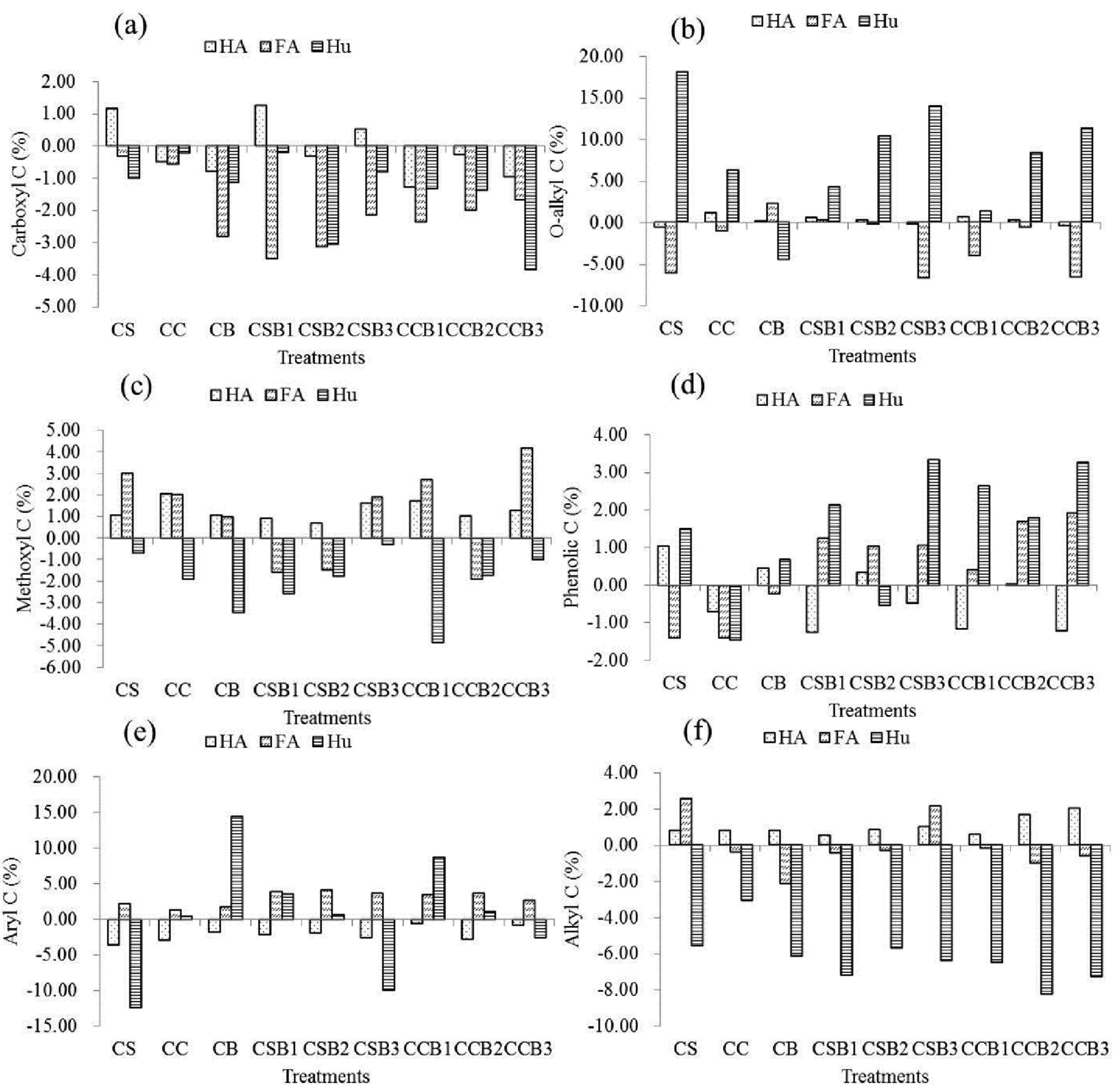


Fig. 2 The increased proportion of functional groups of HA, FA and Hu and for carboxyl C (a), O-alkyl C (**b**), methoxyl C (**c**), phenolic C (**d**), aryl C (**e**) and alkyl C (**f**) incubated for 180 days

There was a significant positive correlation between aryl C and FA's hydrophobicity ($R^2 = 0.69$, $P = 0.003$, $n = 10$) (Fig. 4a).

