The molecular structure and elemental composition of humic substances from Albeluvisols

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The structure of humic and fulvic acids from a Siltic Albeluvisol and a Stagni-Histic Albeluvisol (Siltic) was studied. It was found that the development of hydromorphism results in the enrichment of humic substances with aliphatic fragments. The presence of unoxidised aliphatic fragments in the structure of humic substances, in combination with anaerobic conditions, favours the gley process in the Stagni-Histic Albeluvisol (Siltic). Features of the structural composition of humic substances are related to the humification rate: this process is slowed in northern regions and accelerated in southern regions, where humic substances are more humified.

Keywords: humic and fulvic acids; solid-state ^{13}C NMR; soil moisture; Albeluvisols

1. Introduction

Soil organic matter (SOM) is one of the major carbon pools in the biosphere. Globally, there is substantially more carbon in the top metre of soil than in the overlying vegetation or in the atmosphere. SOM is composed of plants, animals and microbial residues at varying stages of decomposition. Humification and mineralisation processes change the chemical composition of SOM and thus are of great importance to the terrestrial carbon cycle [1,2].

Humic (HA) and fulvic acids (FA) constitute a large portion of the SOM in terrestrial environments [3]. They play an important role in global carbon cycling and in regulating the mobility and fate of plant nutrients and environmental contaminants [3–6]. Despite intensive research on humic substances during the past decades, the chemical nature of HA and FA is not fully understood. Studies using a variety of spectroscopic techniques have led to major advances in understanding the chemical structure of humic substances [7]. The elemental composition and major types of functional groups of HA and FA are well established, but the macromolecular structure and chemical heterogeneity of humic substances in relation to their molecular size distribution remains controversial [8]. The classic view of humic substances states that they are macromolecular, negatively charged, branched polyelectrolytes with mainly carboxylic- and phenolic-type acidic functional groups [9].

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Various factors of soil formation, including moisture, influence the accumulation and transformation of HA and FA [10–14]. In the north of European Russia, excessive moisture under a stagnant water regime, the presence of humic substances and heterotrophic microflora in boggy soils contribute to gley formation. When being developed agriculturally, excessively watered soils usually undergo water-drainage measures, otherwise they will remain low-productive. For this reason, soils that are to be agriculturally developed and dried should be estimated using reliable diagnostic criteria assessing soil moisture. The aim of this research is to study the molecular structure of humic substances from the most widespread soils in the European northeast of Russia: a Siltic Albeluvisol and a Stagni-Histic Albeluvisol (Siltic).

2. Materials and methods

2.1. *Site and soil characteristics*

Samples of HA and FA were derived from the horizons of soils with different degrees of moisture. The soils sampled were: a Siltic Albeluvisol and a Stagni-Histic Albeluvisol (Siltic) [15]. The soils were developed on mantle loams in the middle taiga zone of the Komi Republic, Russia. The sampling site was located on a hill, 6 km west of the city of Syktyvkar. The site was at the Maksimov field station of the Institute of Biology of Komi Scientific Centre of the Ural Branch of the Russian Academy of Sciences (61°39.6′, 50°41.0′).

The Siltic Albeluvisol was located at an altitude of 170 m above sea level (masl), the top of a hill on the interfluve and the 'ridge' of a microhigh of 1.5 m. The site was an aspen–birch–spruce forest with bilberry, cowberry and green mosses, and many dry and rotten tree branches and trunks.

The Stagni-Histic Albeluvisol (Siltic) was located in a microhollow between small flat microelevations. In this space is situated the birch–spruce *Sphagnum–Polytrichum* forest. The *Sphagnum* cover is prominent near the peat.

Morphological descriptions of the studied soils are given in Table 1. Physicochemical properties and the quantity of fungi and bacteria are presented in Table 2.

2.2. *Analytical methods*

FAs were isolated from air-dried soil samples by double-extractions with a mixture 0.1 M NaOH and 0.1 M $Na_4P_2O_7$. A saturated solution of Na_2SO_4 was then added to the alkaline extract (20%

Table 1. Morphological descriptions of soils.

Soil	Horizon	Depth of sampling, cm	Soil horizon description
Siltic Albeluvisol	Oe	0–5	Weakly decomposed peaty, very dark brown litter covered by fallen leaves, needles and branches; pierced by roots and fungal hypha, moist.
	AEh	5–7 (10)	Light gray, light silty loam, friable, many roots.
Stagni-Histic Albeluvisol (Siltic)	Oe	0–12	Sphagnum peat with inclusions: branches, needles, leaves; many roots, weakly moist.
	Ehg	12–20	Brown, stratified, nodules (1–3 mm in size) are common, some vertical cracks with organic coatings. Roots up to 0.5 cm in diameter.

