

Role of mineral matrix composition and properties in the transformation of corn residues

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Abstract

The influence of the composition and properties of the mineral matrix in soils on the humification of corn residues was studied. The substrate (silica sand, loam, silica sand + 10% bentonite, or silica sand + 30% kaolinite) was mixed with 10% corn residues (milled to 3–5 mm) and incubated under stationary conditions for 6–19 months. Sampling for the analysis was performed every month, and a few times in the first month. The dynamics of mineralization and humification of plant residues was studied by applying elemental and bulk analyses of neogenic organic matter (OM), densitometric fractionation of substrates, FTIR spectroscopy, solid-phase ¹³C-NMR spectroscopy, and scanning electron microscopy with an electron microprobe. It was shown that the humification processes had a wavelike character for loam and sand substrates, which could be explained by the transformation of the microorganism populations together with the change in the amount and quality of OM in the system. The main mechanism for the stabilization of neogenic OM was adsorption on a mineral matrix with formation of relatively resistant compounds. This adsorption can be selective, depending on the composition and properties of the mineral matrix. The FTIR and ¹³C-NMR analyses of OM distribution in different substrates and densitometric fractions showed that sand and heavy fractions (HF >2.2 g/cm³) were enriched with compounds of an aromatic nature and polypeptides. Light fractions (LF-2, 1.4–2.2 g/cm³) accumulated compounds that also contained alkyl and carboxyl groups. The sandy substrate and HF have higher aromaticity indices than LF-2. Higher aromaticity index values of humus substances in the sandy substrate and HF in the loamy substrate, compared to LF-2, evidenced the formation of steady aromatic compounds, in which there may be kernels of humic acids (HA). We do not exclude the possibility of the matrix synthesis of the HA-like substances.

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Introduction

The transformation of plant residues (PR) in soils proceeds slowly, owing to two opposing processes: mineralization and humification of organic matter (OM). These are the key processes in the carbon cycle of land ecosystems. There are different opinions on the mechanisms involved in humification, but the most widely held are the condensation and polymerization hypotheses, as well as the hypothesis of oxidative acid formation. The condensation and polymerization hypotheses are based on the concept of decomposition of the plant tissues and the products of microbial metabolism, forming monomers, followed by the synthesis of humic acids (HA) from these monomers through condensation and polymerization (Flaig, 1971; Kononova, 1963). The hypothesis of oxidative acid formation (Aleksandrova, 1980; Schnitzer, 1977) involves three stages: (1) neof ormation of HA through enzymatic oxidation of high-molecular-weight compounds from PR, (2) transformation of newly formed HA through the acquisition of the most typical HA features (formation of

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“mature” HA) with simultaneous hydrolytic splitting and oxidation of fulvic acids (FA), and (3) very slow “inevitable” decomposition of HA until complete mineralization is achieved. Thus, HA represent a peculiar intermediate transformation stage of organic residues during mineralization. These hypotheses are recognized in Russia and abroad; they underpin current concepts for the transformation pathways and mechanisms of nonspecific organic compounds to humic substances. Orlov summarized these studies and showed that both humification pathways probably occur in soils, and the degree of their involvement in OM transformation depends on the pedogenesis conditions (Orlov, 1990). Travnikova et al. (2002) believe that the condensation of PR decomposition products to HA occurs through the formation of mineral–organic compounds. The thus formed HA are similar in composition and molecular weight to HA and FA bound to the clay fraction of soils. These studies imply direct participation of the clay minerals in humus formation.

Finely dispersed mineral components, predominantly clay minerals, play an extremely important role in humus formation and the conservation of humic substances in soils; they ensure the physical and chemical mechanisms for the protection of humic substances from biodegradation. This is due to the decrease in the availability of the organic substances that are strongly bound to active sites on the surface of the soil for microorganisms (Mikutta et al., 2006; Van Lutzow, et al., 2006). Recent theoretical and experimental studies have confirmed the matrix (catalytic) function of clay minerals in the synthesis of humic substances (Birkel et al. 2002; Kaiser and Guggenberger, 2003; Lehmann et al. 2007; Pinskiy and Kurochkina, 2006). Zavarzina (2006) revealed a direct effect of the mineral matrix on the enzymatic synthesis of humus-like substances through simple decomposition products of PR. The consideration of soil humus as a complex of mineral–organic compounds is increasingly attracting the attention of researchers. The aim of this work was to study the effect of mineral substrates with different compositions on the humification of corn PR under laboratory conditions.

