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Distribution of Molecular Weight of Humic Substances Isolated from Soils of Tallgrass Temperate Rainforests (Chernevaya Taiga)

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Abstract: The boreal forests of Russia are one of the largest forest areas on the planet. As a result of climate change, the rate of carbon sequestration and stabilization of organic matter are important indicators of environmental conservation. To understand mechanisms of stabilization and the structure of soil organic carbon, the molecular-weight (MW) distribution of humic acids (HAs) in soils of the central deciduous-forest zone of boreal forests (Chernevaya taiga, transitional ecotone forest, coniferous forest) was studied. Analysis of the MW distribution of HAs was conducted using size-exclusion chromatography. This approach allowed us to obtain the distribution of high-, medium-, and low-molecular fractions of HAs. It has been revealed that the content of the low-MW fraction prevails over the content of the medium- and high-MW fractions of HAs, which indicates the process of stabilization in soil organic matter. The accumulation of low-molecular-weight fraction occurs with the aromaticity increase in HAs, which indicates that HAs with a relatively high proportion of aromatic fragments have smaller hydrodynamic radius and a lower MW. It has been statistically substantiated that the low-molecular-weight fraction correlates with the content of aromatic compounds and carboxyl structural fragments of HAs, which indicates the resistance of the soil organic matter of Chernevaya taiga to biodegradation.

Keywords: humic acids; Chernevaya taiga; molecular weight; NMR spectroscopy; Retisols

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1. Introduction

Soil humus is a product that accumulates in the soils during the decomposition and humification of various organic precursors [1,2]. It is a key component in the global carbon cycle in the atmosphere, pedosphere, and hydrosphere [3]. In various natural zones, humus differs in stability, turnover rate, and duration time [4]. Stabilization refers to processes that increase the resistance of humus to biotic and abiotic influences and increase the humus turnover time in the soil. Increasing the stability of humus is a fundamental function of the soil that plays a critical role in the process of carbon sequestration. During carbon sequestration from the atmosphere, through the vegetation cover, carbon passes into the structure of humus. As a result of the low degree of stability, organic carbon can be quickly transformed by microorganisms and re-enter the atmosphere in the form of carbon dioxide [4]. With relatively high rates of stabilization, the input organic matter can be stored in the soil for a long time. As humus is critical for stabilizing soil structure, regulating plant nutritional patterns, and ensuring water infiltration and storage in the soil; it supports key soil functions and implements such ecosystem properties as climate regulation, nutrient cycling, and primary production [5].

Soil is the largest surface reservoir of organic carbon on Earth [6]. Due to local geogenic features, climatic conditions, and land use, soils show different levels of stabilization of humic substances [4]. Generally, soil organic matter can be divided into three pools, according to the turnover rate. The active pool (3–10 years) primarily undergoes biotic and abiotic transformation and it consists mainly of plant and animal residues (coarse humus), as well as particulate organic matter. The slow pool (10–100 years) is represented by physically protected humus (amino sugars, and fractions of lignin and lipids). The passive pool (>100 years) is represented by biochemical characteristics inaccessible to microorganisms, as well as organic matter physically associated with the mineral fraction (microaggregates, non-hydrolysable organic matter, lipid and lignin transformation products) [4]. Nowadays, an important task is to monitor landscapes that can make the greatest contribution to climate change on our planet, as well as those areas that are responsible for sequestering carbon from the atmosphere [7]. The total carbon stock of the world's forests is 662 gigatonnes (Gt) and the average forest carbon density is 163 t/ha [8]. Soil carbon stocks vary widely between forests, with the highest soil carbon stocks (including litter) being highest in boreal forests (202 Gt), followed by tropical forests (155 Gt), and the lowest carbon stocks in temperate forests (69 Gt) [9]. Over the past 30 years, carbon stocks in the world's forests have decreased by 6 Gt (from 668 to 662 Gt). The boreal forests of Russia are among the largest continuous forests on Earth, occupying 20% of the world's forest area and 70% of the world's boreal forest area [8]. The tall-grass Chernevaya taiga of the foothills and mountains of southern Siberia is one of the largest massifs of tall-grass forests in Russia. In general, within the boreal forest, tall-grass forests have been preserved in the form of isolated areas [10]. The productivity of boreal forests is changing after climate warming [11], as well as the potential for carbon sequestration in boreal forests, which may change significantly in the next century due to climate change [12]. The direction of carbon sequestration will depend on how the stabilization mechanisms of soil organic matter will respond to climate change [13]. It is necessary to predict the resistance of organic matter to transformation processes. A powerful mechanism for investigation of the stability of SOM is the analysis of the qualitative composition of HAs.

