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Influences of Nitrogen Application Levels on Properties of Humic Acids in Chernozem Amended with Different Types of Organic Materials

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Abstract: The objective of this study was to examine the structure changes in humic acids (HAs) in Chernozem after the application of different types of organic materials (OMs) under an indoor simulation condition for plastic mulched drip irrigation, measured with Fourier transform infrared (FTIR) spectroscopy. The biotechnological extract of fulvic acid (BFA), decomposed sheep manure (M), corn straw pellets (Ps) and corn straw powder (Pr) were used as the four OMs for testing, and they were applied to Chernozem at the same amount of actual material; three nitrogen (N) levels (no N, low N, and high N supply) were applied to each type of (OMs), separately. The total culture period was set to 90 days and soil sampling was taken at 0, 30, 60 and 90 days, respectively. The results showed that different types of OMs exerted different effects on Chernozem based on the FTIR spectra of HAs. The application of M combined with high N supply was the best way to fertilize Chernozem, under which the H-bonded OH groups and aromatic compounds were enhanced, resulting in increased soil carbon (C) sequestration; while the carbohydrates in HAs was easily consumed as microbial energy substance. The HAs from the Chernozem amended with BFA became more aliphatic, simpler and younger. High N supply was beneficial for increasing the complexity of HAs from Chernozem amended with Ps, but was not conducive to soil cation retention. Within a short time of incubation, the application of Pr combined with high N was detrimental to the C sequestration in Chernozem, and inhibited the consumption of carbohydrates by microorganisms.

Keywords: humic acid; FTIR spectroscopy; organic materials; Chernozem; oxygen-containing functional group

1. Introduction

Maintaining food security was a major challenge in China. The deterioration in soil quality is now a very severe problem. The decline in soil fertility and productivity due to excessive soil erosion, nutrient run-off, and loss of soil organic matter (SOM) has stimulated the interest in improving soil quality with the addition of organic materials (OMs) from different sources [1]. To maintain or improve the fertility and productivity of arable soils, several types of OMs can be considered, such as crop straw, animal manure and other organic wastes from agricultural processing, as a source of SOM. However, the improper use of these OMs will cause waste of resources and environmental pollution.

Humic substances (HSs) are an important part of the soil organic carbon (C) pool, accounting for approximately 80% of SOM. HSs involve in the whole variety of geochemical processes in the soil [2]. The addition of OMs to the soil is expected to increase the soil HSs pool and also to alter the chemical properties and functions of SOM. As a major component of HSs, humic acids (HAs) reflect

the existing pedo-ecological conditions more precisely than HSs, and thus HAs are a good indicator for characterizing soil biological activity and productivity [3]. The HAs function in various ways in maintaining and improving soil fertility and health, therefore, the quality of HAs is considered as a key indicator for evaluating the effects of different types of OMs on soil fertility and health [4].

As a non-destructive analytical method, Fourier transform infrared spectroscopy (FTIR) is advantageous over ^{13}C nuclear magnetic resonance, ultraviolet-visible spectroscopy and "steady-state" fluorescence spectroscopy in the qualitative and semi-quantitative analysis of various oxygen-containing functional groups of soil HAs. The FTIR technique generates more information about the stretching and deformation vibrations of chemical bonds between atoms, evaluating the improvement in soil fertility and health, and differentiating the humification condition after organic amendment [5,6].

The influences of land use management on the characteristics of soil humic substances could be detected with FTIR spectroscopy of HAs [7]. FTIR spectra of HAs in a specific soil type amended with different types of OMs might have similar characteristics, but the absorption intensity varied [8]. Before and after the addition of OMs to the soil, the chemical characterization of HAs by infrared analysis could provide deep understanding of the effect of OMs conversion to HAs on soil health [9]. Diffuse reflectance infrared Fourier transform spectroscopy was adopted to examine the effects of compost amendment on two grassland soils, indicating that the ratio of carbonyl and carboxyl functional groups to aliphatic methyl and methylene groups increased in the light fractions of the amended soils [10]. Dai et al. [11] applied four types of OMs pig manure, biogas residue, biochar, and crop straw separately to soil at the same nitrogen (N) application rate, and concluded that OMs applied to a sandy loam soil shaped the bacterial community of the soil. Hu et al. [12] evaluated the variations in the structure of HAs after the application of chicken manure, sheep manure, maize straw, fodder grass or tree leaves in a Chernozem under plastic mulched drip irrigation, and found that the amendment of tree leaves was most effective in increasing the accumulation and structure stability of HAs. The HAs extracted from a soil amended with municipal sewage sludge for seven years had a higher concentration of aliphatic and N-containing groups, but a lower concentration of polysaccharides than those from the unamended soil based on the FTIR spectroscopy [13]. Infrared spectroscopy of HAs extracted from the soil amended with date palm compost or sheep manure revealed the enrichment with aromatic structures in HAs [14]. It was seen that the structure of HAs in amended soils might be affected at varying extents depending on the nature and origin of OMs [15].

