

Article

Effects of Organic Material Types on Temporal Changes in Characteristics of Humic Acids Extracted from a Chernozem

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Abstract: Returning organic materials to fields is an effective management practice for improving soil quality in agroecosystems. In the case of scarce organic material resources, choosing the optimal material type for improving the quality of a soil type is an urgent problem to be solved. The objective of this study was to evaluate the temporal variations in the spectroscopic characterization of humic acids (HAs) over a period of 90 days after applying biotechnologically extracted fulvic acid (BFA), well-decomposed sheep manure (M), corn stover pellets (Ps) and corn stover powder (Pr) at equal C amount to a chernozem under laboratory conditions, measured by Fourier transform infrared (FTIR) spectroscopy and ultraviolet-visible spectrophotometry (UV-Vis). The results indicated that the BFA and Pr with higher C contents (68.9 and 59.8 g kg⁻¹, respectively) were more beneficial to the C mineralization of HAs. M, applied in the largest amount, together with the highest available N, P, and K contents (575.4, 160.1, 478.9 mg kg⁻¹, respectively), and its more easily consumed carbohydrates as well as well-decomposed status, was more likely to promote the condensation of HAs. BFA carried more hydrophilic groups into the HAs, while Ps did not introduce high levels of adsorbed H₂O molecules and aromatic C=C (or C=O) groups. Ps did not affect the molecular structure of HAs after incorporation into the chernozem. At the equal C input amount, M had the greatest benefit, whereas the beneficial effect of Ps was the least, in improving the chernozem quality.

Keywords: chernozem; organic material; humic acid; FTIR spectroscopy; E₄/E₆ ratio

1. Introduction

Organic amendment is a suitable strategy for soil restoration in semiarid areas such as the western part of Jilin Province, China, where the soil organic matter (SOM) content and biological quality are low. The application of different organic materials (OMs), such as crop stover, animal manure and other wastes from agricultural processing, is a method to replenish degraded soil quality through improvement of soil fertility. Inputs of raw or mature OMs contribute differently to soil fertility. Raw organic inputs are the most readily utilized by soil microorganisms, while mature inputs provide more recalcitrant polymerized compounds, which are easily incorporated into soil humic substances (HS) [1]. Improper choice of these OMs will not only affect crop yields but will also cause the waste of resources and environmental pollution [2]. Due to the limited resources of agricultural solid waste, it is particularly important to find a suitable OM type that has the best fertilization effect on a certain soil type.

Humus (HS) is the most stable fraction of SOM and plays a central role in the functional biodiversity of terrestrial ecosystems. As the main component of HS, humic acids (HAs) more precisely reflect the existing pedo-ecological conditions and are therefore a good indicator for characterizing the biological activity and productivity of soils [3]. The mineralization of OM returned to soils is controlled by numerous factors—of which, the OM type has long been recognized as an important factor [4]. Different types of OM might have resulted in discrepancies among trials. Dai et al. applied four types of OM, including pig manure, biogas residue, biochar and crop stover, to soil at the same N level and concluded that OM amendment shaped the bacterial community in sandy loam soil by changing the soil pH and SOC [5]. Hu et al. evaluated the variation in the structure of HAs after the application of chicken manure, sheep manure, maize stover, fodder grass and tree leaves to semiarid soil under plastic mulched drip irrigation and noted that the chernozem amended with tree leaves was the most effective for the accumulation and stability of HAs [6]. Lucas et al. reported that HA spectra were not greatly influenced by the maturity of the amendment or by the application dose [7].

Although the effects of the different types and doses of OM on soil biochemical properties were initially clarified, the fertilizing effect of different OM at an equal C amount on the same soil is still unclear. Therefore, this paper aimed to evaluate the spectroscopic characteristics of HAs in a chernozem amended with four types of OM at an equal C amount under laboratory conditions. We hypothesized that the applications of different OM types at an equal C amount might have varying effects on the spectroscopic characteristics of HAs. Then, by comparing the spectroscopic characteristics of HAs extracted from a chernozem which was amended with different types of organic materials at an equal C amount, it would be possible to find the most suitable fertilization scheme for a certain type of soil.