The composition of HAs determines the direction of the processes of stabilization of organic matter in the soil [1,2,14,15]. Humic substances represent a combination of heterogeneous compounds formed as a result of the decomposition of plant and animal residues in terrestrial and aquatic ecosystems [16]. Bioclimatic conditions, the content of humification precursor, as well as the local position in the landscape determine the diversity of the composition and properties of humic substances [17–21].

There are two main concepts for the formation of HAs. Based on the first hypothesis, due to the thermodynamic selection, the formation of the most stable structural fragments in the composition of HAs occurs [1,2,22]. According to thermodynamic selection, an accumulation of aromatic fragments and a decrease in aliphatic ones occur. This process is accompanied by an increase in the molecular weight of HAs and by a decrease in low molecular weight, which is enriched in aliphatic chains, carbohydrates, and amino acids [2,23].

Based on the second hypothesis, HAs are a supramolecular ensemble, a set of molecules with a relatively small molecular weight (from 400 to 1000 Da), self-organizing into large-molecular fragments under the action of weak dispersion forces (van der Waals, π–π, π–CH) [22,24,25]. Stabilization of soil organic matter occurs due to various mechanisms: the influence of microorganisms (soil microorganisms are biotic agents of organic matter transformation); quality of humification precursors; chemical stabilization (condensation of stable supramolecular complexes); physicochemical stabilization (formation of organo-mineral complexes and soil aggregates); and physical stabilization (features of structural microstructure of soil aggregates) [4].

In a previous study [26], we used ^{13}C and ^{1}H–^{13}C NMR spectroscopy to investigate the composition of HAs. We discovered that Chernevaya taiga soils accumulate up to 48% of aromatic structural fragments in the middle of the soil profile, which differs significantly from soils from other boreal forests, indicating a high degree of aromatic compounds in tall-grass forest plant restudies [27–29]. The data obtained in previous work made it possible to reveal information about the structure of carbon and hydrogen atoms and their environment; however, they do not allow us to investigate such a structural indicator as the MW of HAs. Thus, the aim of this work was to study the MW distribution of HAs isolated from soils of boreal forest (Chernevaya taiga, transitional ecotone forest, coniferous forest). According to the aim, the following tasks were set: 1—to identify the features in the distribution of MW of HAs; 2—to estimate the relationship between the molecular composition and the MW distribution of HAs in the studied soils.

2. Materials and Methods

2.1. Study Area

The main part of Chernevaya taiga is located at altitude ranging from approximately 200 to 700/800 m on the western, windward macroslopes of the mountains and foothills of southern Western Siberia (Figure 1).