Although there were some reports on the effects of organic amendation on soil fertility and health, the dynamic changes in the structural properties of HAs were rarely tracked and compared during the soil amending process with different OMs types, especially under the applications of different nitrogen (N) levels. Therefore, this study aimed to evaluate the dynamic variations in the structure of HAs in Chernozem after being amended with four types of OMs (biotechnological extract of fulvic acid, decomposed sheep manure, corn straw pellets, and corn straw powder) separately, at three different N application levels (zero, low, and high) under an indoor-simulation plastic mulched drip irrigation. The specific objectives in the current research were to: (i) Examine whether it is feasible to evaluate the effects of the above four different types of OMs amendments on Chernozem fertility and health with the spectral characteristics of HAs using the FTIR spectroscopy technique; (ii) find a suitable soil amendment measure for the improvement of Chernozem fertility and health.

2. Materials and Methods

The soil samples were collected in 0~20 cm depth from the fourth farm at the town of Tumuji, Jalaid Banner, Inner Mongolia (123°00′ E, 46°17′ N), China, and then passed through a 2-mm sieve before initial analysis, which was classified as Chernozem according to the classification system of the Food and Agriculture Organization (FAO) [12]. The soil chemical and physical properties are shown in Table 1, and the soil profile is presented in Figure 1, showing a thick humus-rich surface horizon with a light-colored lime-rich layer below. The four types of OMs were selected as follows: Biotechnological

extract of fulvic acid (BFA), decomposed sheep manure (M), corn straw pellets (Ps) and corn straw powder (Pr), which contained 68.9, 26.1, 43.1 and 59.8 g kg^{-1} of total organic C, respectively.

Table 1. Chemical and physical properties of Chernozem.

Index	pH	Organic Matter, g kg^{-1}	Available N, mg kg^{-1}	Available P, mg kg^{-1}	Available K, mg kg^{-1}	Sand, g kg^{-1}	Silt, g kg^{-1}	Clay, g kg^{-1}
value	8.2	19.8	84.82	6.55	51.36	679.1	250.1	70.8



Figure 1. Soil profile from the Chernozem sampling site.

Under a simulation condition for plastic mulched drip irrigation, the method of indoor culture was adopted to explore the effects of four different origins of OMs (biotechnological extract of fulvic acid, decomposed sheep manure, corn straw pellets, and corn straw powder) at an equal application rate of actual materials on HAs characterizations of Chernozem at three different N application levels including no N (0), low N (L), and high N (H). A total of 285 g of air-dried Chernozem was mixed with 15 g OMs. The mixed materials (MM) were placed in the plastic flowerpot with a size of 122 mm × 96 mm × 114 mm. According to the requirements of the three N application levels, 0 g, 0.095 g and 0.189 g $(NH_4)_2SO_4$ were applied to 300 g of MM, respectively. The plastic mulched drip irrigation maintained the water content of MM at approximately 60% of water-holding capacity. A constant temperature at (28 ± 1) °C for 90 d was provided with incubators. The sampling times of MM were at 0, 30, 60, and 90 d of incubation for the extraction of HAs.

A total of 2 g of each MM sample was mixed with 20 mL of 0.1 mol L^{-1} NaOH in a 50 mL centrifuging bottle, shaken for 24 h on a horizontal shaker, and centrifuged at 25,900 g for 30 min. The supernatant was decanted and stored. This procedure was repeated twice and then the extracted supernatant was combined. Subsequently, 16 mL of 2.5 mol L^{-1} HCl was added to the extracted solution to precipitate the HAs for 1.5 h in a water-bath oscillator at 70 °C, centrifuged for 20 min, and then the supernatant was discarded. The centrifuged HAs were dissolved in 16 mL of 2.5 mol L^{-1} HCl again, and this procedure was repeated. The collected HAs were dissolved with 0.1 mol L^{-1} NaOH until the pH value reached neutral. Finally, the HAs were rinsed with a little amount of H_2O and

transferred into 50 mL plastic bottles, and freeze-dried (Model: FD-1D-80, Beijing Boyikang Laboratory Instruments Co., Ltd., Beijing, China). The dried HAs samples were stored in a desiccator. Compared to the method of HAs extraction recommended by IHSS, NaOH used as the extractant in this study for FTIR analysis of HAs yielded higher resolved IR spectra, especially in the regions of stretching vibrations including aromatic and aliphatic groups [16].