The correlation between aryl C and hydrophobic C of Hu was found to be significantly positive ($R^2 = 0.95$, $P < 0.001$, $n = 10$) (Fig. 4a).

The significant positive correlation was observed between the O-alkyl C of Hu and the hydrophilic C of Hu ($R^2 = 0.94$, $P < 0.001$, $n = 10$) as well as between the methoxyl/N-C of Hu and the hydrophilic C of Hu ($R^2 = 0.51$, $P = 0.02$, $n = 10$) (Fig. 4b).

Chemical structure of HS investigated by FTIR spectroscopy

The FTIR spectra of HA, FA, and Hu are shown in Fig. S1. Automatic baseline correction was performed in the spectral region of 4000 to 400 cm^{-1}. Peak heights of 3500–3200 cm^{-1}, 2920 cm^{-1}, 2850 $cm^{-1,}$ 1630–1620 cm^{-1}, 1450 cm^{-1}, 1353 cm^{-1}, 1271 cm^{-1}, and 1110 cm^{-1} were used in the creation of the map profiles. The broad intense band at about 3400 cm^{-1} corresponded mainly to stretching vibrations of H-bonded hydroxyl (O-H) groups of phenol and traces of amine (N-H) stretch [22, 44–46]. The weak bands near

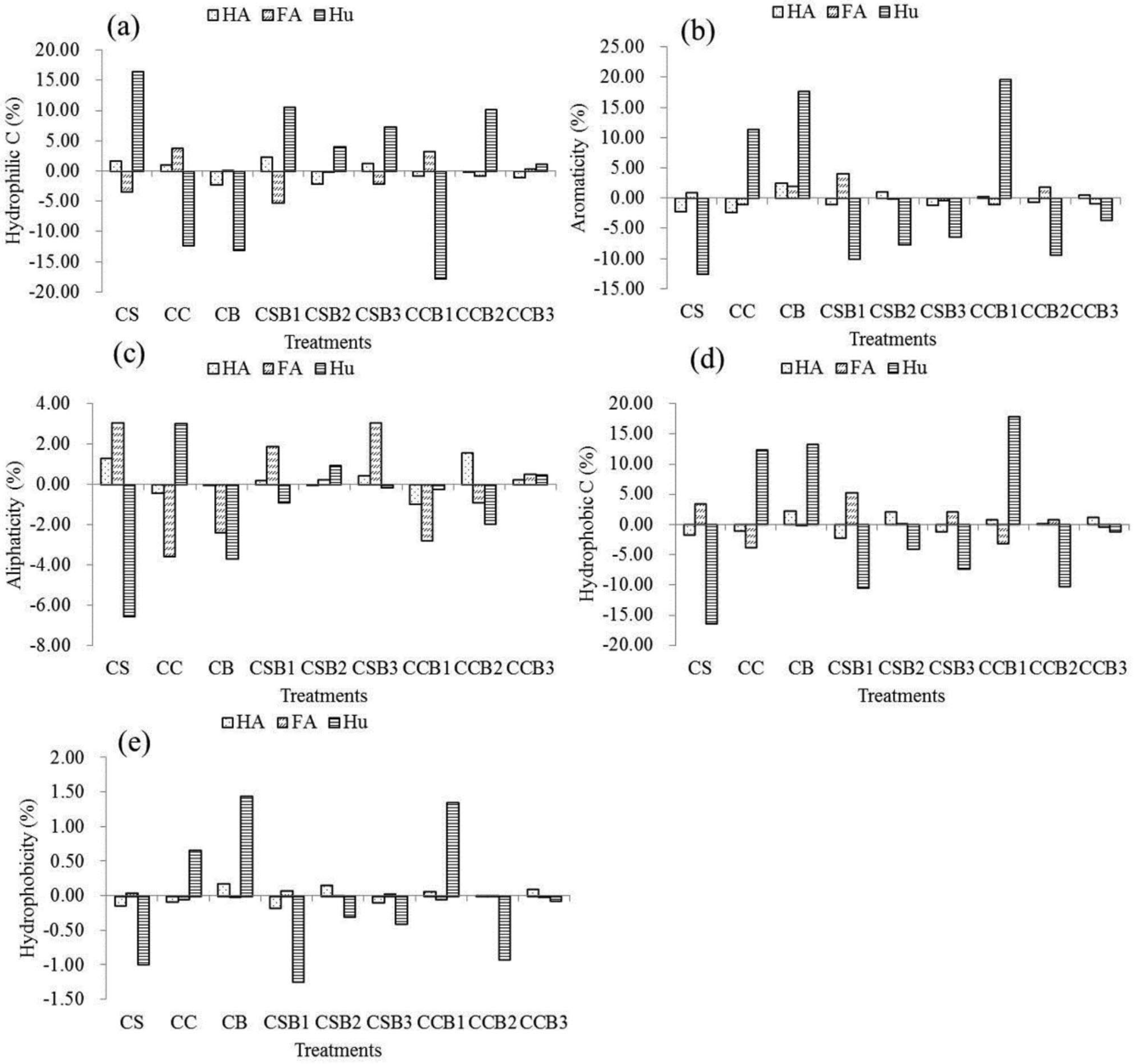


Fig. 3 The increased proportion of hydrophilic C (**a**), aromaticity (**b**), aliphaticity (**c**), hydrophobic C (**d**) and hydrophobicity (**e**) of HA, FA and Hu. Notes: The calculation were as follows: (1) $\text{Hydrophilic} = \text{carboxyl C} + \text{O-alkyl C} + \text{methoxyl C}$,
(2) $\text{Hydrophobic} = \text{aromatic C} + \text{alkyl C}$,