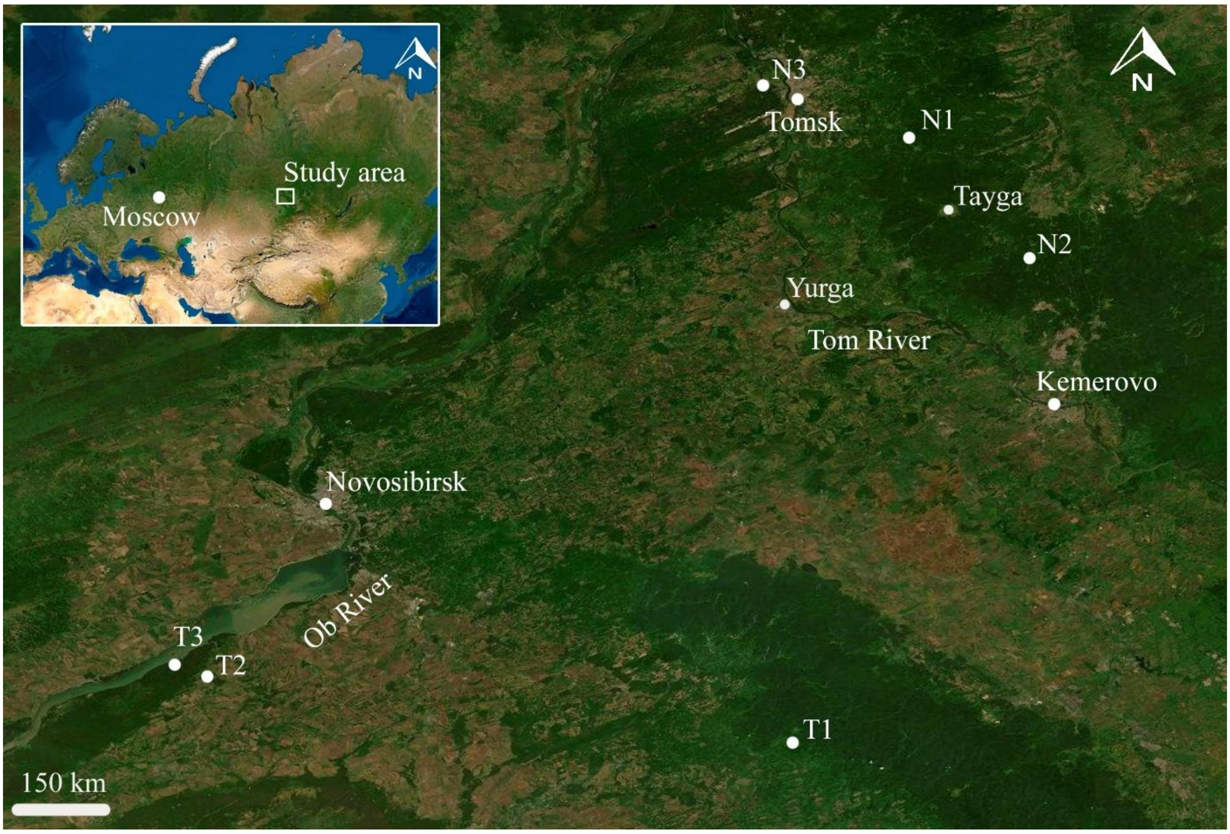

Figure 1. The study area (origin maps form ESRI.ArcGIS.Imagery). The sample ID corresponds to Table 1.

Chernevaya taiga (N1 and T1 profiles) is a type of barrier-rain landscape and is characterized by numerous hydrological and nutrient features dominated by fir and aspen [30–32]; the grass layer is well developed; large shrubs in the undergrowth; quite rich epiphytic bryoflora; the flora includes a complex of relatively heat-loving nemoral species. Deep winter snow cover (from 80 cm to 2 m or more) prevents soil freeze. There is no stratification of the litter; the turnover rate is less than 2 years. The number of earthworms in Chernevaya taiga is one of the highest in Russia. Due to the above-mentioned factors and soil features of Chernevaya taiga, its biodiversity is relatively high in comparison to other zonal ecosystem types. The soils of Chernevaya taiga are formed under unique combinations of geogenic and bioclimatic conditions, under which the soils do not freeze through in winter and are provided with moisture, which contributes to the rapid mineralization of the bedding material and the fixation of mineral nutrients in the upper humus stratum of the soil profile. The accumulation of biophilic elements is the most important properties of Chernevaya taiga soils, which is associated with the phenomenon of gigantism and extremely high plant productivity. The soil boreal pine stands formed in oligotrophic environments due to sublaying of soils by sand, which are initially poor in nutrients. Thus, we compared humification and soil formation in principally different conditions of ecosystems and environments.

Two soil profiles from Chernevaya taiga (N1 and T1 profiles), two soil profiles from transitional ecotone forest (N2 and T2 profiles), as well as two soils from relatively oligotrophic habitats (N3 and T3 profiles) confined to ancient dune aeolian sandy massifs covered with pine forests were chosen (Table 1). The choice of soil horizons is due to the formation of organo-accumulative horizons in the studied soil profiles. In these soil profiles, the most active processes of transformation of organic matter were noted. These horizons demonstrated the darkest color within soil profile and with the highest gravimetric content of organic carbon. Thus, in these horizons we can expect the highest extraction yield of the humic acids and by this highest representability of the organic matter parameter within evaluation by chemical instrumental methods.

Table 1. The description of studied soils.