2. Materials and Methods

2.1. Materials

Soil sampling sites were located in Haositai village in northeastern China (E 123°26'21'', N 46°11'27''). Soil was collected from a depth of 0 to 0.20 m in cultivated corn areas of a chernozem (CH) according to World Reference Base (WRB) 2014. Chernozem samples were air-dried and sifted through a 2 mm mesh sieve; the samples had a pH value of 8.2 and contained 16.2 g kg⁻¹ of organic matter, as well as 104.8, 16.6 and 121.4 mg kg⁻¹ of available N, P and K, respectively, as determined by the methods of potentiometric, potassium dichromate heating oxidation-volumetric, alkali hydrolyzation-diffusion, NaHCO₃ extraction–molybdenum antimony anti-colorimetric, CH₃COONH₄ extraction-flame photometry for determination.

There were four types of OM applied in the experiment. They were a biotechnologically extracted fulvic acid (BFA), well-decomposed sheep manure (M), corn stover pellets (Ps) and corn stover powder (Pr), which contained 68.9, 26.1, 43.1 and 59.8 g kg⁻¹ of total organic C, respectively. Among them, Ps were produced by mechanical compression of Pr, and there was a 6.8-fold decrease in volume. The above OM were all ground and passed through a 2 mm mesh sieve.

2.2. Experimental Design

Plastic mulched drip irrigation was simulated under laboratory conditions and was installed in some polyethylene material flowerpots (size 122 mm × 96 mm × 114 mm) without plants to explore the effects of different origins of OM at an equal C amount on the spectroscopic characteristics of HAs extracted from the soil and OM mixtures. Four types of OM were incorporated into the air-dried chernozem, making the total weight of the mixture in each flowerpot equal to 300 g. Wherein, the amount of C input from the external source was set to 1.0 g, after calculation, BFA, M, Ps, and Pr need to be added in the amounts 14.5 g, 38.3 g, 23.2 g, and 16.7 g, respectively. All the C/N ratios of mixtures from different treatments were adjusted to 25:1 with (NH₄)₂SO₄ solution. Initially, the available N, P, and K contents of the chernozem amended with BFA, M, Ps and Pr were 210.9, 37.2, and 349.8 mg kg⁻¹, 575.4, 160.1, and 478.9 mg kg⁻¹, 260.3, 35.3, and 407.5 mg kg⁻¹ and 283.8, 36.8, and 395.1 mg kg⁻¹,

respectively. The plastic mulched drip irrigation ensured that the water content was maintained at approximately 60% of the water-holding capacity. The incubation period was set at 90 days, and dynamic sampling for HA extraction was performed at 0, 30, 60 and 90 days. In the process, a constant temperature of 28 ± 1 °C was maintained with incubators. Experiments were performed in triplicate and repeated three times with similar results.

2.3. Extraction and Purification of HAs

The extraction and purification of the HAs, developed from the method of Pospíšilová and Fasurová [8], were conducted as follows: 100 g of the soil and OM mixtures obtained in the incubation were extracted with 1000 mL of 0.1 mol L^{-1} NaOH in a 2500 mL wide mouth glass bottle and then dispensed into several centrifuge tubes, shaken for 24 h on a horizontal shaker, and centrifuged at 25,900 g for 30 min. The alkali extract (supernatant) was decanted and stored, and this procedure was repeated twice. Subsequently, 800 mL of 2.5 mol L^{-1} HCl was added to the alkali extract to precipitate the HAs for 1.5 h in a water bath oscillator at 70 °C. The mixture was centrifuged for 20 min, and the supernatant was discarded. The HAs were repeatedly extracted by 2.5 mol L^{-1} HCl. The obtained HAs were dissolved using 0.1 mol L^{-1} NaOH until the pH value reached neutral. Finally, the HA solutions were rotary evaporated, freeze-dried in a freeze dryer (FD-1D-80, Beijing Boyikang Laboratory Instruments Co., Ltd., China), sifted through a 0.10 mm mesh sieve, and stored in a desiccator.

2.4. Instrumental Techniques

The powder sample of HAs should be dried in a vacuum oven at 100 °C for 3 h before measurement. A quantity of 1.5 mg of the HAs was compressed under vacuum with 250 mg of KBr pellets at a pressure of 20 MPa. The ratio of HAs:KBr of all samples was 1:166.7. Fourier transform infrared (FTIR) spectroscopy (FTIR-850, Tianjin Gangdong Sci & Tech Development Co., Ltd., China) was used for the structural characterization of HAs and can provide valuable information [9]. FTIR spectra were measured in the wavelength region from 400 to 4000 cm^{-1} at ambient temperature. The results were analyzed by ZWin software supplied with the FTIR-850 spectrometer. The measurement parameters were set as follows: 32 scans with 4 cm^{-1} resolution, and a data interval of 1.93 cm^{-1} . The triangle was chosen as the apodization mode with the option of collecting background before collecting samples. The DTGS KBr was set as the detector. The brief steps of spectra processing are as follows: choose the absorbance as Y-axis format, select the method of five-point three-time smoothing to smooth the spectra twice, select the option for automatic baseline correction, mark the absorbance peaks' position and calculate their area using the peak area tool. Finally, we saved the processed spectra with the transmittance (%) as Y-axis as a new CSV file and plotted them using Origin 8.0 (OriginLab, Wellesley, MA, USA) software. The statistical tests of FTIR relative intensities were performed using one-way univariate ANOVA (the Duncan test) in SPSS 18.0 (SPSS Inc., Chicago, IL, USA).