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. Fourier transform infrared (FTIR) spectroscopy (Model: FTIR-850, Tianjin Gangdong Sci & Tech Development Co., Ltd., Tianjin, China) was used for structural characterization of HAs [17]. FTIR spectra were taken in the wavelength region from 400 to 4000 cm^{-1} at an ambient temperature. The results were analyzed by ZWin software supplied with the FTIR-850 spectrometer. The set measurement parameters were as follows: 32 scans with 4 cm^{-1} resolution, data interval 1.93 cm^{-1}. A triangle was chosen as the apodization mode and the option of collecting background before collecting samples. The DTGS KBr was set as the detector. The brief steps of spectra processing were 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' positions and calculate their area using the peak area tool. Finally, save the processed spectra with the transmittance (%) as Y-axis as a new CSV file, and plot them using Origin 8.0 software.

3. Results and Discussion

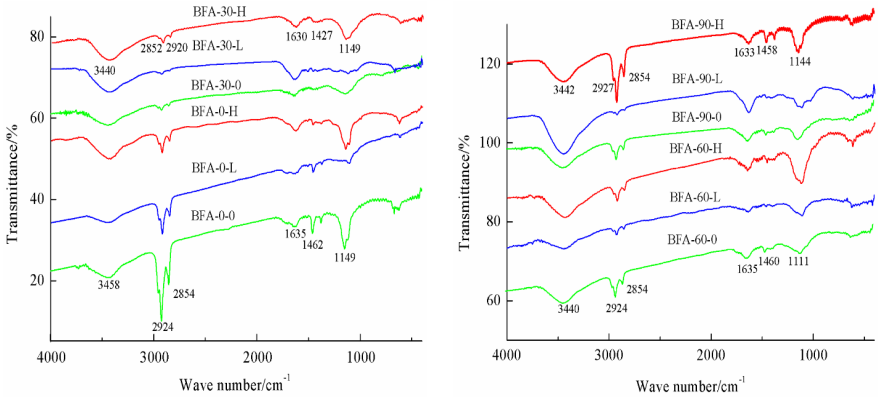
The spectra of HAs extracted from Chernozem amended with four different types of OMs are presented in Figures 1–4, respectively. The main absorbance bands and corresponding assignments obtained for the HAs are listed in Table 2, and their intensities are listed in Table 3. All the FTIR spectra showed the predominance of hydroxyl groups, aromatic compounds (mainly derived from lignin moieties) [4], and carbohydrates in the HAs extracted from Chernozem amended with these four types of OMs.

Table 2. Peak positions and corresponding assignments of humic acids (Has) extracted from Chernozem amended with four types of organic materials (Oms).

Wave Numbers (cm^{-1})	Band Assignment	References
3400–3480 cm^{-1}	H-bonded OH groups (alcohols, phenols, and organic acids) and N-H groups	Rashid et al. [18]
2920–2960 cm^{-1} and 2850–2860 cm^{-1}	Aliphatic C-H stretching of CH_3/CH_2 groups	Xin et al. [19]
1600–1655 cm^{-1}	Aromatic C=C skeletal vibrations and C=O stretching of quinine	Chen et al. [17]
1380–1470 cm^{-1}	-CH deformation of -CH_3 and -CH bending of CH_2	Niemeyer et al. [20]
1090–1170 cm^{-1}	C-O stretching of polysaccharides and polysaccharide-like substances, and/or Si-O of silicate impurities	Wu et al. [21]

Table 3. FTIR main absorption peaks' relative intensity of HAs extracted from Chernozem amended with biotechnological extract oy fulvic acid (BFA), decomposed sheep manure (M), corn straw pellets (Ps) and corn straw powder (Pr). The culture days of 0, 30, 60 and 90 were represented by −0, −30, −60 and −90, respectively. The ratio of d/(a + b + c) (from FTIR spectra) could roughly represent the ratio of aromatic carbon (C) to aliphatic C in the HAs.