Material and Methods

Model experiments were performed with corn, the aboveground mass of which was preliminarily dried and milled to particle sizes of to 3–5 mm. Pure quartz sand, carbonate-free cover loam (soil-forming rocks of gray forest soil from the Experimental Field Station of our Institute), silica sand + 10% bentonite, and silica sand + 30% of kaolinite were used as the mineral substrates. The sand was preliminarily washed with a 10% HCl solution to a negative reaction for Fe^{3+} , PO_4^{3-} , Ca^{2+} , and SO_4^{2-} and it was then washed with distilled water to remove the residual Cl^- ions. The mineralogical composition of the loam included quartz (59%), kaolinite (16%), mica (13%), feldspars (11%), and smectite (2%). The sand and loam were thoroughly mixed with the reduced aboveground corn mass at a ratio of 1:10, placed in 200 cm³ glass vessels, and wetted to 60% of the maximum water capacity. The substrates were inoculated with soil microorganisms by adding a 1 mL soil suspension containing 0.01 g gray forest soil into each vessel. The incubation was performed in a biological thermostat at 20°C. The duration of the experiment was 19 months for the sand and loam substrates and 6 months for the bentonite and kaolinite substrates. Samples were taken after 0.2, 0.5, 0.7, 1, 3, 6, 9.5, and 19 months. The experiments were performed in triplicate. The humic substances (HS) were extracted with a 0.1 M $\text{Na}_4\text{P}_2\text{O}_7$ solution with pH value of about 10 (Aleksandrova, 1970). The organic carbon content (C_{org}) in the total-extract, HA, and FA solutions was determined using bichromate and More salt (the Tyurin method).

The HA preparations were isolated using a twofold extraction with 0.1 N NaOH solution followed by precipitation of the HA with 6 M HCl solution. The preparations were purified by re-precipitation after re-dissolution in an alkaline solution with the addition of KCl and the treatment of the solution with a mixture of 0.1 M HCl and 0.3 M HF (Kholodov et al. 2009). The redistribution of the newly formed humic substances throughout the mineral substrates, as well as their stabilization, was studied by densitometric fractionation using a solution of sodium polytungstate ($\text{Na}_6\text{O}_{39}\text{W}_{12}\cdot\text{H}_2\text{O}$) as the heavy liquid (Magid et al. 1996; Turchenek and Oades, 1979). The ratio between the studied sample and the $\text{Na}_6\text{O}_{39}\text{W}_{12}\cdot\text{H}_2\text{O}$ solution was 1:1. Three densitometric fractions —two light fractions with densities <1.4 (LF-1) and 1.4–2.2 g/cm³ (LF-2) and a heavy fraction with a density of >2.2 g/cm³ (HF)—were successively separated from the samples after different incubation times. For this purpose, ultrasonic treatment (50 W, 15 min), centrifugation, membrane ultrafiltration, and specially prepared $\text{Na}_6\text{O}_{39}\text{W}_{12}\cdot\text{H}_2\text{O}$ solutions of different densities were used. The elemental composition of the OM (C, N) in the samples of the incubated material and the densitometric fractions was determined using an Elementar Vario EL III CHNS analyzer. The chemical structure of the OM was studied with ¹³C-NMR spectroscopy and Fourier-transform infrared spectroscopy (FTIR; Thermo Electron FTIR Nicolet 5700 spectrometer), using the KBr technique at a sample/salt ratio of 1:200 and in the wavelength range 4000–400 cm⁻¹. The obtained spectra were treated using OMNIC 8.1 software.

Results and Discussion

Humification of corn residues in the tested mineral substrates

The dynamics of the humic substances formed during the decomposition of corn PRs in sand, loam, kaolinite, and bentonite-containing substrates are presented in Figure 1a. The undulating character of humification is typical for sand and loam substrates. Maximum amounts of newly formed HS were noted after humification for 1 and 9 months when using these substrates. The wavy character of the humification is more clearly demonstrated by the dynamics of the C/N ratio of the humic substances (Figure 1b). The minimum values of this ratio were observed for both systems after 3 months of incubation. A decrease in the humification rate, which was most significant in the sandy substrates, was simultaneously observed. During the next stages of humification, HA were always dominant in the humic substances of these systems.

