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Urease and β-glucosidase activity enhanced the transformation of functional groups of humin amended by straw and straw-derived biochar

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Abstract

Background: Organic materials carbon is mainly sequestrated in humin. However, the carbon (C) stability of humin with organic materials amendments in different types of soils is mainly affected by organic materials or soil types as well as the enzymatic reaction is important for understanding the C sequestration mechanism of soil.

Results: In this study, straw and straw-derived biochar were incorporated into three different types of soils. Using ^{13}C nuclear magnetic resonance (NMR) spectroscopy, the structural composition of humin (Hu) was analyzed, along with the urease and β-glucosidase activities. The results showed that biochar amendment enhanced aryl C to 49.63%–76.55% while straw amendments increased O-alkyl C of Hu to 33.99%–51.70%. Two-way ANOVA analysis revealed significant differences in the impact of soil types and treatments on enzyme activities and functional groups of humin. A significant positive correlation was found between β-glucosidase activity and O-alkyl C, phenolic C, and methoxyl C of humin. In addition, urease activity showed a significant positive correlation with alkyl C of humin. Compared to tobacco straw, tobacco straw-biochar amendment increased β-glucosidase activity for 22.01 mg kg^{-1} d^{-1}, 26.01 mg kg^{-1} d^{-1} and 14.01 mg kg^{-1} d^{-1} in three types of soils, respectively.

Conclusions: These results showed that β-glucosidase contributed to the transformation of humin functional groups and was influenced by organic materials and soil types, especially the organic materials types. The findings imply that straw or straw-derived biochar application stimulates urease and β-glucosidase activities, which improves the alternation of functional groups of humin in different types of soil.

Keywords: Biochar, Humin, Straw, β-Glucosidase activity, Urease activity, ^{13}C NMR spectroscopy

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Graphical Abstract
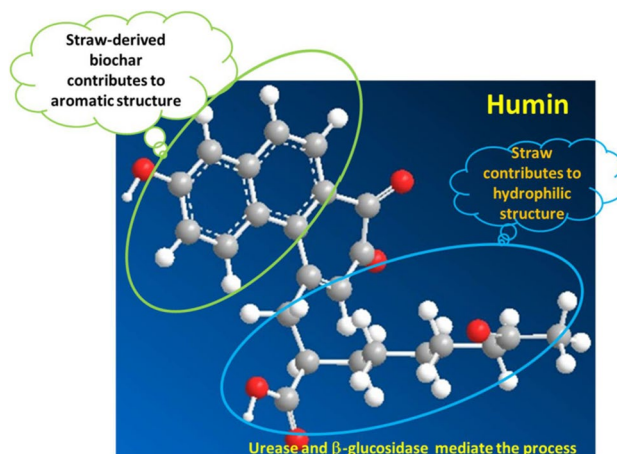
Background

Crop straw returning to the field is an effective way of sequestering soil organic carbon (SOC) in the long run. Crop residue-derived SOC could reach up to 43% [1]. A model's prediction indicated that the removal of straw, as opposed to its incorporation, would result in reduction of carbon stocks by 2.5%–10.9% of the initial SOC after 50 years [2]. However, practice of crop straw returning to the field is limited, and a significant portion of crop straw must be sequestered in soils in order to raise the SOC content.

Biochar is produced from crop straw, organic waste, dead branches and other types of organic material. Biochar is a recalcitrant carbon-based material that can be used to store carbon in the soil in a stable form [3–6]. The ^{13}C NMR spectroscopy revealed that the relative abundance of O-alkyl C and alkyl C increased in aged biochar, while lignin and lipid of SOC increased in maize straw [7, 8]. The structure of SOC varies depending on the type of soil and organic materials used. Even though the concentration of SOC in agricultural soils can be significantly increased by applying crop straw and its derived biochar, more research is still required to determine the stability mechanisms of C sequestration in soils that have been amended with crop straw and biochar derived from the same straw.

Humic substances (HS) including humic acid (HA), fulvic acid (FA) and humin (Hu) are the main components of soil organic matter (SOM) [9]. The transformation of organic materials into humic substances is both affected by organic materials and soil types, while it is mainly affected by organic materials [10]. Biochar directly influenced humic substances level in soils, and the content of fulvic acid and humic acid increased with biochar amendment [11, 12]. Biochar and humic substances combined with manure improve the soil characteristics [13]. The transformation between humic acid and fulvic acid after application of maize straw is revealed by a ^{13}C isotope trace experiment [14]. Moreover, additional research is required to investigate the transformation of functional groups within humic substances fractions. Humin, being the predominant component of soil organic matter, plays an important part in the process of C sequestration [15]. Biochar amendment clearly increased the amount of humin, which was beneficial to the structural stability of the SOC pool [16]. The levels of humic substances found in the two types of soil were also distinct from one another [17]. In addition, C that is derived from biochar is capable of transforming humic substances into humic acid and fulvic acid [18]. In general, application of chemical fertilizer over a prolonged period of time, which was investigated by ^{13}C NMR spectroscopy, led to an increase in the alkyl C of humin [19]. However, the structural changes that take place in humin as a result of crop straw and biochar derived from crop straw amendments in a variety of soil types are not yet completely understood.