	No Nitrogen (N) Application (0)				**Low N Application (L)**				**High N Application (H)**			
Wave numbers/cm^{-1}	BFA −0	BFA −30	BFA −60	BFA −90	BFA −0	BFA −30	BFA −60	BFA −90	BFA −0	BFA −30	BFA −60	BFA −90
3420–3464	40.4	40.9	36.2	39.4	40.3	42.5	38.4	39.7	36.6	38.7	37.7	44.9
2920–2927 [a]	8.2	7.7	7.7	6.5	7.8	10.2	6.9	7.1	8.1	7.9	8.2	6.9
2852–2854 [b]	3.7	2.6	4.0	3.2	3.9	3.2	2.5	2.8	3.9	3.2	2.7	3.7
1626–1653 [d]	22.3	12.7	14.8	19.7	22.2	17.1	17.2	17.3	21.3	13.7	15.9	18.7
1427–1464 [c]	6.1	10.6	11.3	11	7.1	9.2	11.0	8.2	3.6	11.3	9.9	4.2
1108–1161	19.3	25.6	26.0	20.2	18.8	17.9	24.1	24.9	26.5	25.3	25.7	21.7
Ratio of d/(a +b + c)	1.24	0.61	0.64	0.95	1.18	0.76	0.84	0.96	1.37	0.61	0.76	1.26
Wave numbers/cm^{-1}	M −0	M −30	M −60	M −90	M −0	M −30	M −60	M −90	M −0	M −30	M −60	M −90
3417–3468	39.4	42	41.8	43	48.2	38.2	42.6	42.9	34.1	40.1	41.6	42.3
2920–2929 [a]	7.1	9.4	8.0	9.1	8.4	6.9	7.1	7.8	7.7	6.3	8.6	7.1
2850–2854 [b]	3.3	4.4	3.5	4.4	4.6	3.5	3.1	3.6	3.1	3.5	4.0	3.9
1601–1653 [d]	19.4	20.4	22	16.7	18.2	16.2	17.8	21.4	18	20.1	21.6	19.3
1383–1466 [c]	9.4	3.7	3.2	12.2	6.7	16.4	3.9	2.9	11.3	7.7	7.3	5.0
1097–1157	21.4	20.1	21.6	14.6	14.0	18.8	25.6	21.5	25.8	22.3	17	22.4
Ratio of d/(a + b + c)	0.98	1.17	1.50	0.65	0.92	0.60	1.26	1.50	0.81	1.15	1.09	1.21
Wave numbers/cm^{-1}	Ps −0	Ps −30	Ps −60	Ps −90	Ps −0	Ps −30	Ps −60	Ps −90	Ps −0	Ps −30	Ps −60	Ps−90
3406–3473	43.5	40.7	43.3	43.3	40.1	51.2	39.4	49.5	50.7	41.4	38.7	49.3
2922–2960 [a]	9.4	6.9	7.8	8.4	7.6	10	6.8	8.2	10	4.9	7.9	11
2852–2857 [b]	6.3	3.3	3.3	4.2	3.2	4.3	3.4	4.7	5	3.5	3.4	3.8
1630–1653 [d]	23.2	17.3	19.9	18.4	21.2	22.9	18.5	23.5	22.6	20.1	23.5	26.2
1402–1464 [c]	4.1	6	11.8	7.4	3.6	1.1	10.4	4.5	3.4	7.7	9	5.3
1090–1169	13.6	25.7	13.9	18.4	24.4	10.5	21.4	9.5	8.3	22.3	17.5	4.5
Ratio of d/(a + b + c)	1.17	1.07	0.87	0.92	1.47	1.49	0.90	1.35	1.23	1.25	1.16	1.30
Wave numbers/cm^{-1}	Pr −0	Pr −30	Pr −60	Pr −90	Pr −0	Pr −30	Pr −60	Pr −90	Pr −0	Pr −30	Pr −60	Pr −90
3415–3463	37.1	44	52.1	42.2	37.4	37.9	40.5	42	48.6	39.3	36.3	41.1
2922–2927 [a]	7.8	8	8.6	8.4	7.3	6.6	7.4	7.5	8.1	7.5	5.6	7.1
2852–2854 [b]	4.3	3.8	3.6	3.6	3.4	3.6	4.3	3.3	4.7	3.5	3.3	4.4
1618–1653 [d]	17.4	21.6	15.4	18.3	20	19.4	23.1	13.2	24.8	21.6	20	18.9
1452–1464 [c]	2.8	4.9	4	4	6	4.4	5.8	3.8	3.7	11.5	9	7.3
1063–1163	30.7	17.6	16.2	23.4	25.8	28.1	19	30.3	10.1	16.6	25.7	21.1
Ratio of d/(a + b + c)	1.17	1.29	0.95	1.14	1.20	1.33	1.32	0.90	1.50	0.96	1.12	1.01



(**a**) (**b**)

Figure 2. FTIR spectra of HAs extracted from Chernozem amended with biotechnological extract of fulvic acid (BFA). The culture days of 0, 30, 60 and 90 were represented by −0, −30, −60 and −90, respectively. No, low and high N applications were represented by −0, −L and −H, respectively. The spectra obtained at 0 and 30 days were shown in (**a**), and the spectra obtained at 60 and 110 days were shown in (**b**).