(3) $\text{Aromaticity} = \text{aromatic C} / (\text{aromatic C} + \text{O-alkyl C} + \text{alkyl C}) \times 100$,
(4) $\text{Aliphaticity} = \text{alkyl C}/(\text{aromatic C}+\text{O-alkyl C}+\text{alkyl C}) \times 100$,
(5) $\text{Hydrophobicity} = (\text{aromatic C}+\text{alkyl C})/(\text{carboxyl C}+\text{O-alkyl C}+\text{methoxyl C})$

2920 cm^{-1}, 2850 cm^{-1}, 1450 cm^{-1} and 1353 cm^{-1} were assigned to C-H deformations [22, 26]. The broad bands near 1630–1620 cm^{-1} were assigned to aromatic C=C stretching or asymmetric C-O stretching of COO- groups in lignin and other aromatics or aliphatic carboxylates [22]. The absorption at 1271 cm^{-1} was also linked to C-O stretching of phenolic OH [22]. The absorption at 1100–1000 cm^{-1} attributed to C-O band in both polyalcoholic and ether functional groups, such as those found in oligo- and polysaccharide [26].

The absorbance area percentages of the peaks of HA, FA, and Hu investigated by FTIR spectra are shown in Table S1 and S2. Compared to the control treatment, the C-H deformations peak area at 2920 cm^{-1} and 2850 cm^{-1} increased, but decreased in the high ratio of straw/biochar or compost/biochar. It was in line with ^{13}C NMR spectroscopy analysis of alkyl C of HA. The peak area of FA at 1271 cm^{-1} increased in the high ratio of straw/biochar or compost/biochar, which was consistent with methoxyl C of FA analyzed by ^{13}C NMR spectroscopy. In