In general, microbial decomposition of crop straw is an important source of humic substances formation. Microorganisms found in soil play a key role in the transformation of humic substances [20]. For instance, arbuscular mycorrhizal fungus has been shown to accelerate the decomposition of grass leaves [21]. The soil microbiological properties correlate to the functional groups of humin in long-term fertilization [22]. The findings of the ^{13}C isotope tracing analysis show that the plant-derived C of fulvic acid has a significant correlation to β-glucosidase activity [23]. Enzyme improved the hydrolysis of crop straw [24]. Though the extracellular β-glucosidase

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derived from microorganisms is in low levels [25], it plays a key role in the improvement of cellulose degradation rates [26]. One of the key function of β-glucosidase is the release of aromatic compounds of plant-derived materials [27, 28]. In addition, the organic materials derived from plant residues affect the enzyme activities in various soils. Biochar application increased β-glucosidase activity in different soils [29, 30]. Urease originated mainly from plants and microorganisms was affected by the addition of organic matter [31, 32]. There was an increase in urease activity after the addition of organic materials [33]. Even though majority of the organic materials are transformed into humin in soils amended with various type of organic materials [23, 34, 35], it is not entirely clear how soil enzyme activities contribute to humic substances composition and stability.

The main objective of this study was to investigate the effect of soil types and organic materials types on enzymes activities and humic substances fractions, as well as the contribution of urease and β-glucosidase activities to the chemical composition of humin in three soils amended with crop straws and their derived biochars.

Materials and methods

Soil samples

Representative soils (0–20 cm) of Cryids Aridisols (CA, 37º49′32.50″ N, 118º29′18.58″ E), Uderts Vertisols (UV, 36º19′33″ N, 120º24′40″ E), and Ustalfs Alfisols (UA, 36º17′28″ N, 120º36′44″ E) value were collected from Shandong Province in October 2015, the North of China [36]. The soil samples were air dried, free roots or gravels were picked, and then passed through 2-mm sieve. The properties of soils, straw, and straw-derived biochar are displayed in Table 1. The SOC and total organic carbon (TOC) contents of straw and straw-derived biochar were analyzed by wet oxidation method [37]. The total nitrogen (TN) content of soil, straw, and straw-derived biochar was determined using the Kjeldahl method and

titration, and the pH was determined using the potentiometry method in a 1:2.5 soil–water ratio [38].

Organic materials amendment

Wheat straw, tobacco straw, and biochars derived from wheat straw and tobacco straw were all passed through a 1-mm sieve. Wheat straw and tobacco straw were collected after harvest, air dried, and pyrolyzed at 450–550 °C to produce biochar by Shangqiu Sanli New Energy Company, China. The C and TN contents in the crop straw and biochars were determined by using wet digestion method [37]. The basic properties of these organic materials were analyzed by standard method [38].

Laboratory incubation

Soil samples were preincubated and kept at 20 °C for one week at 60% field capacity (FC). The treatments included soil without organic materials (control), soil mixed with wheat straw-derived biochar (WB), tobacco straw-derived biochar (TB), wheat straw (WS), and tobacco straw (TS), all at the same C concentration of 8.73 g C kg^{-1}. The C/N ratio of these samples was adjusted to the native C/N ratio with $(NH_4)_2SO_4$ solution, and the moisture content was adjusted and maintained at 60% FC. The samples were incubated at 20 °C for 180 days.

Extraction and analysis of humic substances

The humic substances (HS) were extracted using the method described by Song et al. [35]. The water-soluble solution (WSS) was first extracted with distilled water, and then extractable humic substances (HE) were extracted using a mixed solution of 0.1 M NaOH and 0.1 M $Na_4P_2O_7$. The residue left after filtration of suspension was used for extraction of humin. Humin was then treated with 30% HCl + 30% HF solution for 24 h, which then repeated for six times. The pH of humin samples was brought to 6–7 by distilled water. The humin samples were air dried and passed through a 0.25-mm sieve before being analyzed for ^{13}C NMR spectra.

Table 1 Basic properties of soils and organic materials

Soils and materials	TOC (g kg^{-1})	TN (g kg^{-1})	pH (H$_2$O)	C/N	WSS (g kg^{-1})	HA (g kg^{-1})	FA (g kg^{-1})	HU (g kg^{-1})
Soil CA	5.89 ± 0.07	0.61 ± 0.01	8.10 ± 0.10	9.59 ± 0.10	0.09 ± 0.04	0.99 ± 0.20	3.86 ± 0.07	4.48 ± 0.30
Soil UV	9.96 ± 0.16	1.02 ± 0.01	6.99 ± 0.10	9.74 ± 0.06	0.09 ± 0.04	0.78 ± 0.12	3.71 ± 0.40	9.00 ± 0.21
Soil UA	17.04 ± 0.13	1.79 ± 0.04	4.03 ± 0.03	9.54 ± 0.20	0.11 ± 0.04	1.87 ± 0.11	2.34 ± 0.17	10.29 ± 0.22
Tobacco straw biochar	356.20 ± 3.11	8.39 ± 0.25	10.86 ± 0.04	–	–	–	–	–
Wheat straw biochar	344.70 ± 1.80	9.42 ± 0.03	10.67 ± 0.04	–	–	–	–	–
Tobacco straw	470.13 ± 1.43	15.96 ± 0.13	–	–	–	–	–	–
Wheat straw	436.93 ± 1.44	8.12 ± 0.07	–	–	–	–	–	–