As seen from Figure 2 and Table 3, compared with 0 d, the intensity of peak at 3420–3464 cm^{-1} of HAs from Chernozem amended with BFA combined with no or low N level was reduced at the end of culture, while the intensity of this peak treated with BFA at high N level was dramatically higher at 90 d. Oppositely, the intensity of the peak located at 1108–1161 cm^{-1} of HAs at no and low N levels were both stronger while this intensity treated by high N level was weaker, at 90 d compared to 0 d. BFA could increase carbohydrates production and feed beneficial microbes. Under the applications of no and low N, the carbohydrates of HAs were produced from the microbial degradation, and then they were accumulated due to the reduced microbial activity caused by N deficiency, while in the case of high N supply, the microbes could continue to reproduce themselves and maintain high activity, and consume the carbohydrates as substrates continuously. The higher N level was more helpful for the reproduction of microbial cells and the mineralization process of carbohydrates. Therefore, no and lower N levels were better for the accumulation of carbohydrates than high N supply.

FTIR spectra showed the presence of aliphatic (bands 2900–2860 cm^{-1}, 1460 cm^{-1}) and aromatic regions (1600 cm^{-1}), thus the ratio of peak intensity at 1600 cm^{-1} to the sum of two peak intensities at 2900–2860 cm^{-1} and 1460 cm^{-1} could roughly represent the ratio of aromatic C to aliphatic C [22]. Wu et al. [21] also found the ratio of the peak area of 1630 cm^{-1} to the peak area of 2930 cm^{-1} was positively correlated with the ratio of stable C to labile C, and this ratio was used as an indicator of SOM stability. According to the above rules, the aliphatic region from Chernozem amended with BFA included three parts: 2920–2927, 2852–2854, and 1427–1464 cm^{-1}, which are represented by a, b and c, respectively, and the aromatic area was at 1626–1653 cm^{-1}, represented by d; then the d/(a + b + c) ratio could roughly indicate the ratio of aromatic C to aliphatic C. During the culture period, the ratio of d/(a + b + c) of HAs from Chernozem amended with BFA decreased first and then increased at the three levels of N application, and finally decreased. The BFA contained the highest C content (68.9 g kg^{-1}) out of the four tested OMs. After the incorporation of BFA into Chernozem, the corresponding C/N ratio was most significantly increased, resulting in inhibited microbial activity, and undergoing extensive degradation followed by a little condensation, thereby making the HAs structure simpler. The condensation and oxidation processes of simple monomeric units into macromolecular polymers were a feature of humification, but this process was governed by nutrient abundance and length of humification [11]. It could be speculated that the HAs in Chernozem affected by N applications were first decomposed and re-synthesized, and then the newly formed HAs molecules did not condense to the initial level, and thus, the overall performance showed a decrease in aromaticity, indicating HAs tended to develop toward labile C. The newly-formed HAs could be defined as "young forms" [23]. In general, the molecular structure of HAs changed after the application of organic manure in that the number of aromatic structures decreased significantly [24]. Zhang et al. [8] also indicated that the horse manure combined with inorganic N, P, and K fertilizer increased the lengths of aliphatic chains in soil HAs. The HAs molecular structure of Chernozem amended with BFA became more aliphatic, simplistic and younger. By the end of culture, the comparisons were made among the three different levels of N application. The high level of N application was more favorable for the formation of hydroxyl groups of HAs. Compared with no and low N levels, the high N level was more conducive to the maintenance of HAs aromaticity. In addition, the N application was beneficial for the synthesis of carbohydrates, however, the effect from the high level of N application was less than the low N level. Carbohydrates were an important nutrient, the high N supply might enhance the microbial activity and utilize more carbohydrates [25].