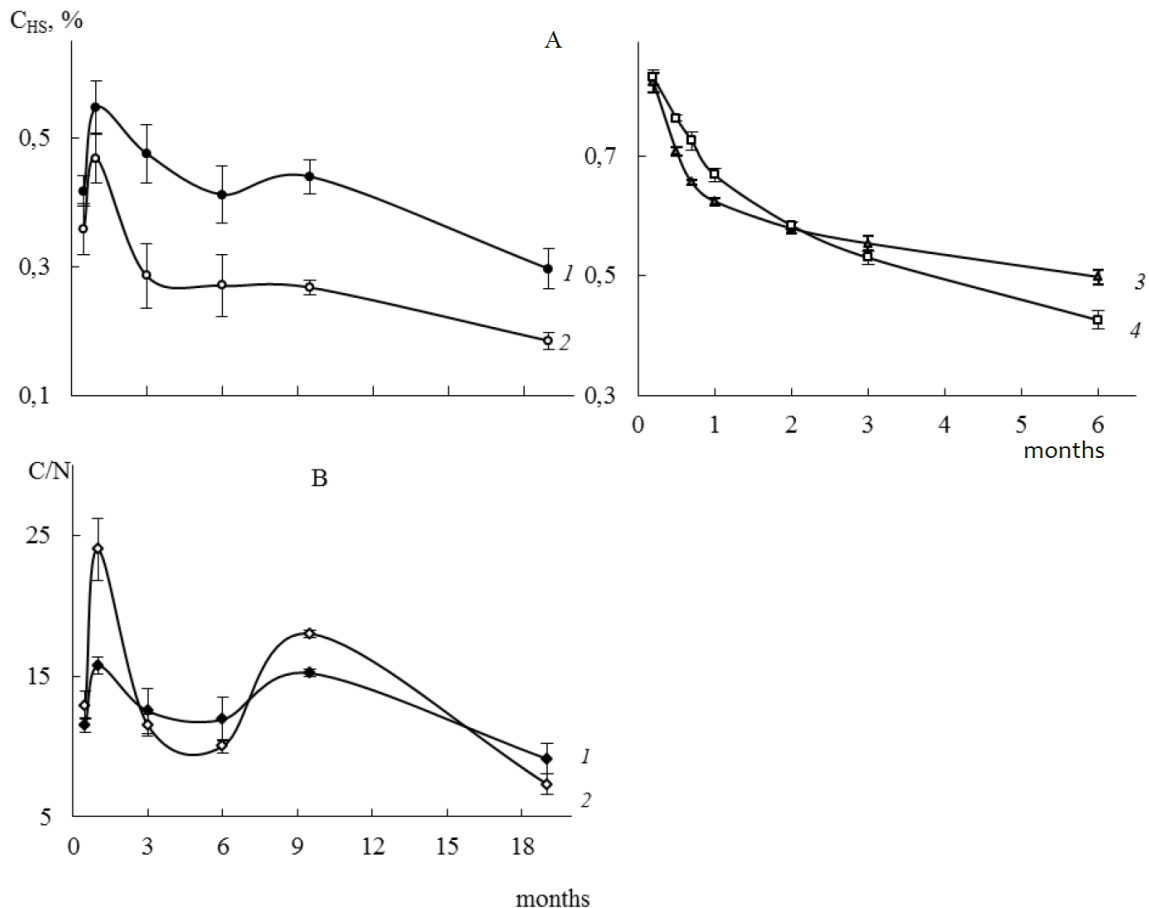


Figure 1. Changes in the content of humic substances (a) in: loam (1); sand (2); sand + 30% kaolinite (3); sand + 15% bentonite (4) and their C/N ratio (b) in loam (1) and sand (2) during the transformation of corn plant residues.

According to [Kononova \(1963\)](#), one month is sufficient to complete the humification of PR for the aboveground mass of most plant species. During the period from 3 to 9.5 months, humification proceeded in an almost stationary manner, with a gradual decrease in the content of humic substances (C_{HS}) to 5.6%. After 19 months, the concentration of HSs in the sand was 3.7%. This can be related to not only the reduced rate of humification, but also to the mineralization of newly formed humic substances. The composition of the humic substances is presented in Table 1. After 6 months, the content of HS in the loam substrate was higher than in the sandy substrate by 2.1%. After 9.5 months, a secondary increase in the content of HS to 8.8% of the initial concentration C_0 was observed. Then, a gradual decrease in the content of the HS was noted until 19 months incubation was reached. Thus, undulating dynamics of the HS were revealed in the loamy substrate. [Aleksandrova \(1980\)](#) noted that the content of nitrogen in the HA is not stable during humification. The undulating humification of clover roots in loam and sand can be noted in the experiments of [Lyuzhin \(1968\)](#). This type of humification can be explained by “the undulating development of the

microbial population" (Semenov et al., 2011). The increase in the nitrogen content towards the end of the incubation period is a common feature in our experiments in the transformation of nitrogen-containing moieties of the humic substances. The lower C_{HS} values in the sandy system and the large dispersion of these values sometimes mask the undulating character of humification, but the dynamics of the C/N ratio clearly reveal it.