The deviation measure indicated standard error (SE, *n* = 3)

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Solid-state ^{13}C NMR spectroscopy

Detailed procedure for application of solid-state ^{13}C NMR spectroscopy was described by Song et al. [39]. Bruker AVANCE III 400 WB spectrometer was used to investigate ^{13}C CPMAS spectra. It was equipped with a 4 mm standard bore CPMAS probehead, while contact time was 2 min and 10,000 scans were recorded.

The chemical shift ranges of ^{13}C CPMAS NMR spectroscopy of Humin were 0–45 ppm (alkyl-C), 45–60 ppm (methoxyl-C or N–C), 60–110 ppm (O-alkyl-C), 110–145 ppm (aryl C), 145–160 ppm (phenol C), and 160–190 ppm (carboxyl- and carbonyl-C).

Urease activity and β-glucosidase activity

The urease and β-glucosidase activities were determined by diffusion method and colorimetric method, respectively [23]. For urease activity measurement, soil samples were treated by 10% urea, methylbenzene, and phosphoric acid (pH 7), then it was incubated at 37 ℃ for 15 h, followed by titration with H_2SO_4 solution. The volume of H_2SO_4 solution was recorded and calculated for urease activity. The β-glucosidase activity of soil was determined by treating with phosphoric acid–citric acid buffer solution (pH 4.8), methylbenzene solution and 4-nitrophenyl-beta-D-glucopyranoside, and then it was incubated for 1 h. The ethyl alcohol was added and filtered after incubation. The filtrate was then mixed with Tris (hydroxymethyl) methyl aminomethane and p-nitrophenol activity was measured at 400 nm by colorimetric method.

Statistical analysis

The variation in functional groups of humin and enzyme activities was tested using a two-way ANOVA with soil types and organic materials as factors. Humin functional groups were subjected to components analysis and cluster analysis. The LSD method of comparison for all pairs was used to determine the significance of the difference. EXCEL2010 software was used for data analysis. Moreover, the JMP statistical software, Version 11.2 (SAS Institute, Cary, NC, USA, 2013) was used for two-way ANOVA test, principal components analysis, cluster analysis and treatments comparison.

Results

Functional groups of humin

The functional groups of crop straw, biochar and humin investigated by solid-state ^{13}C NMR spectra are shown in Table 2 and Fig. 1a–d. The specifics of functional groups assignment were demonstrated by Song et al. [39]. The short-chain polymethylene linked to aryl C was observed at 25 ppm [40, 41]. The amorphous –(CH$_2$)– and crystalline –(CH$_2$)– groups were detected

at 30 ppm and 33 ppm, respectively [40, 42]. The peak at 56 ppm was assigned to carbon singly bonded to O and N heteroatoms [43]. The peak at about 72 ppm was C2, C3 and C5 carbons of sugar, while the signals at about 84 ppm and 105 ppm were C4 carbons and C1 carbon, respectively [44]. The broadband around 130 ppm was attributed to H substituted and C substituted aromatic functional groups [45]. The peak at 152 ppm, on the other hand, was assigned to phenolic C in lignin [46]. The signal at 173 ppm was assigned to carboxyl groups [47, 48].

With the exception of carboxyl C, the two-way ANOVA test of functional groups of humin revealed that most of the functional groups were significantly different in soil types and in treatments (Additional file 1: Table S1). Only soil types showed significant differences in terms of the carboxyl C. In addition, the two-way ANOVA test of functional groups of Hu indicated that the aromaticity of humin was significantly different between treatments, whereas the aliphaticity was significantly different between soil types (Additional file 1: Table S2). However, a two-way ANOVA analysis revealed that the hydrophobicity of humin varied significantly depending on the soil types (Additional file 1: Table S2). The relative contents of functional groups are shown in Table 2. The content of aryl C increased to 49.63%–76.55% and the aromaticity increased to 53.58%–78.39% after biochar amendments. Conversely, the content of alkyl C decreased to 5.68%–31.00% while the aliphaticity decreased to 5.74%–31.06% with biochar amendments. The hydrophobicity of humin increased to 2.81%–38.23%. However, content of O-alkyl C of humin increased to 33.99%–51.70%, and phenolic C increased to 2.34%–7.01% as a result of crop straw treatments. The content of alkyl C of Hu decreased to 19.16%–38.68%, and hydrophobicity of humin decreased to 0.49%–0.89% with crop straw treatments. The reduction in alkyl C of humin that was caused by addition of tobacco straw and wheat straw to the soil was the same in soil UV and soil UA, but it was different in soil CA. The aryl C of humin as result of straw treatment was higher in soil CA, but it was lower in soil UV and soil UA.