Absorbances at 465 and 665 nm ($\text{Abs}_{465 \text{ nm}}$ and $\text{Abs}_{665 \text{ nm}}$) of HAs dissolved in a mixed solution of 0.1 mol L^{-1} $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$ and 0.1 mol L^{-1} NaOH were measured using an ultraviolet–visible (UV–Vis) spectrometer (TU-1901, Beijing Puxi General Instrument Co., Ltd., China). E_4/E_6 ratios of HAs were calculated as the ratio of $\text{Abs}_{465 \text{ nm}}/\text{Abs}_{665 \text{ nm}}$ [8].

3. Results and Discussion

3.1. E_4/E_6 Ratio of HAs

Figure 1 shows the changes in the E_4/E_6 ratios for the HA samples. The E_4/E_6 ratio was used to indicate the degree of humification; the ratio decreased with an increase in the degree of humification [10]. Based on the equal C amount, the four types of OM were applied to the chernozem and their E_4/E_6 ratios of HAs showed different changes. With an increase in the incubation time, the E_4/E_6 ratio of HAs from the chernozem amended with BFA decreased slightly and then increased

greatly. The E_4/E_6 ratio of HAs treated with M gradually decreased, and there was no significant change in the E_4/E_6 ratio of HAs from the Ps treatment. The E_4/E_6 ratio of HAs treated with Pr gradually increased during the period of 0–60 days and then decreased slightly at 90 days. Compared with 0 d, at the end of 90 d, the E_4/E_6 ratios of HAs from the chernozem amended with BFA and Pr increased by 33.3% and 51.5%, respectively, which indicated a poor HA quality and the presence of more aliphatic but fewer aromatic compounds, while it decreased by 24.2% in the M treatment, indicating that it was relatively rich in aromatic structures. However, there were no obvious structural changes in the HA-treated Ps.

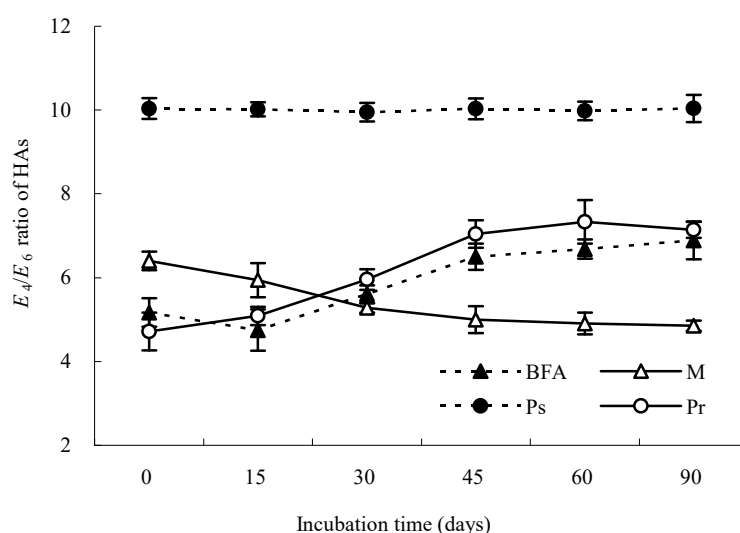


Figure 1. Changes in the E_4/E_6 ratio of HAs extracted from chernozem amended with biotechnologically extracted fulvic acid (BFA), well-decomposed sheep manure (M), corn stover pellets (Ps) and corn stover powder (Pr). The experiment was repeated three times, and the mean was taken for drawing the scatter plot.