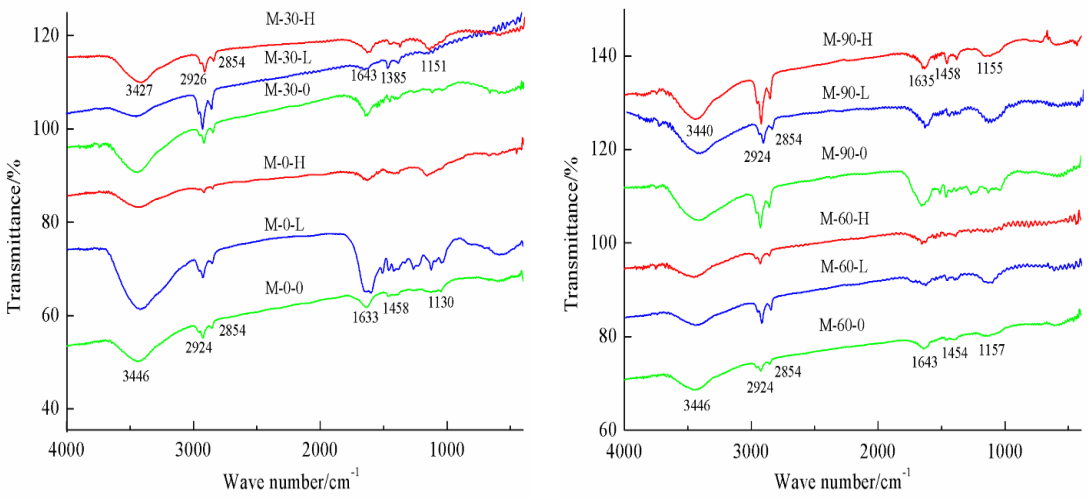
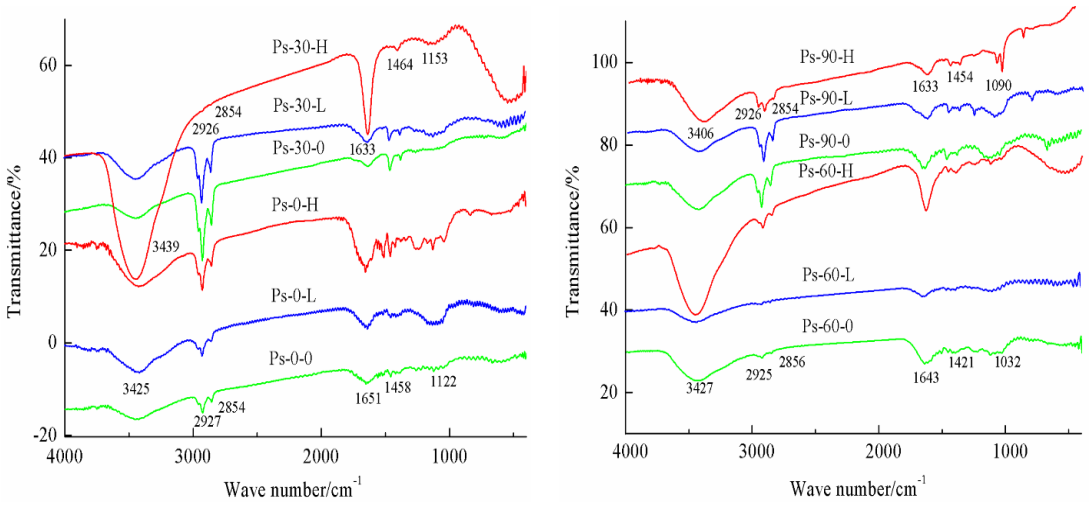


Figure 3. FTIR spectra of HAs extracted from the Chernozem amended with decomposed sheep manure (M). The culture days of 0, 30, 60 and 90 were represented by −0, −30, −60 and −90, respectively. No, low and high N applications were represented by −0, −L and −H, respectively. The spectra obtained at 0 and 30 days were shown in (**a**), and the spectra obtained at 60 and 110 days were shown in (**b**).

Figure 3 showed the FTIR spectra of HAs extracted from Chernozem amended with M. Compared with 0 d, the relative peak areas at 3417–3468 cm^{-1} of HAs were increased in the treatments of no and high levels of N application, while that of HAs was reduced by the low N level, at the end of culture. Contrary to the above trend, the intensities of the absorption band at 1097–1157 cm^{-1} of HAs treated with no and high levels of N application both decreased, while the intensity from low N level of the application was increased at the end of culture relative to 0 d. The microbes in the raw cow manure demonstrated the highest degradation capabilities for carbohydrates among the tested organic materials [26]. Hence, the concentration of carbohydrates in the HAs could be regulated by the application of exogenous N. During the culture, the d/(a + b + c) ratios of HAs from Chernozem amended with M treated by three N levels of the application showed different trends. This ratio under no N level of the application was increased firstly and then decreased. On the contrary, this ratio at a low N level experienced a decrease first and then increased. This ratio in the treatment of high N level increased despite the fluctuations. By the end of the culture, this ratio under no N level was lowered, while the ratios at low and high N levels were enhanced, reflecting the ongoing humification process. In this study, the fully decomposed sheep manure was used, which was equivalent to the bio-fertilizer. Dębska et al. [27] pointed out that the use of a bio-fertilizer that increased the formation of permanent humus compounds provided evidence of an increase in SOM stability. Furthermore, the added N hurt the richness of soil bacteria, which likely reduced SOC mineralization and increased soil C sequestration [28]. Long-term nutrient enrichment might have shifted the mechanisms of C stabilization from physical protection to chemical stabilization via shifting microbial community composition [29]. When M was used as the soil amendment, the application of N was more beneficial for the increase of aromatic C proportion in HAs in Chernozem. However, aromatic C of HAs treated with high N level was lower than that at the low N level. Besides, the low N level was more conducive to the production of carbohydrates. Microbes selectively degraded the less recalcitrant compounds and thus gradually increased the average recalcitrance of the non-respired C, the N supply was easier to promote this trend, but the high N supply more or less promoted the microbial degradation of recalcitrant compounds [30].