Relationship of functional groups of humin

In this study, the correlations of functional groups are shown in Fig. 2. Significantly negative correlations were discovered between aryl C and phenolic C ($R^2 = 0.79$, $P < 0.001$, $n = 12$), O-alkyl C ($R^2 = 0.85$, $P < 0.001$, $n = 12$), methoxyl/N–C ($R^2 = 0.90$, $P < 0.001$, $n = 12$), and carboxyl C ($R^2 = 0.34$, $P = 0.045$, $n = 12$) (Fig. 2a and b). A positive correlation was found between aryl C and hydrophobicity ($R^2 = 0.32$, $P = 0.03$, $n = 15$) (Fig. 2c). Not only did positive correlations exist between humin functional groups, but they could also be divided into different groups using principal component analysis and cluster analysis. The

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

Treatments	160–190	145–160	110–145	60–110	45–60	0–45	Aromaticity (%)[a]	Aliphaticity (%)[b]	Hydrophobicity[c]
Tobacco straw biochar	0	0	95.24	0	0	4.76	95.24	4.76	0
Tobacco straw	4.51	7.54	6.64	69.48	7.31	4.51	14.85	4.73	0.23
Wheat straw biochar	0	0	93.46	0	0	6.54	93.46	6.54	0
Wheat straw	3.30	4.30	3.64	75.55	6.15	7.07	8.21	7.31	0.18
Soil CA									
Control	1.05	0	2.94	0.18	5.42	90.42	2.97	91.37	14.05
TB	0.20	0	64.70	4.09	0	31.00	64.83	31.06	22.28
WB	0.11	0	68.75	1.35	1.08	28.70	68.83	28.73	38.23
TS	1.77	4.97	14.78	33.99	6.08	38.41	20.10	39.10	0.49
WS	2.18	2.34	7.23	42.24	7.34	38.68	9.78	39.54	0.89
Soil UV									
Control	10.98	3.40	25.58	22.94	4.83	32.27	32.55	36.25	1.49
TB	1.16	0	68.68	24.48	0	5.68	69.49	5.74	2.90
WB	2.35	0	76.55	5.69	0	15.42	78.39	15.79	11.44
TS	5.90	7.01	14.18	43.11	7.33	22.47	22.52	23.88	0.65
WS	6.43	6.24	11.06	51.70	5.40	19.16	18.49	20.48	0.48
Soil UA									
Control	4.21	2.82	16.00	27.54	9.64	39.79	19.65	41.54	1.35
TB	2.82	0	62.92	7.20	1.04	26.02	64.75	26.77	8.04
WB	7.36	0	49.63	16.49	2.36	24.15	53.58	26.07	2.81
TS	6.77	6.70	12.12	38.52	9.75	26.13	20.19	28.03	0.69
WS	7.31	6.14	11.55	43.71	6.36	24.93	19.09	26.89	0.64

[a] Aromaticity is calculated as follows: aromaticity = aromatic C/(aromatic C + o-alkyl C + alkyl C × 100

[b] Aliphaticity is calculated as follows: aromaticity = alkyl C/(aromatic C+O-alkyl C+alkyl C)×100

[c] Hydrophobicity is calculated as follows: hydrophobicity = (aromatic C+ alkyl C)/(carboxyl C+ O-alkyl C+ methoxyl C)

aryl C, phenolic C, O-alkyl C and methoxyl C were the first components and all contributed equally to the structural formation of humin amended with organic materials. However, alkyl C was in the second component compared to other functional groups of humin (Table 3 and Fig. 3). The cluster analysis of functional groups of humin revealed that O-alkyl and Alkyl C were in the same cluster, while carboxyl C, phenolic C, and methoxyl C were in a different cluster. Moreover, aryl C was distinct from other functional groups (Fig. 4).

Differences of urease activity and β-glucosidase activity

The urease activity, β-glucosidase activity, and the normal distribution of the enzyme activities are shown in Fig. 5 and S1. The urease activity and β-glucosidase activity were low in straw-derived biochar treatment compared to the straw treatment. Compared to TB treatment, the urease activities increased at 14.93 mg kg^{-1} d^{-1} and 20.53 mg kg^{-1} d^{-1} in soil CA and soil UA, while it decreased at 6.35 mg kg^{-1} d^{-1} in soil UV for WB treatments. Compared to TB treatment, β-glucosidase activities increased at 13.34 mg kg^{-1} h^{-1}