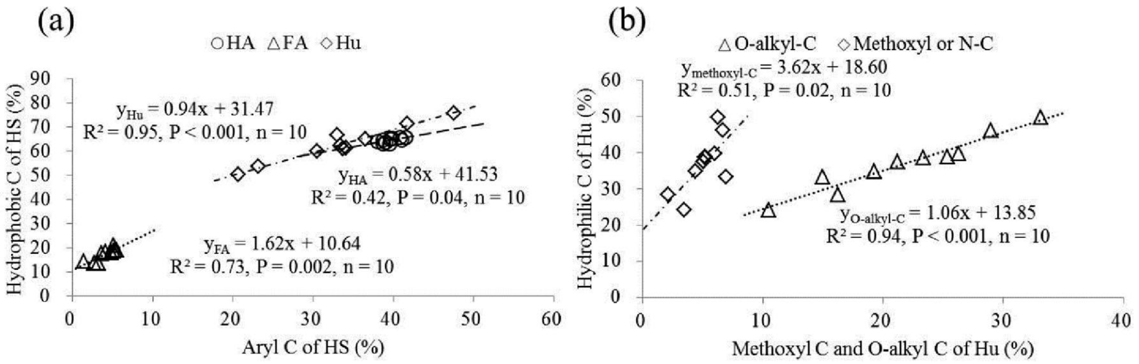


Fig. 4 Correlations between aryl C of HA, FA, Hu and hydrophobicity (**a**) and correlations between methoxyl/N C and hydrophilic C as well as O-alkyl C and hydrophilic C of Hu (**b**)

addition, the peak of FA at 1620 cm^{-1} decreased in the high straw/biochar or compost/biochar ratio. It matched the results of a ^{13}C NMR spectroscopy analysis of aryl C in FA. The H-bonded hydroxyl (O-H) groups of phenol of Hu decreased in biochar incorporated straw or compost treatments, compared to the CB treatment. However, in high biochar/straw ratios, relative content of antisymmetric CH_2 groups of Hu increased, while in low biochar/compost ratios, it decreased.

Two-way ANOVA of different functional groups of the same HS fractions with organic materials treatments analyzed by ^{13}C NMR spectra is shown in Table S3. In addition, the two-way ANOVA for the same functional group of HA, FA, and Hu with different treatments analyzed by ^{13}C NMR spectra is presented in Table S4. There were significant differences in the same HS fraction between different functional groups. For the same functional group of HS, there were significant differences between HA, FA, and Hu.

Discussion

Chemical protection of SOC by hydrophobic functional groups of HS

Humic substances such as HA and FA are naturally occurring organic matter in various environment [47]. Hydrophobic compounds are distributed primarily in large size humic molecules, whereas hydrophilic components are eluted in small size fractions [48]. In this study, the results of two-way ANOVA in this study revealed that there were significant differences in the same HS fraction between different functional groups. For the same functional group of HS, there were significant differences between HA, FA, and Hu. In addition, the aryl C of HA and Hu was in a higher proportion rather than alkyl C of HA and Hu, thus hydrophobicity was significantly correlated to aryl C of HA and Hu. Biochar was mostly composed of aryl C. The aryl C of FA with straw or compost incorporated biochar amendments were mainly affected by biochar, and thus the hydrophobicity of FA in this study was also correlated to aryl C of FA. In addition, the results of FTIR spectra matched the results of a ^{13}C NMR spectroscopy analysis of aryl C in FA too. There was a selective preservation of alkyl C of HA during the compost maturation process [26]. Organic materials, on the other hand, had an effect on the structural properties of SOC [28]. There was more straw or compost-derived C sequestered in HA and FA and transferred to FA [29]. With crop plants added, the HA had a high aliphaticity [49]. In this study, straw, compost, and biochar were found to be beneficial in increasing alkyl C of HA and FA, especially in high ratio of straw/biochar or compost/biochar. The results of FTIR spectroscopy also showed the increase in C-H functional groups and was similar to the results of ^{13}C NMR spectroscopy. Though both aryl C and alkyl C of FA increased with these organic material amendments, the alkyl C of FA was the main hydrophobic C of FA. These studies found that straw or compost were more favorable to alkyl C formation of HA and FA than biochar, but biochar was good for increasing aryl C of HA and FA. Furthermore, the hydrophobic C of HS may be more stable with straw or compost incorporated biochar amendments rather than biochar amendment.