Table 1. Group composition of organic matter after incubation of plant residues of corn in different mineral substrates (% of C_0)

Incubation time, months	0.2	0.5	0.7	1	2	3	6	9.5	19
Substrate	Sand								
HA	–	4.1	–	6.0	–	2.9	3.5	3.7	2.7
FA	–	3.3	–	3.7	–	3.1	2.3	1.9	1.0
HA/FA	–	1.2	–	1.6		0.9	1.5	1.9	2.7
Substrate	Loam								
HA	–	4.2	–	5.7	–	4.3	4.3	5.1	3.4
FA	–	3.6	–	4.7	–	4.7	3.4	3.2	2.1
HA/FA	–	1.2	–	1.2	–	0.9	1.3	1.6	1.6
Substrate	Sand + 15% Bentonite								
HA	9.0	8.7	8.5	8.1	7.8	7.5	6.9	–	–
FA	12.2	10.8	10.1	9.0	7.2	6.0	3.9	–	–
HA/FA	0.7	0.8	0.8	0.9	1.1	1.2	1.8	–	–
Substrate	Sand + 30% Kaolinite								
HA	8.3	7.8	7.9	8.1	8.4	8.4	7.8	–	–
FA	12.8	10.3	8.9	7.9	6.4	5.7	4.9	–	–
HA/FA	0.6	0.8	0.9	1.0	1.3	1.5	1.6	–	–

For the substrates with bentonite and kaolinite, the amounts of newly formed HS gradually decrease until the end of the experiment (6 months). In total, the amount of humic substances is higher in substrates with kaolinite and bentonite than in loam and sand substrates.

The dynamics of the HS in the kaolinite- and bentonite-containing substrates are similar in character. In the first stage, the rate of humification in the kaolinite-containing substrate was higher than in the bentonite-containing one. At the end of experiment (6 months), the content of HS in the kaolinite-containing substrate was also higher than in the bentonite-containing substrate. This can be explained by the stronger adsorption of HA and FA by kaolinite compared with bentonite, loam, and sand. The absence of undulating character of PR humification in the bentonite- and kaolinite-containing substrates may be connected with two factors: 1) the rapid formation of mineral–organic compounds diminishes the possibility of further microbiological transformation of HS and 2) the insufficient duration of the experiments (only 6 months). In the described conditions, the humification coefficient of PR of C_{HS} reached 2.5 in sand, 3.6 in loam, 5.5 in bentonite, and 6.5% in kaolinite per year.

The higher accumulation of HS in the loam and, especially, the bentonite and kaolinite substrates compared to sand is attributed to the following factors. 1) The adsorption of HSs on the surface of dispersed mineral particles forms stable adsorption complexes that are poorly accessible for further mineralization. 2) The presence of minerals with high Al and Fe hydroxides contents in the substrate ensure the positive charge of a significant portion of the finely dispersed particles and, hence, there is stronger adsorption of carboxyl-containing organic molecules (Kurochkina and Pinskiy, 2003, 2004). 3) Aeration of the more finely dispersed substrate is hindered, because of its composition (especially in bentonite- and kaolinite-containing substrates). The sand substrate has the opposite properties and, hence, creates more favorable conditions for the biomineralization of PRs and newly formed humic substances.

The dynamics of the HA in the incubated systems mimic the dynamics of the humic substances, that is, they have undulating character for loam and sand, with a tendency to gradually decrease in content during the experiment for the bentonite- and kaolinite-containing substrates. FA are the most active with a higher content of oxygen-containing functional groups and more accessibility for microorganisms. For this reason, the C_{HA}/C_{FA} ratio after one month of humification, as a rule, remained greater than 1. For bentonite- and kaolinite-containing substrates, the C_{HA}/C_{FA} ratio was less than 1 for the first month of incubation, owing to the stronger stabilization of HS on the clay minerals (Table 1).

Stabilization of humic substances

Evidently, the adsorption of OM by mineral soil components, with the formation of stable surface mineral-organic complexes, plays the deciding role in the stabilization of the soil HS, because this is a quick process (Chenu and Plante, 2006; Kaiser and Guggenberger, 2003; Lehmann et al. 2007; Mikutta et al., 2006). The content of newly formed HS increases in the order sandy < loam << bentonite \approx kaolinite throughout the incubation. This is related to their accessibility for microorganisms. The more intense formation and stabilization of aromatic compounds and decomposition products at different degrees of oxidation occur on the loamy, bentonite, and kaolinite substrates, which correlates with specific surface composition and properties of solid and liquid phases.

To study the stabilization of HS by different densitometric fractions of substrates, two light fractions (LF-1 and LF-2) and a heavy fraction (HF) were separated from the loam; the LF-1 and HF fractions were separated from the sandy systems (Table 2). According to some authors, the light fractions include postmortem residues of plant, animal, and microorganisms, as well as some humic substances at the early stages of humification (Vanyushina and Travnikova, 2003).