and 22.68 mg kg^{-1} h^{-1} in soil CA and soil UA, but decreased at 7.34 mg kg^{-1} h^{-1} in soil UV for WB treatments. Compared to TB treatment, TS treatment increased the urease activities at 115.73 mg kg^{-1} d^{-1}, 18.67 mg kg^{-1} d^{-1} and 5.69 mg kg^{-1} d^{-1} in soil CA, soil UV, and soil UA. TS treatment increased β-glucosidase activities at 22.01 mg kg^{-1} h^{-1}, 26.01 mg kg^{-1} h^{-1} and 14.01 mg kg^{-1} h^{-1} in soil CA, soil UV, and soil UA compared to TB treatment (Fig. 5). The two-way ANOVA analysis of urease and β-glucosidase activities showed that urease and β-glucosidase activities differed significantly between soil types and treatments ((Additional file 1: Table S3). The interaction effects of soil types and treatments on urease and β-glucosidase activities were significant. There was a significantly positive correlation between β-glucosidase activity and O-alkyl C ($R^2 = 0.56$, $P = 0.005$, $n = 12$), phenolic C ($R^2 = 0.39$, $P = 0.03$, $n = 12$), methoxyl/N–C ($R^2 = 0.42$, $P = 0.02$, $n = 12$), but a negative correlation between β-glucosidase activity and aryl C ($R^2 = 0.52$, $P = 0.008$, $n = 12$) (Fig. 2d and e). The significantly positive

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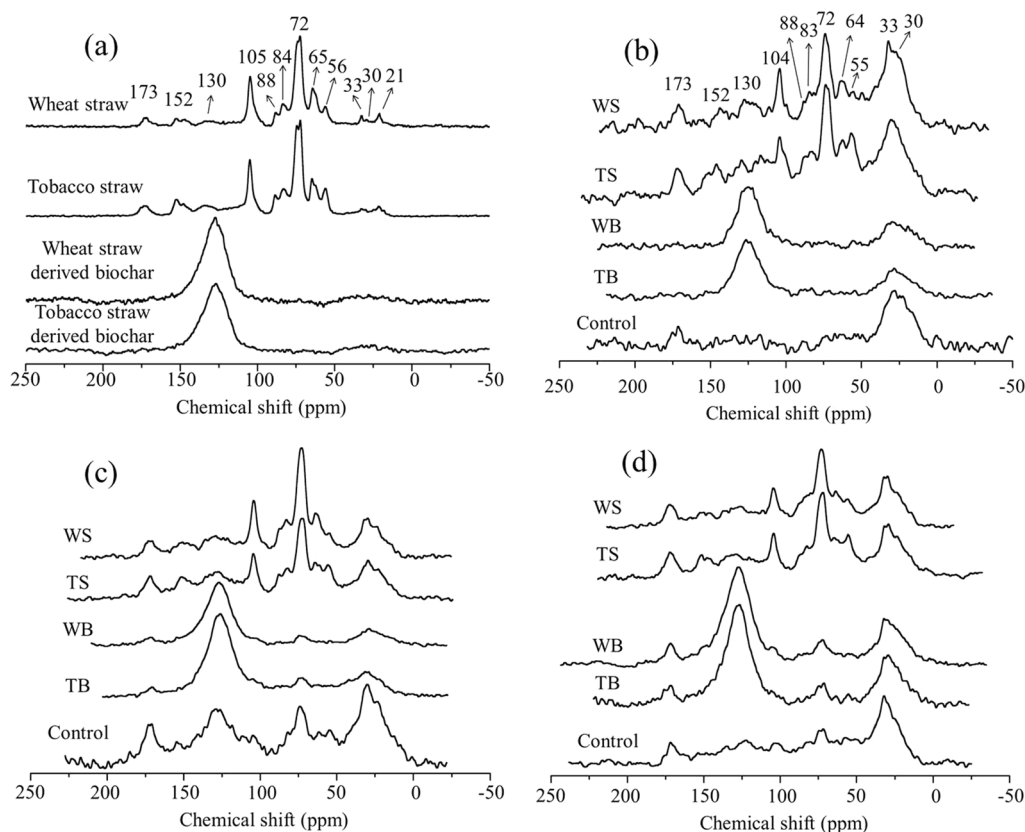
Fig. 1 ^{13}C NMR spectra of Hu of straw and biochar (**a**), soil CA (**b**), UV (**c**), and UA (**d**) incubated for 180 days

correlative was found between urease activity and alkyl C ($R^2 = 0.63$, $P = 0.002$, $n = 12$) (Fig. 2f).

Discussion

Structural changes of hydrophobic C of humin

Humin is an important humic fraction and may account for a larger proportion of SOM than previously thought [49]. Alkyl C is the predominant hydrophobic component of Hu [50–52]. Organic materials may have an effect on the structure of SOC [53]. In this study, biochar and straw amendments increased the aryl C and O-alkyl C of humin, respectively. Organic materials influenced the structure of humin because biochar and straw were high in aryl C and O-alkyl C. In addition, ^{13}C isotope tracing revealed that biochar was mostly sequestered in humin rather than humic acid and fulvic acid [23]. Actually, biochar made at different temperatures increased the content of humin [54]. Biochar's resistance to microbial attack may increase the stability of humin and SOC. The ^{13}C NMR spectra analysis of humin indicated that the functional groups of humin were similar to SOC [51]. Biochar and straw have the potential to increase the aryl C and O-alkyl C of SOC, respectively [53]. The principal