Table 2. Physicochemical properties and quantity of fungi and bacteria in soils.

Horizon	Deposit of moisture, mm	Redox potential, mV	Bulk density g · cm^{-3}	pH		TOC (w/w), %	Quantity of fungi, 1000 cells · g soil^{-1}	Quantity of bacteria, 1000 cells · g soil^{-1}
				Water	1 M KCl			
Siltic Albeluvisol								
Oe	13.4	541	0.10	5.3	4.5	33.5	208	374
AEh	29.2	532	n.d.	4.1	3.3	1.77	448	31
Stagni-Histic Albeluvisol (Siltic)								
Oe	16.5	496	0.15	4.5	3.6	37.6	56	41
Ehg	37.7	359	1.67	4.8	3.9	0.73	2	3

Note: n.d., not determined.

of the extract volume) to coagulate the colloid particles. Centrifugation was performed for 1 h at 13,000 rpm. HA were then precipitated using 10% H_2SO_4, and FA were purified with activated carbon (AG-3 type) according to Kaurichev [16] and salted out on cationite KU2 in the H^+-form. The ash content of the samples was determined by ignition at 600 °C.

The C, N and H contents of humic substances were estimated with a Carlo Erba CHNS-O-EA1108 microanalyser (CE Instruments, Italy).

Solid-state ^{13}C NMR spectra of HA and FA were recorded on a Bruker CXP100 pulsed NMR spectrometer using the cross-polarisation magic-angle spinning (CPMAS) technique. The spectra were obtained at 2.3 T (25.18 MHz). The spectrometer was equipped with a commercial 7mm CPMAS probe at a rotation frequency of 4 kHz. At a contact time of 1 ms and a recycle delay of 0.3 ms, ~ 5000 scans were accumulated for each spectrum. An exponential function with 25 Hz line broadening was multiplied with the free induction decay. After Fourier transformation, the spectra were zero-order phase and baseline corrected. The ^{13}C chemical shifts were reported relative to tetramethylsilane (TMS = 0 ppm).

3. Results and discussion

3.1. ^{13}C NMR profiles

All ^{13}C NMR spectra of humic substances showed similar sets of characteristic bands, which differed only in their intensities (Figure 1, example spectra). For spectral interpretation, the following ranges and preliminary signal allocations were considered [17–19]:

- 0–46 ppm, carbons of aliphatic structures;
- 46–105 ppm, carbons of O,N-alkyl fragments;
- 105–164 ppm, carbons in aromatic rings;
- 164–183 ppm, typical of carbons of carboxylic groups;
- 183–190 ppm, carbons in aromatic rings of quinine fragments;
- 190–204 ppm, typical of carbons of aldehyde and ketone groups.

Semiquantitative data were obtained using the integration routine of the spectrometer. The results obtained are given in Table 3.