Table 2. Content and elemental composition HS in the densitometric fractions

Fraction	Incubation months	time, % ω	C	N	C/N
corn – loam					
LF-1	1	7.4	31.92	0.49	64.91
	3	4.2	35.17	0.58	61.01
	6	3.1	38.93	0.87	44.86
	9.5	2	33.12	0.96	34.34
	19	1.7	36.41	1.07	34.04
LF-2	1	4.6	6.09	0.46	13.12
	3	4.2	6.56	0.49	13.30
	6	3.3	10.26	0.61	16.76
	9.5	3.4	8.79	0.57	15.30
	19	5.9	8.37	0.47	17.84
HF	1	88.0	0.30	0.07	4.54
	3	91.6	0.42	0.06	6.66
	6	93.6	0.41	0.06	6.42
	9.5	94.6	0.40	0.06	6.60
	19	92.3	0.47	0.05	8.47
corn – sand					
LF-1	1	7.5	35.63	0.55	64.48
	3	5.0	29.29	0.81	36.03
	6	3.8	26.66	0.71	37.44
	9.5	3.1	23.66	0.85	27.90
	19	2.5	11.87	0.64	18.44
HF	1	92.5	0.20	0.08	2.49
	3	95.0	0.21	0.02	12.11
	6	96.2	0.31	0.02	12.62
	9.5	96.9	0.49	0.09	5.65
	19	97.5	–	0.04	–

The densitometric fractionation of the loam substrate demonstrated that the mass portion (ω) of LF-1 decreased during the humification of PR from 7.4% in the first month of incubation to 1.7% at the end of the experiment. The relative content of carbon in the LF-1 of the loamy substrate, which remained constant throughout the entire process, indicated that this fraction consists of plant biomass. The increase in the nitrogen content in LF-1 with increasing incubation time can be attributed to the increase in microbial biomass in the plant material, as well as the condensation of nitrogen-containing decomposition products by carbon-nitrogen bonds (Bambalov et al. 1990).

The decrease in the amount of LF-2 in the loamy substrate almost doubled compared to the minimum values at the end of the experiment, which could be related to the fixation of the humic substances formed on the surface of the fine silt and clay particles, which form the major part of LF-2. The carbon content in the LF-2

of the loamy substrate gradually increased from 6 to 8%, with increasing incubation time. The nitrogen content did not change significantly. This fraction was almost absent in the sandy substrates, because of the relative monodispersity of the phase.

A higher content of adsorbed carbon and nitrogen was observed in the loam HF than in the sand HF, which is related to the heterogeneous mineralogy of the loam HF. The adsorption of the cationic forms of organic compounds is especially typical for silicon–oxygen-containing surfaces, which have an overall negative charge under the usual pedogenesis conditions (Pinskiy, 1997). Nitrogen-containing compounds (amines, amides, pyrroles, etc.) are susceptible to strong adsorption by the minerals present in this fraction (Sollins et al., 2006, 2009). The C_{org} values and the weight fractions indicate that the LF-1 carbon in the loamy substrate is appreciably reduced compared to the results of decomposition of PR, whereas the LF-2 and HF carbon pools increased to 30 and 25%, respectively, after 19 months of incubation. In the loamy substrate, 12% of the carbon added to the corn PR remained in the LF-1 for the entire experiment; 8.5% of the carbon was found in the HF, bound to the present minerals, and 9.7% of the carbon accumulated in the LF-2. The dynamics of the LF-2 content correlated with the content of HA. Notably, the sum of the carbon portions in the densitometric fractions at the end of the experiments almost coincided with the relative content of residual carbon in the incubated systems. The difference between the values was 7.8% for the loamy substrate and 11% for the sandy substrate, which corresponded to the errors of the determination in similar experiments.

The elemental composition of LF-1 in the sandy substrate is non-uniform, because the decomposition of the PR in sand is rapid; therefore, the content of carbon decreases and nitrogen-containing products are immobilized on the negatively charged surface of the quartz. The relative content of elements in LF-1 after 19 months of incubation is similar to the composition of LF-2 in the loamy substrate at the end of the incubation. The content of LF-1 in the loamy substrate is closely related to the dynamics of humification. The first LF-1 maximum was noted during 1–3 months of incubation. The period from 6 to 9 months was characterized by the minimum content of the fraction. This period was characterized by a significant decrease in the formation rate of HA.

The mineral composition of LF-2 mainly included the clay aluminosilicates kaolinite, mica, and smectite, as well as primary minerals in HF (Sollins et al., 2006). Quartz prevails among the mineral components of the HFs from both the loamy and sandy substrates. The fixation of HS on the different mineral matrices occurs with direct participation of carboxyl- and nitrogen-containing groups in the protein compounds. The carboxyl-containing OM components interact with positively charged surfaces (e.g., kaolinite) or active sites on the surface of quartz (angles, facets, dislocations); nitrogen-containing groups bearing positive charges interact with the negatively charged surfaces of quartz, which are present in both fractions.