Premised at the equal C amount, as soil amendments, OM with a higher C content, such as BFA and Pr containing total organic C contents of 68.9 and 59.8 g kg^{−1}, respectively, were more beneficial for the C mineralization of HAs. Moreno-cornejo et al. noted that C mineralization of OM was proportional to the doses of their applications [11]. This conclusion did not conflict with this paper. It could be inferred that the inclusion of OM with a higher C content was more likely to promote C mineralization. To ensure an equal C amount, 38.3 g of M was added to the soil, which was the largest amount of OM to be tested in this study. Meng et al. concluded that an increased amount of OM amendment could inhibit its decomposition [12]. M also enriched the chernozem with the highest amounts of available N, P, and K (575.4, 160.1, and 478.9 mg kg^{−1}, respectively), and its well-decomposed status is more likely to promote the condensation of HAs [1]. Therefore, more aromatic C structures were produced in the HAs due to the incorporation of M into the chernozem, which promoted the complexity of HAs. However, Ps were more difficult to degrade due to the high-intensity compression during the production process, so it did not affect the aromaticity of HAs after its incorporation into the chernozem.

3.2. Comparison in FTIR Spectra of HAs

The spectroscopic characterization of HAs is considered an important evaluation indicator of the fertilization effects of different types of OM on the soil [13]. Zhang et al. noted that the FTIR spectra of HAs in the same soil type amended with different types of OM might have similar characteristics, but the absorption intensity varied [14]. Spectra of HAs from the chernozem amended with four types of OM are presented in Figures 2–5. Absorption peak positions and corresponding assignments obtained for the HAs are listed in Table 1, and the variations in relative intensities are listed in Table 2.

Table 1. Peak positions and corresponding assignments of humic acids (HAs) extracted from chernozem amended with different types of organic materials (OMs) at an equal C amount.

Wavelength (cm ⁻¹)	Assignments	References
3400–3480	H-bonded O–H stretching vibrations of hydroxyl groups from alcohols, phenols, organic acids and H-bonded N–H groups	Lucas et al. [7]
2920–2960 and 2850–2860	Aliphatic C–H stretching of CH ₃ /CH ₂ groups	Xin et al. [15]
1600–1655	Aromatic C=C skeletal vibrations and C=O stretching of quinone	Chen et al. [9]
1380–1470	–CH deformation of –CH ₃ and –CH bending of CH ₂ or typical lignin pattern	Niemeyer et al. [16] Li et al. [17]
1090–1170	C–O stretching of polysaccharides and polysaccharide-like substances	Wu et al. [18]

The highest hydroxyl content appeared in the HAs extracted from the chernozem amended with BFA, while the lowest hydroxyl content was observed in the Ps treatment. The HAs extracted from the chernozem amended with Ps had the fewest aromatic C=C vibrations or C=O stretching but the highest percentage of carbohydrates. BFA, a concentrated bio-stimulant, was derived from leonardite and can carry more hydrophilic groups into the HAs. However, Ps were derived from the compressed material of corn stover, and there were few pores in Ps. Therefore, the amount of adsorbed H₂O molecules was very small in Ps, resulting in very few hydroxyl groups being present in the HAs.

3.3. FTIR Spectra of HAs Extracted from the Chernozem Amended with BFA

Souza and Bragança noted that the FTIR spectra showed the presence of aliphatic (bands 2900–2860 cm⁻¹, 1460 cm⁻¹) and aromatic components (1650 cm⁻¹) [19]; thus, the ratio of the intensity at 1650 cm⁻¹ to intensities at 2900–2860 cm⁻¹ and 1460 cm⁻¹ (from FTIR spectra) represented by the ratio $c/(a + b + d)$ could roughly represent the ratio of aromatic C to aliphatic C. Wu et al. also showed that the ratio between the integrated peak areas of 1650 and 2930 cm⁻¹ positively correlated with the ratio of stable C to labile C and was used as an indicator of soil organic matter (SOM) stability [18]. As shown in Figure 2 and Table 2, with the incubation going on, the $\gamma/(\alpha + \beta + \delta)$ ratio of HAs increased gradually and then decreased, and finally, it was reduced. The aromatic C groups in BFA might preferentially enter the HA component of the chernozem with an increase in the incubation time, which could gradually enhance the proportion of aromatic C in HAs, and then the proportion of aromatic C in HAs decreased with the nutrient release and degradation of BFA. The overall performance of HAs showed a decrease in aromaticity, also indicating that HAs became more labile C. The intensity of the HA peak located at 1130–1161 cm⁻¹ had varying degrees of increase compared to 0 d. The incorporation of BFA into the chernozem could enhance the carbohydrate production of HAs and feed beneficial microbes. Rezaeova et al. showed that the molecular structure of HAs changed after the application of organic manure in that the number of aromatic structures decreased significantly [20]. Zhang et al. also indicated that horse manure combined with inorganic N, P, and K fertilizers could increase the lengths of aliphatic chains in soil HAs [14]. The above studies were similar to the conclusion of this article. The HA molecular structure of the chernozem amended with BFA became more aliphatic, simpler and younger.