component analysis and cluster analysis in this study revealed that aryl C of humin differed from other functional groups and was important for the structure of Hu as the first principal component. Similarly, phenolic C and methoxyl C of humin were found in the same cluster and first principal component, and they made an equal contribution to the structure of humin. The principal component analysis results showed that O-alkyl C was more important for the structural changes that occurred in humin as a result of organic amendments, despite the fact that O-alkyl C and alkyl C were found in the same cluster,. Moreover, a positive correlation between aryl C or aromaticity and hydrophobicity demonstrated that biochar had the greatest impact on the hydrophobic components of humin. The modified conditions of soil organic matter transformation resulted in the increase of the hydrophobicity [55]. The addition of organic materials amendments can be an effective way to alter the conditions for transformation of various soil organic carbon fractions. Not only the aryl C of humin, but also the alkyl C of humin, could be affected by organic materials; this effect may be exacerbated by long-term organic fertilizer application [22]. Moreover, the two-way ANOVA

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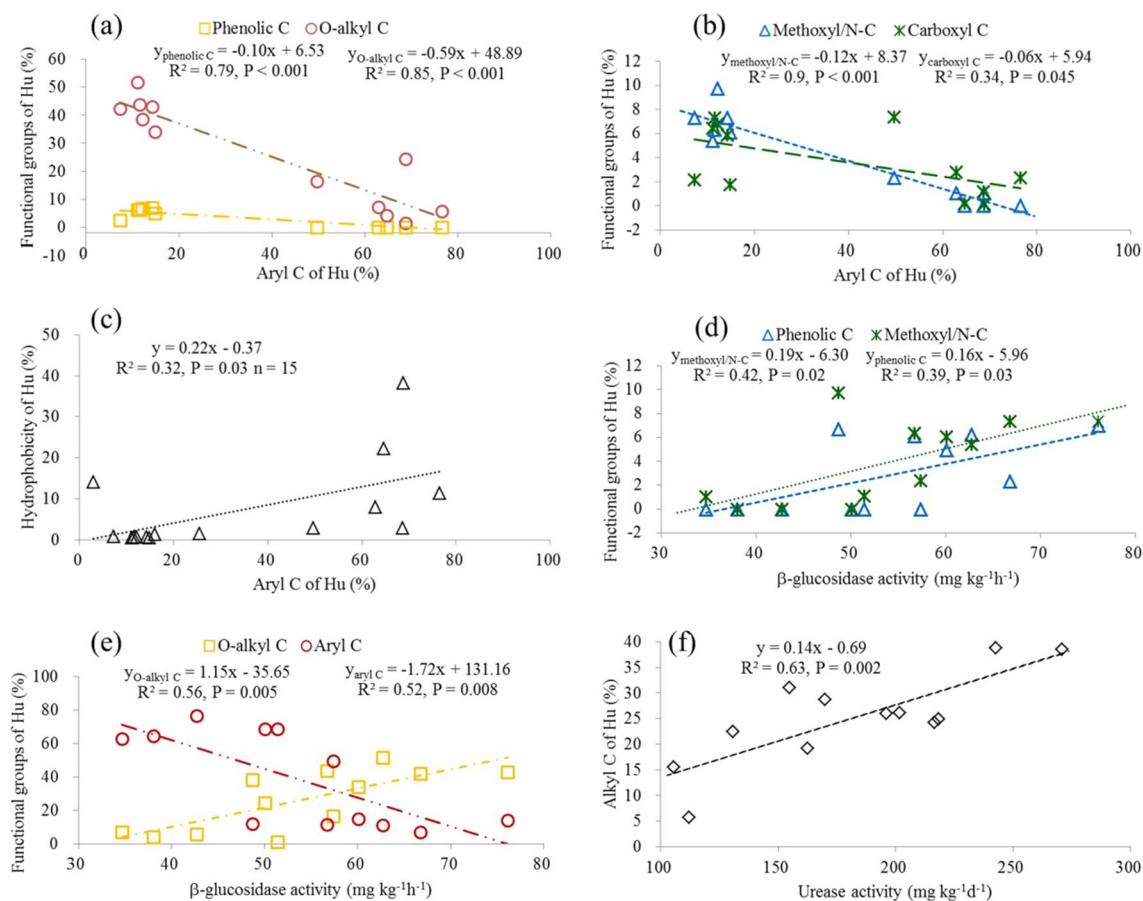
Fig. 2 Relationships of aryl C and other functional groups (**a** and **b**) and hydrophobicity of Hu (**c**), β-glucosidase activity and functional groups of Hu (**d** and **e**), urease activity and alkyl C of Hu (**f**). Notes: All the figures were $n = 12$, except the figure c was $n = 15$

**Table 3** Principal components on correlations of functional groups of Hu

	Principal components 1	Principal components 2	Principal components 3
Eigenvalues	3.65	1.53	0.57
Percent (%)	60.83	25.44	9.50
Cum percent (%)	60.83	86.27	95.77
Eigenvectors			
Carboxyl C	0.34	0.30	0.89
Phenolic-C	0.48	0.17	− 0.18
Aryl-C	− 0.47	0.35	0.02
O-Alkyl-C	0.46	0.27	− 0.37
Methoxyl C	0.47	− 0.24	− 0.07
Alkyl C	0.04	− 0.79	0.20

of functional groups of humin indicated the significant effect of soil types and various organic materials on functional groups of humin. This meant that the hydrophobic

components of humin were affected by organic materials and soil types, thereby affecting SOC stability.