The formation of mineral–organic complexes in LF-2 is mainly caused by the presence of finely dispersed clay minerals. The higher content of HS in bentonite and kaolinite substrates support this point of view. The sufficiently high concentration of carbon in LF-2, and the smaller portion of this fraction compared to that of the HF, indicates not only the formation of surface-adsorbed mineral–organic complexes, but also the coagulation of heteropolar complex salts of humic substances that are not bound to the mineral matrix by strong chemical bonds. The formation of mineral–organic compounds in this fraction is likely to involve carboxyl groups interacting with the surface of secondary minerals.

Structural elements of OM in the substrates according to FTIR and ^{13}C -NMR spectroscopic data

The FTIR spectra of two densitometric fractions of loam (LF-2 and HF), bentonite and kaolinite substrates, in which the OM adsorbed on the mineral surfaces is characterized by different degrees of biological protection, are given in Figure 2. Two peak maxima at 2920–2935 and 2850–2856 cm^{-1} (stretching vibrations of aliphatic CH_2 groups) are observed. It should be noted that the OM of LF-2 is characterized by a significant content of terminal methyl groups, which are manifested in the spectra as an absorption band of significant intensity at about 2875 cm^{-1} . More clearly, this peak is expressed in bentonite and kaolinite substrates (Bambalov et al. 1990; Orlov and Osipova, 1988).

The absorption band in the 1685–1665 cm^{-1} range corresponds to the amide or quinone groups. The highest intensity of this peak is seen in the kaolinite substrate. For other samples, this band is expressed as a shoulder. The absorption bands in the interval 1640–1620 cm^{-1} , corresponding to the stretching vibrations of C=C (aromatic), C=O, and COO^- groups, is broadened for bentonite, HF, and particularly for LF-2, probably

because of the overlapping of the amide I band. The shoulder at approximately 1725 cm^{-1} is likely to belong the COOH groups and appears as a result of the oxidative decomposition of OM in LF-2 and formation of mineral-organic compounds of lower stability (Dick et al. 2003). For the HF in the bentonite and kaolinite spectra, the absorption at $1730\text{--}1725\text{ cm}^{-1}$ is not observed, which indicates the strong coordination of the humic substances with the mineral matrix through COOH groups.

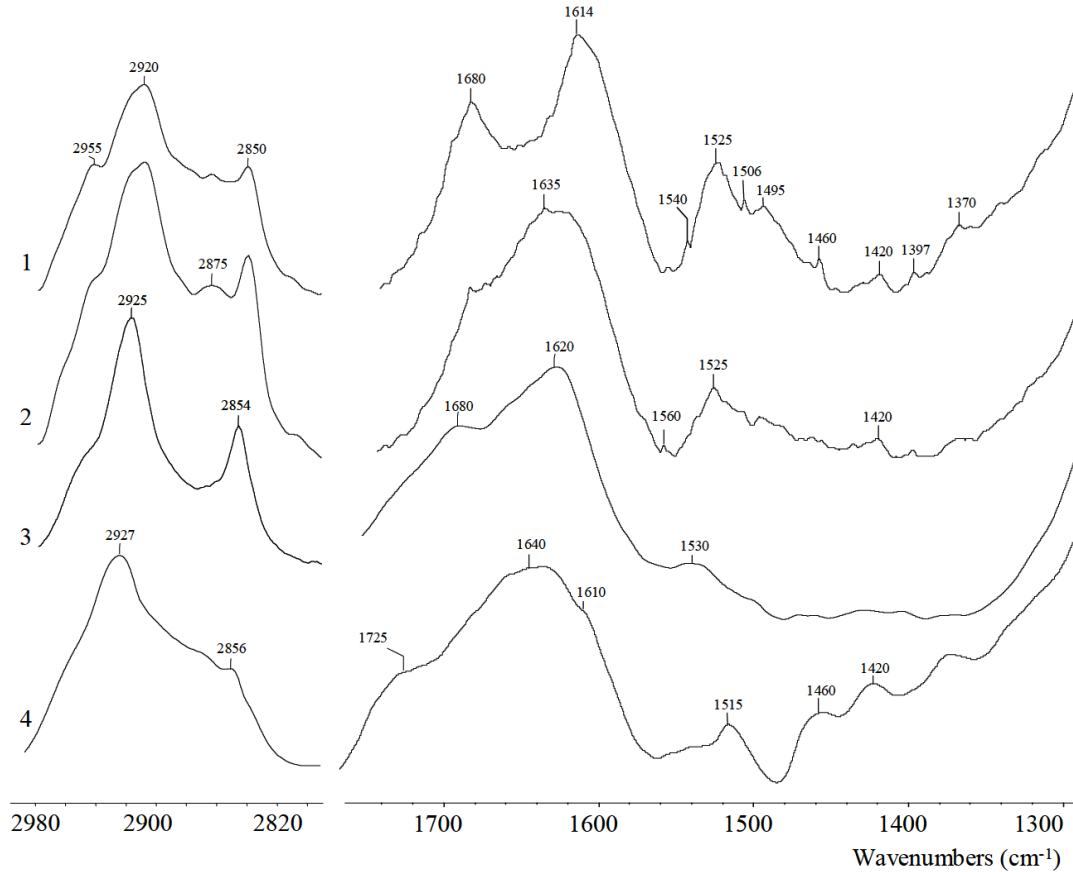


Figure 2. FTIR spectra of a organo-mineral substrates and densitometric fractions: sand + 30% kaolinite (1), sand + 15% bentonite (2), $> 2.2\text{ g/cm}^3$ (3), $1.4\text{--}2.2\text{ g/cm}^3$ (4)