The ratio between the aromatic and aliphatic carbon contents is suitable for use as a formal index for standardising the quantitative parameters of macromolecules [20]. In this case, signals from aromatic structures were summed in the ranges 105–164 and 183–190 ppm; signals from aliphatic structures were summed in the ranges 0–105, 164–183 and 190–204 ppm; signals from oxygen-containing functional groups were summed in the ranges 46–105 and 164–204 ppm.
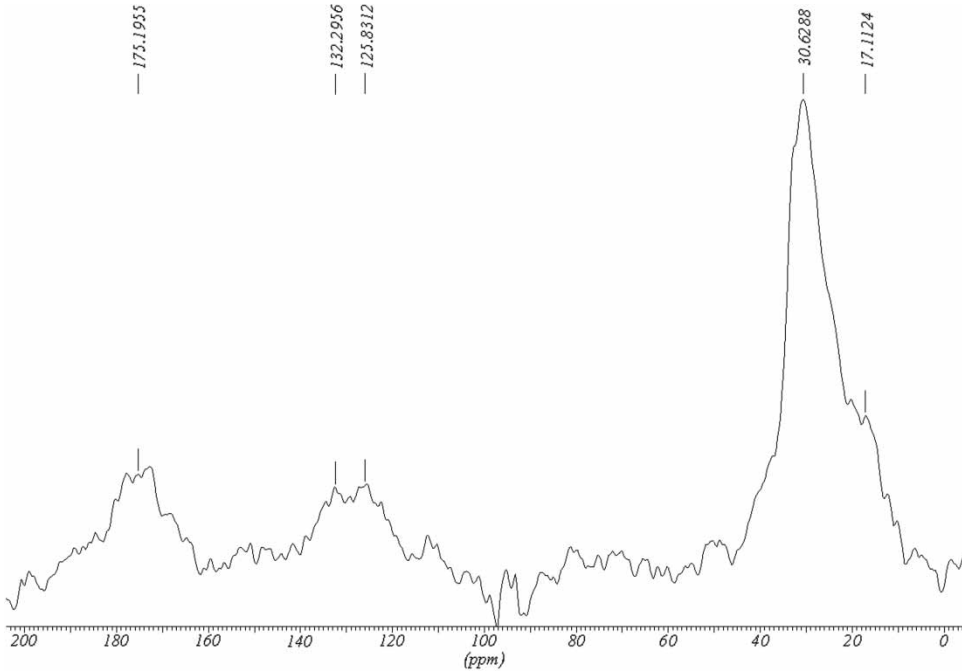
Figure 1.   Typical solid-phase CPMAS ^{13}C NMR spectrum of humic substances – an HA sample from the Ehg horizon of Stagni-Histic Albeluvisol (Siltic).

Table 3.   Content (%) of HA and FA molecular fragments (according to ^{13}C NMR data).

Soil, horizon	Chemical shift, ppm						$\dfrac{AR}{AL}$	Aromaticity, %
	0–46	46–105	105–164	164–183	183–190	190–204		
Humic acids								
Siltic Albeluvisol, Oe	22.4	20.9	43.5	11.3	0.5	1.4	0.79	44.0
Siltic Albeluvisol, AEh	40.7	17.5	25.9	12.1	1.7	2.1	0.38	27.6
Stagni-Histic Albeluvisol (Siltic), Oe	23.2	17.8	31.2	17.0	4.3	6.5	0.55	35.5
Stagni-Histic Albeluvisol (Siltic), Ehg	50.5	10.1	21.6	12.8	2.7	2.3	0.32	24.3
Fulvic acids								
Siltic Albeluvisol, Oe	23.3	29.8	26.0	19.6	0.6	0.7	0.36	26.6
Siltic Albeluvisol, AEh	25.1	25.0	14.5	25.0	3.2	7.2	0.22	17.7
Stagni-Histic Albeluvisol (Siltic), Oe	19.5	22.6	17.1	26.3	5.1	9.4	0.29	22.2
Stagni-Histic Albeluvisol (Siltic), Ehg	31.2	20.6	12.0	27.1	2.5	6.6	0.17	14.5

Comparative analysis of the molecular structures of HA and FA samples showed that FA are enriched with oxygen-containing fragments, which explains their higher solubility in water and higher migration capacity [21]. The high content of carboxylic groups (up to 27.1%) determines the acid aggressiveness of FA with respect to soil minerals and their capacity to produce complex compounds and thus solubilise cations of iron, aluminium and other metals [22]. The high content of carbohydrate fragments (up to 23.2%) makes FA molecules more preferable as nutrients for

soil microorganisms compared with HA, in which molecules are more than half composed of aliphatic chains and aromatic fragments, so their oxidation is less profitable in energy terms [23].

The estimated content of functional groups and molecular fragments indicates an essential difference in their composition when going from the organic to the mineral horizon in all of the soils studied. A high degree of aromaticity of humic substances was noted in the upper horizons (Oe) of the Siltic Albeluvisol and Stagni-Histic Albeluvisol (Siltic).

On comparing the molecular structures of HA from soils with different degrees of hydromorphism, it should be noted that HA extracted from the Siltic Albeluvisol are enriched with aromatic fragments compared with HA from the Stagni-Histic Albeluvisol (Siltic). In addition, an increase in the relative content of oxygen-containing functional groups [-COOR(H), $C_{ar} = O$, C_{al}-OR(H) and $-C = O$] was noted in HA molecules when going from the Oe to AEh horizon of the Siltic Albeluvisol. By contrast, the content of these groups in the HA from the Stagni-Histic Albeluvisol (Siltic) decreases from the Oe to Ehg horizon (Table 3). This is explained by the development of aerobic conditions in the Siltic Albeluvisol, which favour oxidation processes; anaerobic conditions are typical for the Stagni-Histic Albeluvisol (Siltic), where reduction processes occur (Table 2).