3.4. FTIR Spectra of HAs Extracted from the Chernozem Amended with M

Figure 3 shows the FTIR spectra of HAs extracted from the chernozem amended with M. With an increase in the incubation time, the $\gamma/(\alpha + \beta + \delta)$ ratio of HAs gradually increased. In this test, well-decomposed sheep manure was applied—the aromatic C structures of which could preferentially enter the HA component of the chernozem, making the structure of HAs more complex and indirectly improving the humus quality. The highest amount of available nutrients also made the microorganisms that drive the humification process more active. This was similar to the conclusion that complementing

manure with inorganic N fertilizers in integrated nutrient management would improve the soil quality [21].

Table 2. Fourier transform infrared (FTIR) relative intensities (% of total area) for the HAs extracted from chernozem amended with different types of OM s at an equal C amount.

Treatments	BFA-0	BFA-30	BFA-60	BFA-90
Wavelength (cm ⁻¹)				
3419–3445	41.1 ± 0.2 Ab	41.3 ± 0.3 Ab	42.4 ± 0.6 Aa	41.0 ± 0.1 Ab
2923–2925 ^α	6.5 ± 0.2 Cc	7.8 ± 0.3 Ca	7.1 ± 0.2 Db	7.8 ± 0.4 Ca
2854 ^β	3.4 ± 0.1 Bc	3.9 ± 0.1 Ba	3.6 ± 0.0 Cb	3.7 ± 0.1 Bb
1629–1643 ^γ	18.7 ± 0.3 Bb	15.3 ± 0.1 Cd	19.2 ± 0.4 Ba	16.3 ± 0.2 Cc
1456–1463 ^δ	11.6 ± 0.8 Ba	6.3 ± 0.2 Cc	6.2 ± 0.2 Dc	8.8 ± 0.6 Cb
1130–1161	18.8 ± 0.5 Dd	25.4 ± 0.8 Ba	21.6 ± 0.3 Bc	22.4 ± 0.4 Bb
Ratio of $\gamma/(\alpha + \beta + \delta)$	0.87 ± 0.01 b	0.86 ± 0.09 b	1.14 ± 0.09 a	0.80 c
Treatments	M-0	M-30	M-60	M-90
Wavelength (cm ⁻¹)				
3419–3448	36.5 ± 0.2 Cd	40.3 ± 1.0 Ba	38.1 ± 0.3 Bb	37.0 ± 0.2 Cc
2923–2925 ^α	7.3 ± 0.1 Bc	8.6 ± 0.2 Ba	8.3 ± 0.1 Cb	7.1 ± 0.2 Dc
2852–2854 ^β	3.4 ± 0.2 Bc	3.9 ± 0.1 Bb	4.0 ± 0.2 Ba	3.8 ± 0.1 Bb
1635–1645 ^γ	15.8 ± 0.3 Cd	17.2 ± 0.1 Bc	21.1 ± 0.4 Aa	18.8 ± 0.3 Bb
1382–1457 ^δ	12.8 ± 0.2 Ab	10.6 ± 0.2 Ad	14.9 ± 0.4 Aa	11.8 ± 0.1 Ac
1112–1157	24.2 ± 0.6 Ba	19.5 ± 0.4 Dd	22.0 ± 0.2 Bb	21.5 ± 0.1 Cc
Ratio of $\gamma/(\alpha + \beta + \delta)$	0.67 ± 0.02 d	0.74 ± 0.01 c	0.78 ± 0.01 b	0.83 ± 0.02 a
Treatments	Ps-0	Ps-30	Ps-60	Ps-90
Wavelength (cm ⁻¹)				
3422–3445	23.9 ± 0.2 Da	23.1 ± 0.4 Db	21.4 ± 0.2 Cc	20.9 ± 0.3 Dd
2921–2925 ^α	9.7 ± 0.2 Ab	13.0 ± 0.2 Aa	13.1 ± 0.1 Aa	9.3 ± 0.1 Ac
2852–2854 ^β	19.3 ± 0.5 Aa	14.2 ± 0.2 Ad	15.1 ± 0.3 Ac	18.1 ± 0.2 Ab
1631–1652 ^γ	3.2 ± 0.1 Dc	3.3 ± 0.2 Db	3.6 ± 0.2 Ca	3.6 ± 0.1 Da
1430–1461 ^δ	7.5 ± 0.0 Db	7.4 ± 0.1 Bb	7.3 ± 0.0 Cc	8.1 ± 0.3 Da
1122–1128	36.5 ± 0.7 Ac	39.0 ± 0.2 Ab	39.4 ± 0.3 Ab	40.0 ± 0.2 Aa
Ratio of $\gamma/(\alpha + \beta + \delta)$	0.09 ± 0.01 a	0.10 ± 0.00 a	0.10 ± 0.01 a	0.10 ± 0.01 a
Treatments	Pr-0	Pr-30	Pr-60	Pr-90
Wavelength (cm ⁻¹)				
3419–3455	38.3 ± 0.3 Bb	35.6 ± 0.8 Cc	38.3 ± 0.3 Bb	40.3 ± 0.6 Ba
2923–2925 ^α	6.6 ± 0.2 Cd	6.9 ± 0.3 Dc	11.2 ± 0.7 Ba	8.2 ± 0.5 Bb
2854 ^β	3.1 ± 0.0 Cc	3.5 ± 0.1 Cb	3.4 ± 0.0 Db	3.8 ± 0.1 Ba
1635–1650 ^γ	22.2 ± 0.5 Aa	19.2 ± 0.1 Ab	19.4 ± 0.1 Bb	19.3 ± 0.1 Ab
1459–1463 ^δ	10.0 ± 0.1 Cc	10.9 ± 0.2 Ab	12.2 ± 0.7 Ba	10.0 ± 0.2 Bc
1066–1141	19.9 ± 0.2 Cb	23.9 ± 0.7 Ca	15.5 ± 0.4 Cd	18.4 ± 0.3 Dc
Ratio of $\gamma/(\alpha + \beta + \delta)$	1.13 ± 0.05 a	0.90 ± 0.01 b	0.72 ± 0.03 c	0.88 ± 0.00 b