Different effects of crop straw and biochar on the hydrophilicity of humin

Long-term fertilization of organic fertilizer or mineral fertilizer in Calcaric Fluvisol increased the O-alkyl C of Hu while decreasing alkyl C/O-alkyl C ratio and aromaticity [22]. However, long-term application of nitrogen (N) and phosphorus (P) fertilizer amendments increased alkyl C/O-alkyl C ratio and hydrophobicity in Typic Hapludoll [19]. These findings demonstrated that not only organic or inorganic amendment materials, but also soil types, influenced the structure of humin. Straw amendments increased the O-alkyl C of humin in this study. Wheat straw had a high relative content of O-alkyl C of humin when compared to tobacco straw amendment. Although tobacco straw and wheat straw amendments reduced alkyl C, the effect was different in these three soil types. This meant that the effects of organic materials

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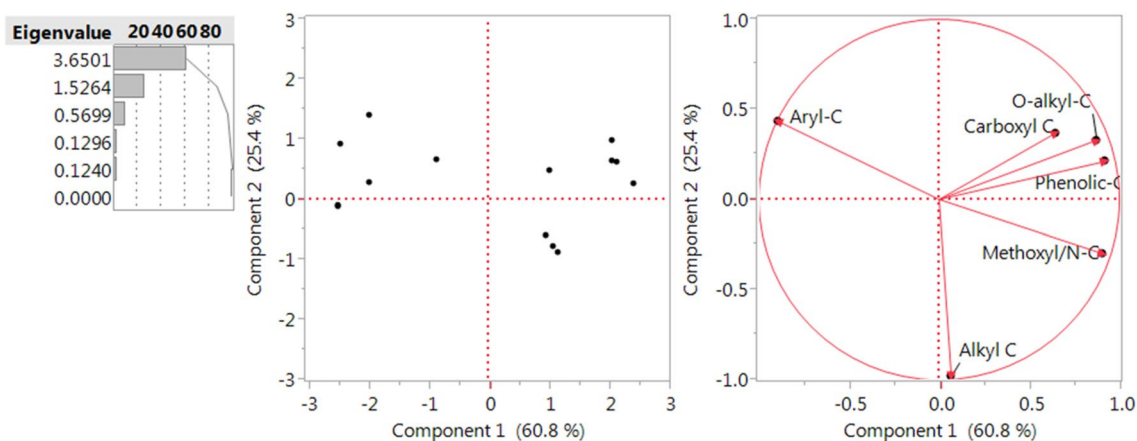
Fig. 3 Principal components analysis of functional groups of Hu
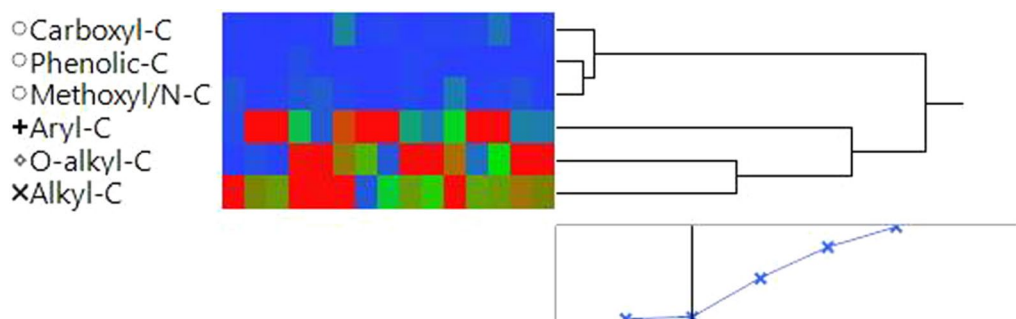
Fig. 4 Cluster analysis of functional groups of Hu
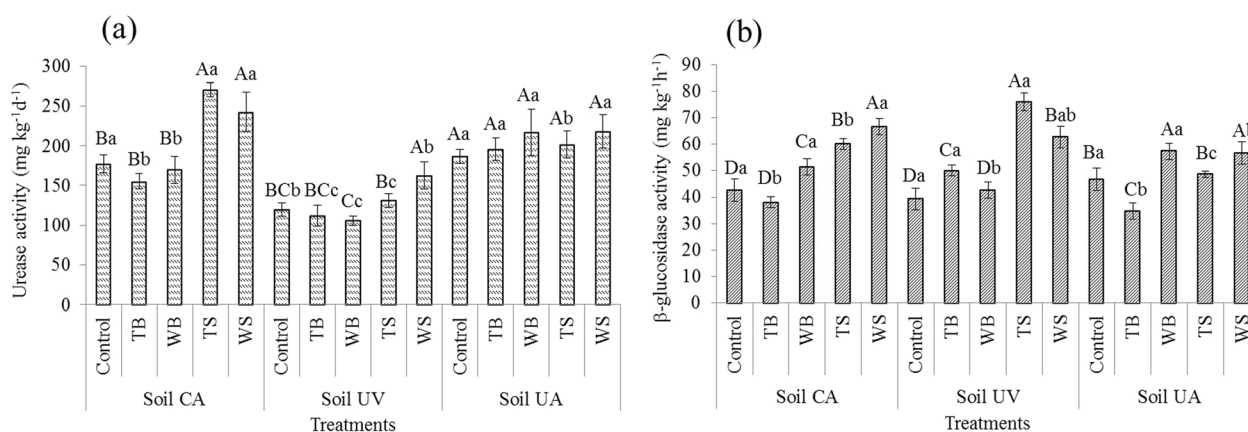
Fig. 5 Urease activity (**a**), β-glucosidase activity (**b**). Notes: Different capital letters in a single column indicate significant difference ($P < 0.05$) between treatments; different low case letters in a single column indicate significant difference ($P < 0.05$) for the same treatment between soil types