Even more pronounced changes are observed in the composition of FA when going from automorphic to hydromorphic soils. The tendency toward an increase in the degree of aromaticity of FA when going from the Stagni-Histic Albeluvisol (Siltic) to the Siltic Albeluvisol remains. The content of oxygen-containing groups also increases by 14.5% when going from the Oe to the AEh horizon in the Siltic Albeluvisol and decreases by 5.2% from the Oe to the Ehg horizon in the Stagni-Histic Albeluvisol (Siltic).

Thus, the humification of plant residues is more intensive in the Siltic Albeluvisol, which favours the formation of the most acidic humic substances enriched in oxygen-containing functional groups with a high aromatic fragment content. Humic substances of the Stagni-Histic Albeluvisol (Siltic), by contrast, show a higher degree of aromaticity and contain mainly unoxidised aliphatic fragments, which favour the gley process under anaerobic conditions.

A comparative analysis of the results obtained for humic substances from Albeluvisols of northeastern European Russia and the structural composition of humic substances extracted from soils of other climatic zones showed that the HA preparations under study are more enriched with aromatic structures than Spodic Cryosols and less enriched with them than the soils of southern regions such as gray forest soils and chernozems [23]. These features of the structural composition of humic substances are related to the humification rate: this process is slowed in northern regions and accelerated in southern regions [24,25].

3.2. *Elemental composition*

The results of the ^{13}C NMR spectroscopic study were compared with the elemental composition of humic substances (Tables 4 and 5). It was found that HA contain 52.6–58.8% C, 5.68–8.24% H, 2.62–3.78% N and 30.3–38.5% O; FA contain 41.8–45.6% C, 3.56–4.52% H, 1.21–2.42% N and 47.7–52.9% O.

Analysis of the elemental composition of humic substances showed that the most humified FA and HA are in the Siltic Albeluvisol, which determine their low $(H/C)_{cor}$ and high O/C atomic ratios. A decrease in the $(H/C)_{cor}$ ratio indicates an increase in the share of aromatic fragments in the HA structure when going from organic to mineral horizons in all of the soils studied. Calculation of the degree of oxidation showed that HA of Albeluvisols are weakly reduced (ω is between -0.91 and -0.20). FA show a degree of oxidation between $+0.40$ and $+0.46$; i.e. they are weakly oxidised compounds. It was found that the degree of oxidation of humic substances in mineral horizons increases with the degree of humification. A decreased degree of oxidation

Table 4. Distribution and elemental composition of humic acids (weight portion and atomic portion are given above and below the line, respectively; all results are given for ash-free preparations).

Horizon	HA content, g · kg soil^{-1}	Ash, %	Content, %				Atomic ratio			$(H/C)_{cor*}$	Degree of oxidation** (ω)
			C	H	O	N	H/C	O/C	C/N		
Siltic Albeluvisol											
Oe	5.6 ± 0.28	11.24 ± 0.11	54.5 ± 0.5	5.68 ± 0.11	36.4 ± 0.4	3.45 ± 0.17	1.25	0.502	18.2	1.92 ± 0.06	−0.25
			35.6 ± 0.4	44.6 ± 0.9	17.88 ± 0.18	1.96 ± 0.10					
AEh	6.0 ± 0.3	4.16 ± 0.21	53.8 ± 0.5	5.69 ± 0.11	36.7 ± 0.4	3.78 ± 0.19	1.27	0.511	16.6	1.95 ± 0.06	−0.25
			35.2 ± 0.4	44.7 ± 0.9	18.00 ± 0.18	2.12 ± 0.11					
Stagni-Histic Albeluvisol (Siltic)											
Oe	11.0 ± 0.6	4.75 ± 0.24	52.6 ± 0.5	5.68 ± 0.11	38.5 ± 0.4	3.24 ± 0.16	1.30	0.548	19.1	2.04 ± 0.06	−0.20
			34.5 ± 0.3	44.8 ± 0.9	18.91 ± 0.19	1.81 ± 0.09					
Ehg	8.5 ± 0.4	1.44 ± 0.07	58.8 ± 0.6	8.24 ± 0.16	30.3 ± 0.3	2.62 ± 0.13	1.68	0.388	25.7	2.20 ± 0.07	−0.91
			32.2 ± 0.3	54.1 ± 1.1	12.48 ± 0.13	1.25 ± 0.06					

Notes: *$(H/C)_{cor} = (H/C) + 2 \times (O/C) \times 0.67$; **$\omega = (2O − H)/C$, where O, H, C is the number of these atoms [21].