Sample ID	Soil ID	Horizon *	Depth, cm	Description	Coordinates and Terrestrial Biome	Soil Name *
N1	1	A_1	0–10	Organo-mineral horizon is characterized by active accumulation of organic matter	N 54.14070° E 84.9495° Boreal forest (Chernevaya taiga), Altay region	Umbric Retisols
	2	A_2	20–30	Organo-mineral horizon is characterized by active accumulation of organic matter		
	3	B/E	30–40	Transitional horizon with loss of silicate clay		
N2	4	A	10–20	Organo-mineral horizon is characterized by active accumulation of organic matter	N 54.37083° E 82.4393° Boreal forest (transitional ecotone forest), Novosibirsk region	Albic Retisols
N3	5	A	3–10	Organo-mineral horizon is characterized by active accumulation of organic matter	N 54.40810° E 82.18420° Boreal forest (Coniferous forest), Novosibirsk region	Albic Retisols
T1	6	A	0–15	Organo-mineral horizon is characterized by active accumulation of organic matter	N 56.30693° E 85.47063° Boreal forest (Chernevaya taiga), Tomsk region	Umbric Retisols
	7	Bt	70–110	Mineral horizon with accumulation of illuvial clay particles		
T2	8	A	0–3	Organo-mineral horizon is characterized by active accumulation of organic matter	N 55.88619° E 86.00433° Boreal forest (transitional ecotone forest), Kemerovo region	Albic Retisols
T3	9	A	10–20	Organo-mineral horizon is characterized by active accumulation of organic matter	N 56.48106° E 84.79860° Boreal forest (Coniferous forest), Tomsk region	Albic Retisols

* Soil names are indicated according to the soil classification WRB [33,34].

2.2. MW Distribution of HAs

HAs were isolated from organic and mineral horizons of soils according to the method recommended by the International Society for the Study of Humic Substances (IHSS) [35]. HAs were isolated from air-dry soil samples by 2-fold extraction with 0.1 mol/dm^3 NaOH, after which a saturated solution of Na_2SO_4 was added to the alkaline extract to coagulate colloidal particles. Then, HAs was precipitated with sulfuric acid. MW distributions of HA preparations were obtained on an AKTAbasic 10 UPS chromatographic system (Amersam Biosiences, Uppsala, Sweden) using a SuperdexTM 200 10/300 GL column (with cross-linked dextran gel, fractionation range for globular proteins 10–600 kDa), aliquot volume HA solutions 0.1 cm^3, elution rate 0.5 cm^3/min., filter wavelength 254 nm. The determination of the working capacity of the gel was carried out using solutions of blue dextran and potassium dichromate at a concentration of 1 mg/cm^3 and the column was calibrated by globular proteins. Previously, solutions of HAs at a concentration of 0.1 mg/cm^3 in 0.1 mol/dm^3 NaOH were purified from "mineral salts" and low-molecular-weight individual organic compounds (carbohydrates, amino acids, etc.) by passing through a column filled with Sephadex G-10 gel (crosslinked dextran, fractionation range for globular proteins up to 700 Da). Tris-HCl buffer with pH 8.2 [36] containing sodium dodecyl sulfate (0.1%) was used as an eluate to prevent specific adsorption of humic substances on the gel, sodium azide (0.02%) as an antibacterial agent, and sodium chloride (0.05 mol/dm^3) for constant ionic strength and to prevent overexclusion. The original Unicorn 5.10 program was used to process chromatographic data and calculate the MW distribution of fractions of HA preparations. HA preparations are polydisperse, i.e., have a set of different MW fractions. As a result, they are described by the MW distribution, which is used to calculate the average MW. Depending on the method of calculating the average, three types of average molecular weights are used: number average (M_n) (1), weight average (M_w) (2), and average (M_z) (3) [37].

Number average MW—averaging over the number of molecules in a biopolymer:

$$M_n = \frac{\sum n_i M_i}{\sum n_i}, \tag{1}$$

where n_i is the number of molecules with molecular mass M_i.

Weight average MW—averaging over the mass of molecules in a biopolymer:

$$M_w = \frac{\sum n_i M_i^2}{\sum n_i M_i} \tag{2}$$

where n_i is the number of molecules with molecular mass M_i.

The average MW is calculated by the equation:

$$M_z = \frac{\sum n_i M_i^3}{\sum n_i M_i^2} \tag{3}$$

where n_i is the number of molecules with molecular mass M_i.

The polydispersity index (4) was calculated from the ratio of M_w to M_n. Polydispersity—a quantitative characteristic of the degree of deviation in MW distribution from monodisperse, consisting of molecules of the same size—is a consequence of the random nature of the reactions in the formation of organic substances, and in some cases, a consequence of the destruction or combination of macromolecules.

The polydispersity index:

$$\text{Polydispersity index} = \frac{M_w}{M_n} \tag{4}$$

where M_w is weight average MW and M_n is number average MW.

3. Results

The parameters of the MW distribution of HAs from the organo-mineral horizons of the studied soil tallgrass temperate rainforests were determined by size-exclusion chromatography. Three regions of gel chromatograms were associated with three molecular fractions of HAs (Figure 2): high-MW region (A), medium-MW region (B), and low-MW region (C). Regions were isolated for all presented gel chromatograms, based on the distribution of the optical density of various HA fractions. The regions belonging to different fractions have different areas, which confirms the distribution of HAs in three areas [38].