Biotechnologically extracted fulvic acid, well-decomposed sheep manure, corn stover pellets and corn stover powder were represented by biotechnologically extracted fulvic acid BFA, M, Ps, and Pr, respectively. The number in the name of treatment represented the days of incubation. The ratio of $\gamma/(\alpha + \beta + \delta)$ (from FTIR spectra) could roughly represent the ratio of aromatic C to aliphatic C in the HAs. Different lowercase letters indicate a significant difference among the different incubation days under the same OM type, and different uppercase letters indicate a significant difference among the different OM types under the same incubation day ($P < 0.05$). Data expression: the mean ± standard deviation.

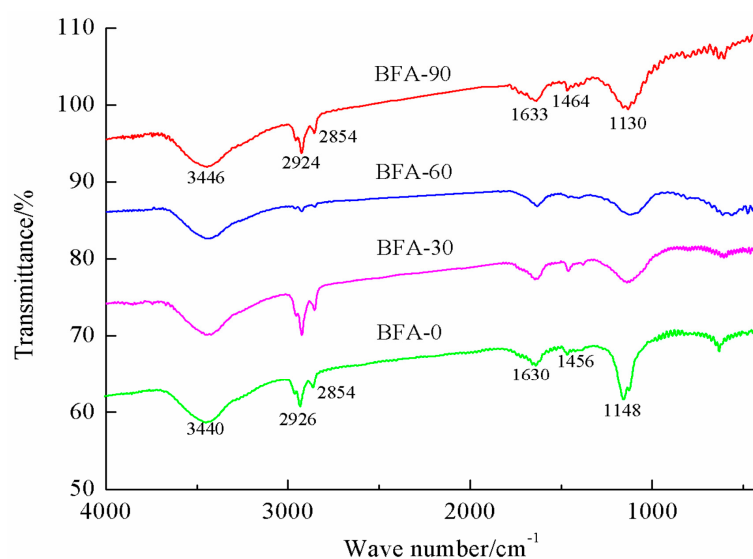


Figure 2. FTIR spectra of HAs extracted from chernozem amended with biotechnologically extracted fulvic acid (BFA). The number in the name of treatment represented the days of incubation.