Table 5. Distribution and elemental composition of fulvic acids (weight portion and atomic portion are given above and under the line, respectively; all results are given for ash-free preparations).

| Horizon | FA content, g · kg soil^{-1} | Ash, % | Content, % | | | | Atomic ratio | | | $(H/C)_{cor}$ | Degree of oxidation (ω) |
			C	H	O	N	H/C	O/C	C/N		
Siltic Albeluvisol											
Oe	18.2 ± 0.9	6.04 ± 0.12	45.6 ± 0.5 33.3 ± 0.3	4.46 ± 0.22 39.1 ± 0.8	47.7 ± 0.5 26.16 ± 0.26	2.27 ± 0.11 1.42 ± 0.07	1.17	0.786	23.4	2.23 ± 0.07	$+0.40$
AEh	5.50 ± 0.28	13.24 ± 0.13	45.3 ± 0.5 33.0 ± 0.3	4.52 ± 0.23 39.5 ± 0.8	49.0 ± 0.5 26.75 ± 0.27	1.21 ± 0.06 0.76 ± 0.04	1.20	0.811	43.4	2.29 ± 0.07	$+0.42$
Stagni-Histic Albeluvisol (Siltic)											
Oe	18.3 ± 0.9	8.98 ± 0.18	45.6 ± 0.5 33.9 ± 0.3	4.24 ± 0.21 37.9 ± 0.8	47.8 ± 0.5 26.65 ± 0.27	2.42 ± 0.12 1.54 ± 0.08	1.12	0.786	22.0	2.18 ± 0.07	$+0.46$
Ehg	10.5 ± 0.5	7.39 ± 0.15	41.8 ± 0.4 31.9 ± 0.3	3.56 ± 0.18 39.8 ± 0.8	52.9 ± 0.5 27.24 ± 0.27	1.73 ± 0.09 1.08 ± 0.05	1.25	0.854	29.5	2.39 ± 0.07	$+0.46$

was noted for FA from the Siltic Albeluvisol, and an increased degree of oxidation was observed for FA from the Stagni-Histic Albeluvisol (Siltic); the opposite situation was typical for HA. This can be explained on the basis of microbiological activity as the decisive factor of humic substance formation. Rapid decomposition of nonspecific substances and the simplest humic substances occurs at increased biological activity [21]. Therefore, the higher biochemical activity of the Siltic Albeluvisol compared with the Stagni-Histic Albeluvisol (Siltic) [26] favours the oxidation of HA molecules with the accumulation of the most stable oxygen-containing products. FA are easily available for soil microorganisms under similar conditions; therefore, they are rapidly consumed and renewed. As a result, FA are composed of the youngest and less-oxidised compounds. Evidence for the increase in the oxygen content during the humification of HA is also available in the literature [24].

The elemental composition of HA and FA from Albeluvisols is relatively well understood, and our results agree well with the available data for cold acid soils [27] and Albeluvisols [22].

4.   Conclusions

The following functional groups and molecular fragments are identified in the structure of humic substances: carboxyl, carbonyl, aliphatic, alcohol, esters, hydrocarbons, phenol, quinone and aromatic. Analyses of the molecular structure of HA showed that macromolecules of HA contain significant amounts (up to 44.6%) of framework-forming aromatic fragments versus a relatively small proportion of a carbohydrate periphery; this is evident from the higher Ar/AL ratios of HA compared with those of FA. An excess of moisture affects not only the amount of accumulated HA and FA, but also their molecular structure; namely, it induces the accumulation of humic substances enriched with aliphatic structures in semihydromorphic Stagni-Histic Albeluvisol (Siltic). The presence of unoxidised aliphatic fragments in the structure of humic substances, in combination with reducing conditions, favours the gley process in the Stagni-Histic Albeluvisol (Siltic). Data on the elemental composition of humic substances confirm the ^{13}C-NMR results. Analyses show that the HA and FA isolated from the Siltic Albeluvisol are more humified than those isolated from the Stagni-Histic Albeluvisol (Siltic). Both the HA and the FA from the Siltic Albeluvisol demonstrate low H/C (adjusted) and high O/C ratios.

Acknowledgements

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