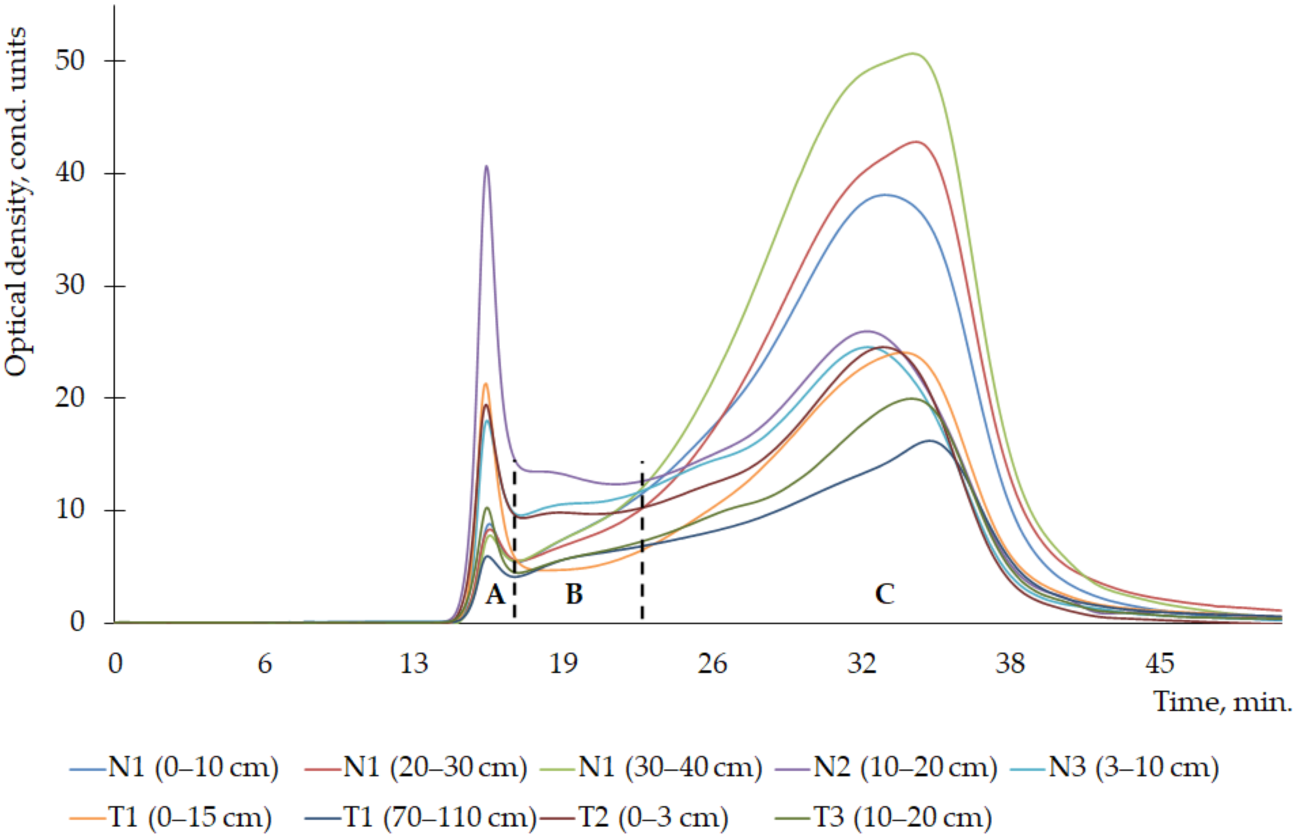

Figure 2. MW distribution of HAs isolated from soils of boreal forest. Soil ID corresponds to Table 1. A—high-MW area; B—medium-MW area; C—low-MW area.

The curves for the MW distribution of HAs have one pronounced maximum in the low-MW region. The proportion of low-MW fractions of HAs in the studied soils varies from 50.2 to 70.1% with *Mr* 1.13–1.61 kDa; the-low MW fraction is dominant in the studied soils, while the maximum values correspond to the organo-mineral horizons in Chernevaya taiga soil (N1 and T1) (Table 2). The medium-MW fraction varies from 26.1 to 36.1% with *Mr* 18.8–30.2 kDa; the maximum content corresponds to the coniferous forest (N3), the lowest content is noted in the soils of Chernevaya taiga (N1 and T1 profiles). The high-MW fraction varies from 3.3 to 16.1% with *Mr* 312–376 kDa; the maximum values correspond to transitional ecotone forest (N2), the minimum to Chernevaya taiga area (N1). In the intraprofile distribution of the soils of Chernevaya taiga (N1), there is an increase in the content of the low-MW fraction from 66.7 to 70%, with *Mr* 1.36–1.38 kDa.