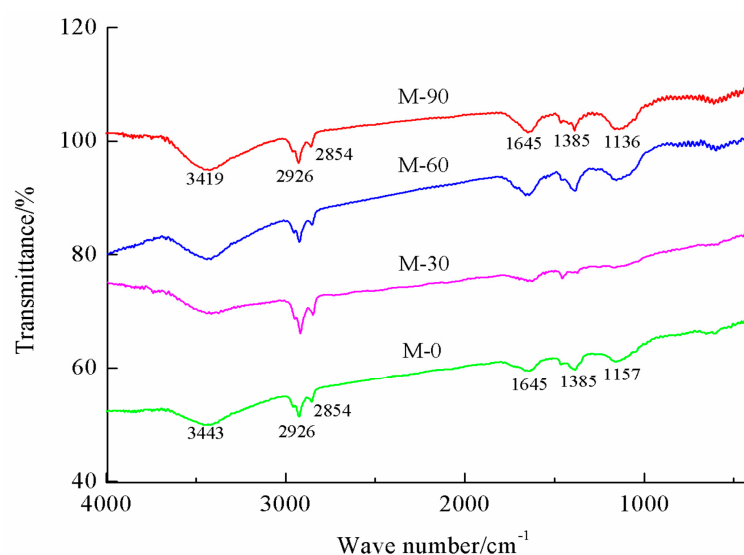


Figure 3. FTIR spectra of HAs extracted from the chernozem amended with well-decomposed sheep manure (M).

Compared with 0 d, the intensity of the absorption band at 1112–1157 cm^{-1} of HAs under different incubation days had different degrees of reduction. The well-decomposed sheep manure itself contained many microorganisms, and its incorporation into the chernozem would inevitably lead to a greater reproductive capacity of microorganisms. In the process, the microorganisms in the mixture demonstrated the highest degradation capabilities for carbohydrates of HAs [22].

3.5. FTIR Spectra of HAs Extracted from the Chernozem Amended with Ps

As a fuel for home heating or cooking, the corn stover pellets were derived from the smashing and compression molding of corn stover in a pellet mill. In this test, Ps were also selected as OM for fertilizing the chernozem. Figure 4 and Table 2 show that there was almost no change in the $\gamma/(\alpha + \beta + \delta)$ ratio of HAs throughout the incubation process. The proportion of aromatic C in HAs from the chernozem amended with Ps was the lowest among the four tested organic amendments. Unlike other OMs, after the incorporation of Ps into the chernozem, their HAs had the highest proportion of

carbohydrates—even higher than their hydroxyl content. This might be related to the glue used in the compression molding of corn stover. Compared with 0 d, the carbohydrates of HAs had varying degrees of increase at 30, 60 and 90 d. The reason for this was that after the corn stover pellets entered the chernozem, the microbial activity and degradation was inhibited due to a more limited void space or a higher density of these pellets, and the carbohydrates were not consumed to support the reproduction of microorganisms. There was no obvious change in the proportion of aromatic C and aliphatic C during the incubation process. Therefore, the corn stover pellets did not promote the quality of the chernozem and were not suitable to regenerate the soil.

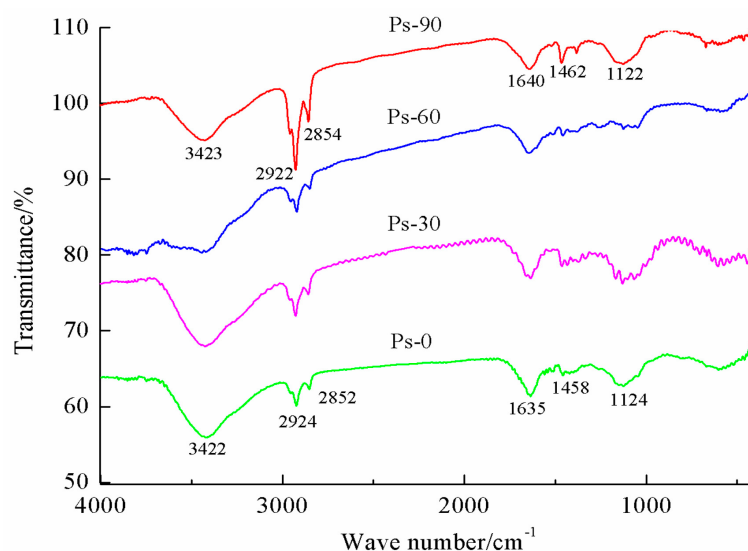


Figure 4. FTIR spectra of HAs extracted from the chernozem amended with corn stover pellets (Ps).