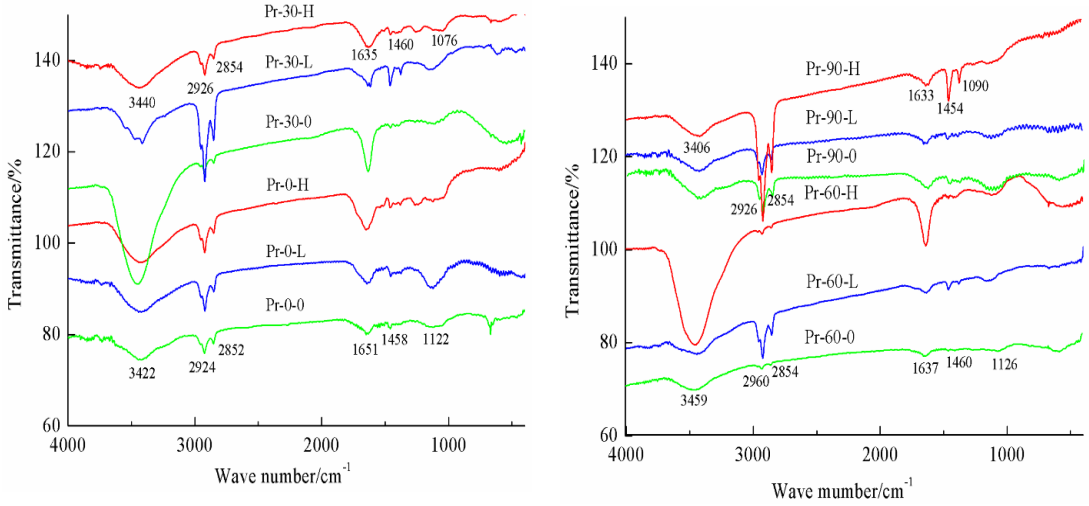
(**a**) (**b**)

Figure 4. FTIR spectra of HAs extracted from the Chernozem amended with corn straw pellets (Ps). The culture days of 0, 30, 60 and 90 were represented by −0, −30, −60 and −90, respectively. No, low and high N applications were represented by −0, −L and −H, respectively. The spectra obtained at 0 and 30 days were shown in (**a**), and the spectra obtained at 60 and 110 days were shown in (**b**).

It was shown in Figure 4 and Table 3 that the intensity of absorption band at 3406–3473 cm^{-1} of HAs from Chernozem amended with Ps was increased under the low N level of application, but the opposite result was obtained under the high N level. There was no significant change in the intensity of this absorption band under no N level of application. Compared to 0 d, the intensity of absorption band at 1090–1169 cm^{-1} of HAs under the no N level of the application was enhanced, while the intensities of this absorption band under low and high N levels of the application were both reduced, by the end of culture. The ratio of d/(a + b + c) at the no N level was first decreased and then increased, while this ratio under low and high N levels of applications underwent an increased, decreased and re-increased process; finally, the ratios with no and low N levels of application decreased, while this ratio from high N level increased. A small amount of N supply could only support the partial microbial degradation of Ps; in this process; the aromatic C content of HAs was reduced. Microbial degradation of HAs was an important part of humus turnover [31]. However, a higher amount of N supply could better support the microbial cell reproduction, release of enzymes, and re-condensation of organic molecules from Ps during the later stage of culture. Therefore, the stable aromatic groups in HAs were increased under the high N level of application. By the end of culture, although both low and high N levels favored the formation of aromatic C in the HAs relative to no N, aromatic C content of HAs treated with a high N level was lower than that of the low N level. The application of N was more helpful for the consumption of carbohydrates than that of the no N application. Corn straw provided a source of energy and nutrients for microbial growth and it was then converted into SOM. In this process, the microbes metabolized the corn straw and generated the degradable products, which were incorporated into soil mineral fractions, and thereby became a part of stable SOM pool. With the application of N, the humification process in Chernozem amended with Ps was more likely to occur.

It was shown in Figure 5 and Table 3 that the intensity of absorption band at 3415–3463 cm^{-1} of HAs from Chernozem amended with Pr was improved under no and low N levels of application but the intensity of this absorption band of HAs under the high N level of application was reduced at the end of culture compared with 0 d. Compared to 0 d, the intensity of absorption band at 1063–1163 cm^{-1} of HAs at no N level was reduced, while those of low and high N levels were both enhanced at the end of the culture. Hsu et al. [32] utilized the FTIR technique to examine the organic matter transformations during composting of pig manure and pointed out that there was a decrease in carbohydrates of HAs

as decomposition proceeded, which was slightly different from the result of Chernozem amended with Pr under the application of N in this study. The absorbance in the range of 1000–1100 cm^{-1} might partly arise from C–O vibration, but it could also originate from Si–O vibration [4]. The explanation for this phenomenon was that the Si–O group produced from the corn straw degradation, and then entered into the HAs, overlapping and disturbing the intensity of peak at 1063–1163 cm^{-1}. Therefore, an increase in the intensity of this peak did not necessarily indicate an increase in the content of carbohydrates in the HAs.