The HAs isolated from the soils of transitional ecotone and coniferous forests differ from each other to a lesser extent. A peak was noted in the region of the high-MW fraction in the sample from transitional ecotone forest (N2), while among HAs of the soils of coniferous forests (T3), a maximum was noted in the low-MW fraction (61.7%).

Table 2. The molecular weight distribution of humic substances in studied soils. Soil ID corresponds to Table 1.

Soil ID	High Molecular Fraction				Medium Molecular Fraction				Low Molecular Fraction				M_n, kDa	S	M_w, kDa	S	M_z, kDa	S	M_w/M_n
	Mr, kDa	S	Molar Fraction, %		*Mr*, kDa	S	Molar Fraction, %		*Mr*, kDa	S	Molar Fraction, %								
			x	S			x	S			x	S							
1	319	4	4.3	0.3	21.5	0.1	29.1	1.3	1.38	0.04	66.7	1.6	20.8	1.3	216	4	310	4	10.4
2	319	1	3.9	0.2	19.8	0.2	26.3	1.8	1.31	0.04	69.7	2.1	18.7	1.2	220	1	311	1	11.8
3	308	1	3.3	0.4	18.8	0.4	26.8	1.8	1.36	0.05	70.1	2.2	16.1	1.7	200	4	299	2	12.4
4	360	3	16.1	0.5	30.2	0.3	33.8	0.4	1.59	0.04	50.2	0.1	68.5	2.1	307	4	355	3	4.4
5	329	1	10.1	0.4	27.9	0.3	36.1	0.3	1.61	0.03	54.3	0.1	43.8	1.3	254	3	321	1	5.8
6	376	5	10.3	0.3	21.1	0.1	26.1	0.8	1.36	0.04	63.5	1.1	45.2	1.9	326	5	373	5	7.2
7	312	3	7.1	0.7	29.1	1.6	33.2	1.2	1.13	0.01	59.7	2	32	3	221	2	301	1	6.8
8	344	3	12.1	0.7	28.9	0.4	32.5	0.5	1.49	0.02	55.5	0.2	51.6	2.2	281	4	338	1	5.5
9	347	3	7.5	0.1	28.5	0.1	31.1	0.4	1.27	0.03	61.7	0.2	34.6	0.25	267	1	340	1	7.7

S—standard deviation, M_n—number average MW, M_w—weight average MW, M_z—average MW, M_w/M_n—polydispresity index.

From the ^{13}C NMR spectroscopy analysis, a relatively high content of aromatic structural fragments (34–48%) was shown in Chernevaya taiga samples (N1), as well as the highest content of the low-MW fraction (66.7–70%). In samples N2 and N3, the content of the aromatic fraction was 24 and 31%, while the content of the low-MW fraction was 50 and 54%, respectively. Thus, the low-MW fraction of HAs increases with an increase in aromaticity (Table 3).

Table 3. The Pearson correlation of different MW fractions and structural fragments of HAs by ^{13}C NMR spectroscopy in studied soils. $N = 9$. $r_{cr} = 0.59$. The data of ^{13}C NMR spectroscopy analysis based on previously obtained data [26].

n/n	C,H-Alkyl ((CH_2)$_n$/CH/C and CH_3)	O,N-Alkyl (NCH/OCH_3)	C_{COH}	Aromatic Compounds (C=C/C–H, C=O)	Carboxyl (COO/N-C=O)	Quinone (R(C=O)R′/RCHO)
Low molecular fraction	−0.64	−0.79	−0.52	0.75	0.78	−0.14
Middle molecular fraction	0.45	0.66	0.35	−0.54	−0.64	−0.01
High molecular fraction	0.69	0.76	0.56	−0.79	−0.76	0.08

Pearson's correlation was used to analyze the correlation between the MW distribution and the content of structural fragments by ^{13}C NMR spectroscopy (Table 3). It was determined that the increase in the content of the low-MW fraction of HAs is associated with an increase in aromatic compounds (C=C/C–H, C=O) $r = 0.75$, as well as carboxyl (COO/N-C=O) $r = 0.78$. It was found that there is a statistical relationship between the content of high-MW fraction and O,N-alkyl (NCH/OCH_3) $r = 0.76$.