3.6. FTIR Spectra of HAs Extracted from the Chernozem Amended with Pr

Figure 5 and Table 2 show that the ratio of $\gamma/(\alpha + \beta + \delta)$ initially experienced a significant reduction, followed by a rebound with the incubation time and, finally, the ratio decreased. This result was consistent with Lucas et al.'s research [7]. Wang et al. noted that stover addition could increase SOM mineralization regardless of N addition [22]. Masunga et al. also reported that microorganisms were more abundant in clover-amended soils than in soils amended with manure [23]. It was implied that the incorporation of corn stover powder into the chernozem could produce a significant increase in microbial biomass size and could increase the microbial mineralization, and then undergoing a humification or HA polymerization process. However, the newly formed HAs did not condense to the initial complexity of HAs, and the final proportion of aromatic C was still reduced. Microbial degradation of HAs is an important part of humus turnover [24], which could provide precursor substances for the humification process. Li and Wu found that the aliphatic C–H group stretching of FA in black soil amended with herb residue was greater than that from animal excrement, while the aromatic C=C and C=O stretching of FA varied in the order: animal excrement > herb residue [4]. In this study, the chernozem amended with corn stover powder had a higher aliphatic C structure in HAs compared with well-decomposed sheep manure. There was a series of fluctuations that showed an increase, decrease and then an increase in the intensity of the absorption band at 1066–1141 cm^{-1} of HAs throughout the incubation. During the period of 0–30 d, the microorganisms utilized the labile C of corn stover to reproduce faster, and more carbohydrates were produced from the degradation of corn stover. At this stage, the production of carbohydrates was greater than the microbial consumption, and then during the period of 30–60 d, the microorganisms encountered the refractory organic C. More carbohydrates were consumed as energy, and then degradation recurred or the microbial cells were autolyzed, resulting in an increase in carbohydrates. Overall, there was a net loss in the carbohydrates of HAs. Hsu et al. utilized the FTIR technique to analyze the organic matter transformations during

the composting of pig manure and noted that there was a decrease in the carbohydrates of HAs as decomposition proceeded [25].

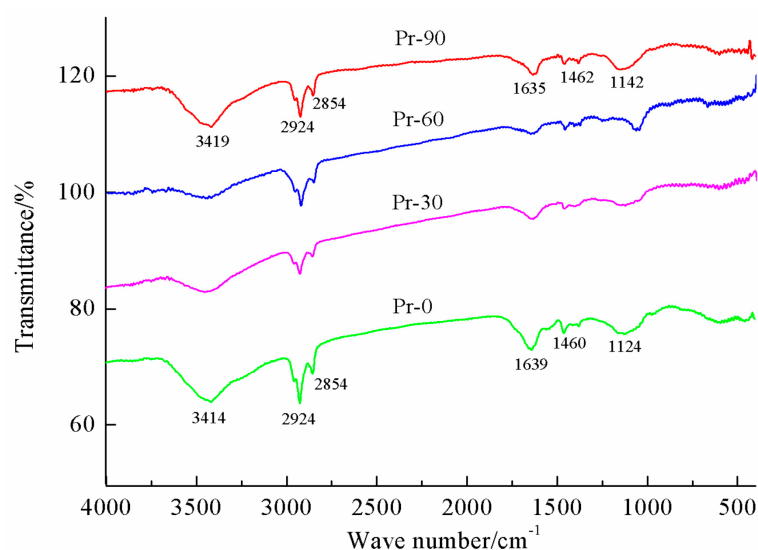


Figure 5. FTIR spectra of HAs extracted from the chernozem amended with corn stover powder (Pr).

In conclusion, at an equal C amount, as soil amendments, BFA and Pr with higher C contents were more beneficial to the C mineralization of HAs, making their molecular structures more aliphatic, simpler and younger. M with the most actual addition, the greatest available N, P, and K contents, and its more easily consumed carbohydrates, together with its well-decomposed status, was more likely to promote the condensation of HAs. BFA carried more hydrophilic groups into the HAs, while Ps did not introduce high levels of adsorbed H₂O molecules and aromatic C=C (or C=O) vibrations into the HAs. The molecular structure of the HAs did not show any significant change after the incorporation of Ps into the chernozem. In this study, with respect to the equal C input amount, the well-decomposed sheep manure had the greatest benefit, whereas the corn stover pellets provided the least beneficial effect, in improving the chernozem quality. Since the amount of extracted HAs from chernozem in this test was less than 20 mg, which was not enough for more analyses except FTIR spectra. In the follow-up work, we will continue to extract a larger amount of HAs used for elemental composition and ¹³C NMR analysis, which will provide more valuable information.

4. Conclusions

The amendments of BFA and Pr were more beneficial to the C mineralization of HAs from the chernozem, while M was more likely to promote the condensation of HAs. Ps did not affect the molecular structure of HAs after incorporation into the chernozem. At the equal C input amount, M had the greatest benefit, whereas the beneficial effect of Ps was the least, in improving the chernozem quality.

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