The absorption band in the $1685\text{--}1665\text{ cm}^{-1}$ range corresponds to the amide or quinone groups. The highest intensity of this peak is seen in the kaolinite substrate. For other samples, this band is expressed as a shoulder. The absorption bands in the interval $1640\text{--}1620\text{ cm}^{-1}$, corresponding to the stretching vibrations of C=C (aromatic), C=O, and COO^- groups, is broadened for bentonite, HF, and particularly for LF-2, probably because of the overlapping of the amide I band. The shoulder at approximately 1725 cm^{-1} is likely to belong the COOH groups and appears as a result of the oxidative decomposition of OM in LF-2 and formation of mineral-organic compounds of lower stability (Dick et al. 2003). For the HF in the bentonite and kaolinite spectra, the absorption at $1730\text{--}1725\text{ cm}^{-1}$ is not observed, which indicates the strong coordination of the humic substances with the mineral matrix through COOH groups.

The peaks at approximately 1560 and 1540 cm^{-1} , corresponding to the bending vibrations of the amide II band, occur in the bentonite and kaolinite substrates; peaks at 1530 and 1525 cm^{-1} occur in the loam (HF), sand (HF), bentonite, and kaolinite substrates and correspond to amide II band N-H bending vibration, asymmetric stretching of COO^- , and aromatic C=C groups stretching vibrations. The peaks at 1515 and 1506 cm^{-1} indicate the presence of lignin-containing aromatic components, which can directly pass into these substrates and densitometric fraction from the original plant material. After 6 months of incubation, peaks at 1460 , 1420 , and 1370 cm^{-1} began to appear, and a peak at 1515 cm^{-1} , corresponding to the stretching vibrations of aromatic C=C bonds, became more distinct; the shoulder at 1540 cm^{-1} (amide II) remained the

same. Consequently, the contents of both aromatic and aliphatic components in the newly formed humic substances of the light fractions increased with the decomposition of the PR.

The presence of absorption bands at 2875 cm⁻¹ (CH₃ groups) and 1460–1370 cm⁻¹ (aliphatic components), as well as a shoulder at about 1725 cm⁻¹ (free carboxyl groups) indicates a significant content of FA in LF-2, which are best protected from mineralization in the loamy, bentonite, and kaolinite substrates. In the opinion of Aleksandrova (1980), the fractionation of the formed humic substances occurs upon interaction with the mineral phase.

The analysis of the solid-state ¹³C-NMR spectra of bentonite-containing (1) and kaolinite-containing (2) substrates, LF-2 (3), and HF (4) of loam are presented in Figure 3. The diagram shows that most of the OM is represented by carbohydrates (polysaccharides, cellulose, etc.), whose content increased from 50 to 67% in the order bentonite < kaolinite < HF < LF-2. The content of aromatic components (lignin-containing aromatic components from the original plant material and newly formed HSs) varied in the reverse order from 13 to 18%. The content of alkyl groups gradually increased from 12 to 27% in the reverse to the order stated above for carbohydrates, but the carboxyl groups decreased from 10 to 4% in the order kaolinite < bentonite < LF-2 < HF. The concentration of compounds containing C=O groups (amides, ketones, aldehyde, etc.) was extremely low, corresponding to the data of a previously published article (Lehmann et al. 2007).