4. Discussion

4.1. Carbon Sequestration to the Soil Organic Matter

Carbon sequestration is one of the key soil properties. In the course of the formation of HAs, high-, medium-, and low-MW compounds are formed, which are available to varying degrees for the biotic and abiotic factors of transformation [39]. Forest ecosystems are one of the largest stores of organic carbon (vegetation and soil cover, microbial biomass), so stabilization issues in these ecosystems play an important role in terms of climate change [8]. The HAs isolated from the soils of Chernevaya taiga have a relativity fast turnover time, which is associated with the composition of humification precursors, as well as microbiological activity [40]. This is indicated by an increase in the proportion of low-MW fragments of HAs since microorganisms transform primarily easily accessible forms of organic matter. A large amount of plant biomass (factor of plant gigantism) provides the soil with a sufficient amount of plant residues, which contributes to the stabilization of organic matter in the soils of Chernevaya taiga. The soils of transitional ecotone and coniferous forests are characterized by a relatively low content of the low-MW fraction in HAs, which may be due to the quality of humification precursors, as well as the active transformation of plant residues in the soil. The formation of a pool of organic carbon in the vegetation and soil cover of the forest is an important ecosystem service that affects climate change [7]. Due to the formation of a network of carbon polygons and carbon farms in Russia, the data obtained can be used to correct data from carbon polygons, as well as an example for creating favorable conditions for carbon farms and carbon sequestration. Carbon polygons are a place where emissions and accumulation of CO_2 are monitored under conditions of various types of relief and soil cover [41]. This network will make it possible to study the planetary carbon cycle, as well as create prerequisites for assessing carbon sequestration and mitigating the effects of climate change on the territory of the Russian Federation [42]. Based on data from carbon polygons, carbon farms can be created to organize a sequestration industry in forest areas [43].

4.2. Statistical Relationship between the MW Distribution and the Content of Structural Fragments by ^{13}C NMR Spectroscopy

A characteristic feature of the HAs isolated from the soils of Chernevaya taiga is an increase in both aromaticity [26] and the content of the low-MW fraction with depth. This may be due to more active processes in the transformation of organic matter in the underlying horizons.

According to temperature studies [27], the underlying horizons are less susceptible to significant temperature changes, which favorably affects the activity of the soil microbiota. Among the studied soils, HAs of Chernevaya taiga (T1 and N1) showed a decrease in the average number and weight average MW values (Figure 3). This is related to a decrease in the proportion of high- and medium-MW fractions in the composition of HAs and the accumulation of stable low-MW fractions of HAs. The formation of the low-MW fraction in the soils of Chernevaya taiga may be associated with a number of factors affecting the humification of organic matter: more diverse microbial community, vegetation cover, and climatic parameters. The increase in the content of aromatic fragments is associated with the content of the low-MW fraction of HAs due to more compact condensed assemblies compared to long aliphatic chains (C,H-alkyl and O,N-alkyl).

From the data obtained, the highest polydispersity index (M_w/M_n) is observed in the HAs isolated from soils of Chernevaya taiga (N1) and maximum of this indicator is observed in the middle of the soil profile. Apparently, this is due to the active processes of humic substance formation in the middle part of the soil profile, while the highest microbial diversity corresponds to the upper soil horizons, with a gradual decrease with depth [27].

An increase in the proportion of aromatic fragments is associated with the stabilization of organic matter in soils and makes them less susceptible to biotic and abiotic degradation. The highest content of low-MW fragments in the composition of HAs is associated with the soils of Chernevaya taiga, while the highest amount of the low-MW fraction (up to 70.1%) is observed in the middle part of the soil profile. This is apparently related to the active transformation of organic matter in the middle part of the soil profile. For HAs isolated from the soils of the coniferous forest and transition ecotone forest, a correlation of the medium-MW fraction with aliphatic structural fragments (r = 0.90) and the low-MW fraction with a carboxyl group (COO/N-C=O) r = 0.99 is noted. Thus, we confirmed the hypothesis about how aromatic fragments are associated with the content of the low-MW fraction of HAs due to more compact condensed ensembles and the high- and medium-MW fractions with a branched network of aliphatic structural fragments (C,H-alkyl and O,N-alkyl).