(**a**) (**b**)

Figure 5. FTIR spectra of HAs extracted from the Chernozem amended with corn straw powder (Pr). The culture days of 0, 30, 60 and 90 were represented by −0, −30, −60 and −90, respectively. No, low and high N applications were represented by −0, −L and −H, respectively. The spectra obtained at 0 and 30 days were shown in (**a**), and the spectra obtained at 60 and 110 days were shown in (**b**).

The ratios of d/(a + b + c) of HAs from Chernozem amended with Pr under no and low N levels increased during the culture of the first 30 d, and then they experienced a series of fluctuations and eventually decreased, while the ratio under high N level got a sharp decrease in the short term, and the trend of decrease continued until the end of culture. Ultimately, the ratios under different N levels were all reduced by the end of the culture compared to 0 d. Referring to the degree of degradation of HAs, the following ranks existed: High N level > low N level > no N level. After BFA, the Pr contained higher C content (59.8 g kg^{-1}) than M and Ps (26.1 and 43.1 g kg^{-1}). Therefore, the higher C/N ratio of Chernozem amended with Pr might inhibit microbial activity; in the process less N could only promote early degradation, but could not support the subsequent condensation, thus making the HAs hold a less resistant structure [33]. The lack of exogenous N supply was difficult to satisfy fast microbial growth, which made mineralization inhibited. The microbial biomass pool needed to proliferate quickly over time, and hence N addition was required to maximize microbial activities [34]. Therefore sufficient N supply could better support the C mineralization of corn straw in Chernozem. Compared with no N supply, low N application was beneficial for the decomposition of aromatic C and the formation of carbohydrates in HAs, which had a greater degree of degradation than that under high N supply. High N supply tended to promote the consumption of carbohydrates relative to no and low N supplies [25].

A broad hump around 3300–3400 cm^{-1} was the characteristic of hydrogen-bonded O-H and methylene stretching existed at 2920, 2850 cm^{-1}, which suggested the presence of organic matter [35]. The BFA was a concentrated bio-stimulant derived from leonardite, and the M was a fully decomposed organic material. The corn straw powder was obtained by mechanical crushing of corn straw, while the corn straw pellets were obtained from the mechanical compaction of corn straw, which were not

subjected to any chemical or biological process and were undecomposed natural OMs. The BFA and M themselves had a certain content of organic acids; their incorporations in Chernozem under the high N supply were more beneficial for the improvement of H-bonded OH groups of HAs than the incorporations of the other OMs. Moreover, high N application could have also promoted the production of N–H groups in HAs. For Ps and Pr, the high N supply could have promoted the mineralization of corn straw; in this process the decrease of hydrophilic groups was caused by the loss of organic acids. Admittedly the high N supply was more beneficial for the formation of N–H groups, but it still could not compensate for the loss of H-bonded OH groups, and finally it reduced the intensity of this peak. Another explanation was that high N supply drove microbes to break H bonds on the corn straw surface, and in this way, more cellulose was exposed to the surface because of the broken H bonds and this destroyed the surface of the corn straw [36].

4. Conclusions

It was feasible to evaluate the effects of different types of OMs on Chernozem fertility and health according to the spectral characteristics of HAs using the FTIR spectroscopy technique. Different types of OMs showed different effects on Chernozem. When Chernozem was amended with BFA or M, the H-bonded OH groups of HAs were reduced at a low N level, but were enhanced with a high N supply. The influences of Ps or Pr on the H-bonded OH groups of HAs were exactly the opposite; BFA and Pr held a higher content of organic C (68.9 and 59.8 g kg^{-1}); thus after Chernozem was amended with BFA or Pr, the aromatic compounds in HAs decreased regardless of the N level. The proportions of aromatic compounds in HAs from Chernozem amended with M or Ps were enhanced by a high N supply but were reduced with no N supply. The high N supply significantly promoted the consumption of carbohydrates in HAs from Chernozem amended with BFA, M or Ps. It was not clear whether the increase in the peak intensity at 1063–1163 cm^{-1} of HAs treated by Pr was caused by high N application or the interference of Si-O bond. Considering comprehensively, the combined application of high N with M was the best way to improve the fertility and health of Chernozem.

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