amendments on different functional groups of humin were varied; the straw structure influenced the selective preservation of hydrophilic functional groups. Straw mulching may increase SOC content by increasing non-labile C [56]; whereas, in another study, Song et al. [23] reported that organic materials amendments increased humin more than humic acid and fulvic acid. In this study, straw amendments increased

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O-alkyl C of humin. It was discovered that that O-alkyl C of Hu contributed to the increase in humin.

Contribution of urease and β-glucosidase activities to chemical composition of humin

The humic substances is the main component of SOC. Humin is the primary fraction of humic substances, and due to its heterogeneous structure and stability in soils, it makes a significant contribution to the reduction of greenhouse gases (GHGs)s [57]. Soil microbes and several enzymes facilitate the transformation of SOC and humic substances in various soils. Nevertheless, variations in composition and activity of soil microbial communities may be explained by differences in the functional groups of SOC, such as O-alkyl C, aryl C, alkyl C and carbonyl C [58]. The addition of biochar led to an increase in the β-glucosidase activity [29]. In this study, addition of biochar made from wheat straw resulted in increased β-glucosidase activity (13.34–22.68 mg kg^{-1} d^{-1}), as compared to tobacco straw-biochar amendment. Furthermore, β-glucosidase activity increased at 14.01–26.01 mg kg^{-1} d^{-1} when tobacco straw (non-pyrolyzed material) was used as the treatment, in comparison to using tobacco straw-biochar (pyrolyzed material) treatment. It is possible that the straw stimulated the enzyme activity more than biochar did because it was easier for soil microorganisms to decompose straw rather than biochar. The ^{13}C isotope trace analysis indicated that biochar and straw were mainly transformed to humin [23]. Most of the variations in soil microbial community composition and activity could explain by alkyl C, O-aryl C, aryl C and carbonyl C [58]. Not only the soil microbes, but also the enzymes contributed to the transformation of functional groups of SOC. In this present investigation, the methoxyl C ($R^2 = 0.42$, $P = 0.02$), phenolic C ($R^2 = 0.39$, $P = 0.03$) and O-alkyl C ($R^2 = 0.56$, $P = 0.005$) were positively correlated to β-glucosidase activity. Lignin-degrading enzymes play important role in degradation of lignin part of organic materials [59] and lead to humic substances formation. The enzymes such as β-glucosidase and urease mediate polymerization, decomposition, immobilization, and protection processes involved in the soil organic matter transformation [58]. The close relationship between the functional groups of humin and urease and β-glucosidase activities demonstrated that the chemical composition of humin was determined by enzyme activity. It is obvious from current study that organic materials influenced urease and β-glucosidase activities, which played an important role in C sequestration and humin stability.

Conclusions

The aryl C of humin was the primary functional group affecting the stability of humin when organic materials were added. In addition, soil types influenced the effects of organic materials on structure of humin. Biochar is useful for enhancing aryl C of humin, whereas crop straw is useful for enhancing O-alkyl C of humin. Compared to tobacco straw-derived biochar treatment, wheat straw-biochar increased β-glucosidase and urease activities at 13.34–22.68 mg kg^{-1} d^{-1} and 14.93–22.53 mg kg^{-1} d^{-1}, while wheat straw increased at 14.01–26.01 mg kg^{-1} d^{-1} and 5.60–115.73 mg kg^{-1} d^{-1}. Wheat and tobacco straw increased urease and β-glucosidase activities more effectively than biochars derived from wheat and tobacco straw. Crop straw and biochars derived from it are effective in stimulating urease and β-glucosidase activities, resulting in the transformation of functional groups of humin in soils.

Supplementary Information

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> **Additional file 1.** Additional tables and figure.

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Declarations

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