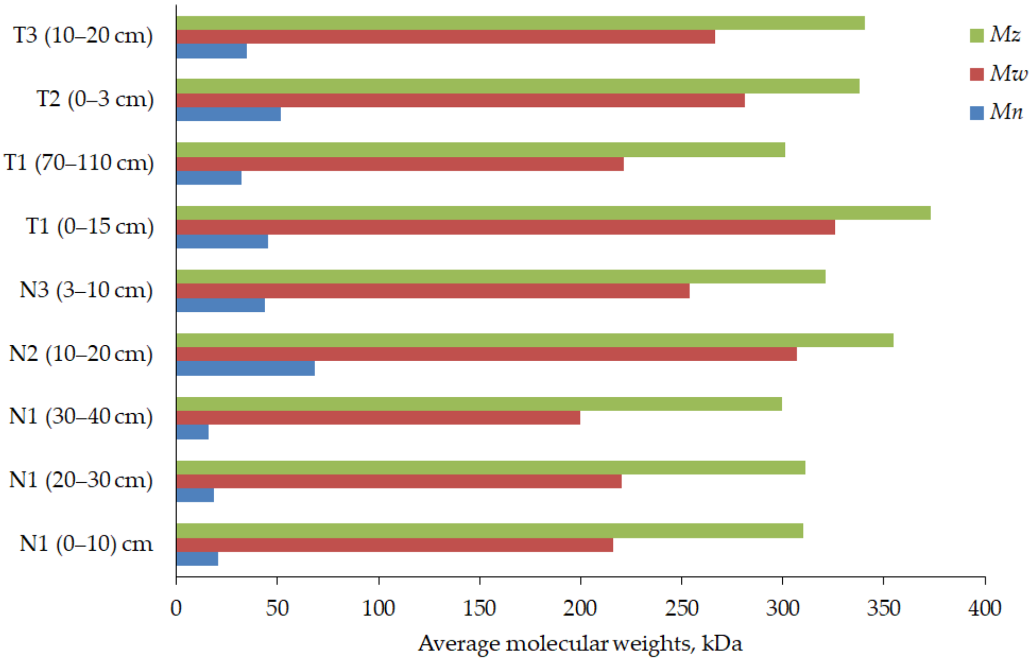

Figure 3. The number average MW distribution of studied soils. Soil ID corresponds to Table 1.

The studied soils of boreal forests demonstrate relatively low MW of HA fractions. Thus, in the tundra soils of the European northeast of Russia, it was found that the fraction of high-MW compounds has a mass of $\geq$600 kDa (up to 3% of molar fraction), the medium-MW fraction is 61.6–83.1 kDa (18.1–33.4% molar fraction), and the low-MW fraction is 2.55–5.27 kDa (63.6–80.8% molar fraction) [44]. The relatively high MW of HAs in tundra peat soils is due to the low temperature and low biological activity of these soils during the climatic summer. This contributes to the preservation of branched carbohydrate and paraffin fragments in the HA composition, which increases the hydrodynamic dimensions of HA molecules. At the same time, the content of low-MW fractions of HAs is higher than in the soils of boreal forests and reaches 80.8%. An increase in the proportion of low-MW compounds in tundra soils is associated with the long-term agricultural use (more than 50 years) of the territory and an increase in the biological activity of soils [44]. It was also noted that cryogenic processes can influence the formation of low MW of HAs fractions. However, the soils that are formed in the peat soils of the European northeast of Russia have a relatively low share of medium MW 14.55–22.0 kDa (16.7–32.9% of molar fraction) and low MW 1.05–1.57 kDa (64.51–82.56% of molar fraction) relative to the soils of boreal forests [38]. Apparently, the processes of hydrolysis of organic substances significantly affect the MW of various fractions, for example, in tundra and peat soils, due to the relatively high degree of soil hydromorphism, low-molecular fractions have a significantly low MW [44].

5. Conclusions

Analysis of the MW distribution of HAs isolated from soils of Chernevaya taiga, transitional ecotone, and coniferous forest revealed that soils of Chernevaya taiga accumulate up to 70.1% of low-MW fraction, whereas this indicator decreases in soils of transitional ecotone and coniferous forest. HAs of transitional ecotone and coniferous forest contain a relatively high content of high- and medium-MW fractions. The highest content of the medium-MW fraction with *Mr* 21.1–28 kDa was noted in humic substances extracted from coniferous forest. The highest content of the high-MW fraction with *Mr* 308–360 kDa corresponds to transitional ecotone forest. HAs formed in the soils of the Chernevaya taiga have significant differences from HAs formed in the zonal soils of Siberia. It was found that the low-MW fraction of HAs contains more aromatic fragments while high MW consists mainly of branched aliphatic chains, with a predominance of amino acids, alkyl, and carbohydrate fragments.

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