Despite the fact that Hu accounted for more than 50% of HS [14], understanding of Hu is limited. Majority of solid NMR spectra studies of Hu revealed that Hu had similar functional groups in various soils [25]. Hu was more stable in biochar treatment rather than straw or compost treatment in this study due to chemical protection of hydrophobic functional groups and it was mainly affected when biochar was applied in combination with straw or compost. Moreover, biochar promoted aryl C and aromaticity in Hu, but straw and compost were effective in increasing aliphaticity and alkyl C. The Hu was the main fractions of HS and represented a large proportion of SOC [15, 29], so the results were similar to the structural characteristics of SOC as well [28]. All components of SOM eventually decompose, but at different rates, and Hu is relatively resistant to decomposition

[19]. In comparison to straw and compost, biochar contained more aryl groups, and it was also difficult for soil microbes to decompose. In addition, more biochar-derived C was sequestered in Hu [29]. Biochar was stable and difficult to be decomposed by soil microbes. Therefore, lots of biochar as solid materials was kept as Hu in soil. Biochar stabilized recalcitrant C of manure organic carbon [50]. The results of this study also showed that the increasing aryl C improved the structural stability of Hu, HA, and FA in a high ratio of biochar/straw or biochar/compost. Moreover, the hydrophobicity of HA, FA, and Hu increased by the application of straw or compost incorporated biochar. With the decomposition of straw and compost, however, more phenolic C and alkyl C were formed in Hu. The forms of organic materials have a large impact on not only the Hu, but also the SOC structure [28]. In this study, the hydrophobic C of HA was increased by the transformation of alkyl C contributed by straw and compost, but the hydrophobic C of Hu and FA was increased by the transformation of aryl C contributed by biochar.

Chemical protection of SOC by hydrophilic functional groups of HS

Hydrophilic functional groups of HS were not as stable as hydrophobic functional groups in general. The O-alkyl C region resonances were assigned to monomeric units in oligosaccharide and polysaccharide [42, 51]. Straw return was an effective method for improving SOC accumulation [52, 53]. Spaccini et al. [42] investigated the molecular characterization of compost materials in various stages of maturity as well as transformation of labile carbohydrates and glycosidic bonds of polysaccharides. Carbohydrates, including polysaccharides, were found to be involved in a number of oxidative and condensation reactions thought to occur during humification [54]. In general, straw decomposes faster than compost and biochar. Thus, there was more O-alkyl C of Hu, as well as polysaccharides formed during the decomposition of organic materials, particularly straw. The O-alkyl C of HS could rise as result of straw returning to the field [55]. In this study, not only HA and FA, but also Hu, increased hydrophilic C by increasing O-alkyl C and methoxyl/N C primarily through straw and compost amendments. The changes of FTIR spectroscopy peak area of FA at 1271 cm^{-1} was consistent with methoxyl C of FA analyzed by ^{13}C NMR spectroscopy. Therefore, the hydrophilic C of Hu was positively correlated to O-alkyl C or methoxyl/N C. In comparison, the straw or compost amendments could increase carboxyl C content of HA, FA, and Hu because they decomposed more easily than biochar. In general, it was easier to change the O-alkyl C of HA [56]. However, the increase in O-alkyl C of HA and FA with biochar amendment suggested that biochar could improve the stability of hydrophilic C of HA and FA in this study. All of these findings suggested that organic materials influenced the transformation of HA, FA, and Hu functional groups and that there were differences between HS fractions. The HA, FA, and Hu in soil amended with straw had more hydrophilic functional groups, but the Hu in soil amended with biochar or compost had more hydrophobic C and were more stable than the straw amendment. Biochar favored of aryl C formation of FA and Hu, whereas straw or compost favored alkyl C formation of HA and Hu.

Conclusion

Organic material types affected the transformation of functional groups of HA, FA and Hu and there were differences between HS fractions. Biochar affected the structural stability of HS through hydrophobic C protection and incorporated straw or compost improved hydrophilic C of Hu protection. Biochar favored the aryl C formation in FA and Hu, whereas straw or compost favored the formation of alkyl C formation in HA and Hu. Straw and compost increased more hydrophilic functional groups of HS at the same time. It is better to apply biochar incorporate straw or compost together and increase the stability of HS in soil.

Supplementary Information

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Supplementary Material 1

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Author contributions
XS contributed to manuscript writing, data curation and finished 13 C NMR spectroscopy analysis. YF prepared the samples and assisted to 13 C NMR spectroscopy analysis. JL assisted to manuscript revision. YZ provided the biochar. XL assisted to the 13 C NMR spectroscopy analysis. QH contribute to the manuscript revision and figure drawing. JZ contributed to the statistical analysis. DC contribute to the funding and data analysis. All authors read and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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