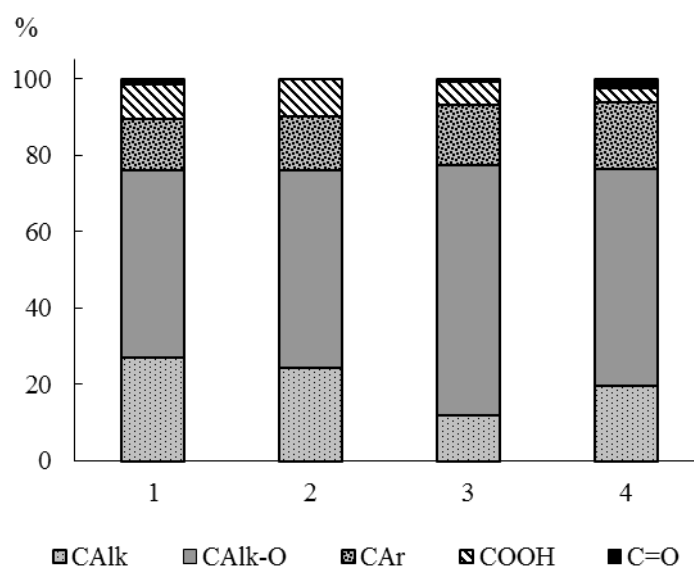


Figure 3. The content of structural fragments of organic matter in organo-mineral substrates and densitometric fractions according ¹³C-NMR spectroscopy: sand + 15% bentonite (1), sand + 30% kaolinite (2); LF-2 (3), and HF (4) of loam.

Aromaticity index of PRs humification products

The formation of a stable OM structure was quantitatively assessed by the aromaticity index (I_{AR}) which was calculated by formula:

$$I_{AR} = C_{Ar} \cdot 100 / C_{Ar} + C_{Alk}$$

where C_{Ar} is the surface area of the peak with a chemical shift associated with aryl groups, and C_{alk} is the surface area of the peak with a chemical shift associated with alkyl groups. The degree of humification of PR was calculated as the ratio: CH_{Alk}/CO_{Alk} (Table 3).

Table 3. Values of OM aromaticity and degree of PR humification calculated from the ¹³C-NMR spectra

Variants	Original PR	PR after incubation	PR + bentonite	PR + kaolinite	OM in LF-2	OM in HF
I_{AR} , %	7.2	12.4	15.0	15.4	16.7	18.5
CH_{Alk} / CO_{Alk}	0.19	0.23	0.57	0.68	0.18	0.35

According to the results, after 6 months incubation, the aromaticity of OM increased in the series: original PR < PR without solid phase < PR + bentonite substrate < PR + kaolinite substrate < PR + LF-2 < PR + HF. The degree of humification PR changed in the same manner, except for the densitometric fractions of the loam LF-2 and HF. This is because of the heterogeneity of the particle size and the composition of the fractions. The increase in the I_{AR} value coincides with the specificity of humification and is, probably, related to the synthesis of HA on the mineral matrices (Kononova, 1963; Zavarzina, 2006).

Conclusion

The content of newly formed HS increases in the range sandy < loam << bentonite \approx kaolinite throughout incubation. This is related to their accessibility for microorganisms. The rate of humification decreases with the time of the experiment. The humification of PR is of wavy character, which is mostly manifested in the loamy substrate. The dynamics of the humic substances in the systems, the coefficients and degrees of humification, and the C_{HA}/C_{FA} ratio are of wavy character. The wavy character of the humification is more clearly demonstrated by the dynamics of the C/N ratio in the humic substances. This can be related to the changes in the total abundance and activity of the microorganisms, as well as the structure of microbial cenosis during the experiment, because of the varying quality of the organic material in the systems.

The higher content of humic substances in the loamy substrate and, in particular, in the bentonite- and kaolinite-containing substrates is related to the formation of stable mineral-organic compounds with finely dispersed substrate components. These compounds are poorly accessible for microorganisms and ensure the preservation of not only HA, but also many FA. The sandy substrate has weak protecting properties with respect to the newly formed humic substances, which favors further mineralization. Thus, the loamy, bentonite, and kaolinite substrates ensure some fulvatization of humic substances compared to the sandy substrate. Similar values of I_{AR} for the sandy and loamy substrates at the early stage of incubation indicate the predominance of primary decomposition products retaining some features of the original material in the OM.

Analysis of the FTIR and solid-state ^{13}C -NMR spectra shows that the HF ($>2.2 \text{ g/cm}^3$), the mineral phase of which mainly consists of primary minerals with a predominance of quartz, is enriched with aromatic compounds and polypeptides. The light fraction (LF-2, $1.4\text{--}2.2 \text{ g/cm}^3$) accumulates, along with aromatic compounds, substances containing alkyl and carboxyl groups to a higher extent than the HF. This is related to the physicochemical nature of the surface of the mineral particles in the substrates, their densitometric fractions, and the properties of the OM transformation products. Catalytic processes, which are more typical for the loamy substrate and the bentonite- and kaolinite-containing substrates, can also play a significant role.

The spectroscopic data for the OM of the sandy substrate and the loam HF reveal a similarity between their humification products throughout the experiment. Apparently, the mineral matrices of the sand and the loam HF have similar properties and can ensure the stabilization of HA in adsorption complexes directly on the mineral surfaces. The higher I_{AR} values for the humic substances in the HF of the loamy substrate compared to that in the LF-2 ($1.4\text{--}2.2 \text{ g/cm}^3$) indicate the formation of the most stable aromatic compounds, which can form the basis of the HA nucleus.

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