Environmental aspects of molecular composition of humic substances from soils of northeastern European Russia

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Abstract: Data on the molecular structure of humic substances (HSs) of zonal soils for the southern, middle, northern taiga and southern tundra of northeastern European Russia have been obtained. This was accomplished using solid-state ^{13}C nuclear magnetic resonance (^{13}C NMR) technique. The soils under study vary in the point of genesis and degree of hydromorphism. The impact of environmental factors (temperature and humidity) on qualitative and quantitative composition of humic (HAs) and fulvic acids (FAs) has been determined. Excess moisture significantly affects HS accumulation and HS molecular structure: hydromorphic taiga soils accumulate HSs enriched by unoxidized aliphatic fragments, tundra soils – the ones enriched by carbohydrate fragments. Various conditions of soil genesis predefine the specific character of structural and functional parameters of HSs in the southern taiga to southern tundra soils, as is expressed through the increased portion of labile carbohydrate and amino acid fragments and methoxyl groups within the structure of HSs. The tundra humification is characterized by levelling-off of structural and functional parameters of major classes of specific organic compounds of soils – HAs and FAs.

Key words: Arctic, humic acids, fulvic acids, soil, permafrost, tundra, taiga.

Introduction

Low temperature, high precipitation rate and appearance of continuous or discontinuous permafrost in cold boreal and polar environments result in favourable conditions for huge amounts of soil organic matter (SOM) accumulations (Jackson *et al.* 2017; Vasilevich *et al.* 2018). At the same time, the climate warming and other global changes having occurred over the last decades result in biodegradation and mineralization of soil and permafrost organic matter for the first time in the millennia. In order to assess the implications of global climate changes on SOM sequestration rate and its stability, both the amount and structural stability of SOM should be investigated for polar and cold boreal environments (Routh *et al.* 2014; Fritz *et al.* 2015).

Here, the content and stock of soil organic matter are very important for assessing the carbon balance, but the stability of humus in changing conditions is also a very informative indicator, especially for soils of cold environments. There are a few indices for assessment of humus stability: ratio of humic acids (HAs) to fulvic acids (FAs), degree of aromaticity, content of free radicals in molecules (Sartakov *et al.* 2015; Abakumov and Alekseev 2018) and elemental composition of both groups of humic substances (HSs) (Lodygin *et al.* 2017). According to dominant view, humic substances are self-organizing systems building persistent substances of stochastic nature under nonequilibrium environmental condition in the natural selection process (Ostenberg *et al.* 1996; Kholodov *et al.* 2015). The variety of specific components of organic substance of soil is related to a wide range of climatic conditions, vegetation, fauna and microflora patterns, and soil formation processes (Kholodov *et al.* 2011; Dergacheva *et al.* 2012).

Humic acids possess the ability to accumulate, because they do not migrate within the profile or in the landscape (Dergacheva 2003). It is the accumulative nature of HAs, their ratio versus FAs due the bioclimatic conditions, the ability of the system to organize and regulate itself as well as preserved key properties of humus in diagenesis that allow for using humic substances as markers of soil formation stages and phases (Kallas and Dergacheva 2011; Kukuļs *et al.* 2019). The humus profile captures everything, even short-term environmental changes, and reflects soil development stages and phases which are diagnosable even (Dergacheva *et al.* 2018; Rodrigues *et al.* 2019). A number of articles have been published in Russia so far stating major regularities for the change of structural and functional parameters of humic substances as affected by bioclimatic and zonal factors (Orlov 1995; Kholodov *et al.* 2011; Dergacheva *et al.* 2012). Based on consolidation of a large data set, the authors have outlined descriptors of HSs composition reflecting environmental and geographic conditions. The authors are attempting to assess such zonal features as total carbon, hydrogen, their molar ratio, extinction coefficients, (non)hydrolysable fraction of humic acids, etc. One of the most reliable integral parameters in this context would be atomic ratio (H/C), which reflects indirectly a degree of condensation of molecules and truly

decreases towards south. This indicator is directly connected with one of the most meaningful, for humic substance formation, values – biologically active soil temperatures ($\Sigma t > 10$ °C) characterized by clear latitudinal zonation (Orlov 1995; Dergacheva *et al.* 2012). The H/C index correlates well with the data on humic substance aromaticity obtained through ^{13}C-NMR spectroscopy (Kholo-dov *et al.* 2011; Vasilevich *et al.* 2018).

NMR spectroscopy has significantly expanded the knowledge on SOM composition and structure, enriched understanding of humification and transformation mechanisms, and made it possible to identify the impact of xenobiotics on the qualitative composition of humic substances (Uyguner *et al.* 2004). All these achievements enabled a wide use of the NMR method to analyze the molecular composition and structure of heterogeneous SOM. The study of the molecular structure demonstrates clear interrelation between distribution of non--oxidized aliphatic and carbohydrate fragments, with their share decreasing in going to the south, and aromaticity based on the ^{13}C-NMR spectroscopy data, which (on the contrary) increases in passing from Eutric Albic Retisol (Loamic, Ochric) to Albic Luvisol and Chernozem soils. (Kholodov *et al.* 2011). The changed structural and functional parameters in the zonal soil types reflect the kinetic theory of humification (Orlov 1995), which expresses accumulation of biothermodynamically stable fragments of aromatic character. It is worth mentioning that the zonal soil types analyzed by the authors cannot be defined as the final range, as it borders with southern taiga in the north. Therefore, the aim of this work is to identify environmental and geographic regularities of the formation of HAs and FAs molecular composition in the taiga and tundra soils of northeastern European Russia. The specific objectives were to (i) investigate the molecular composition and carbon species distribution in HAs and FAs of mineral and peat soils based on the application of CP/MAS ^{13}C NMR spectroscopy and to (ii) identify regularities of HA and FA structural composition changes in soils of various degree of hydromorphism in the taiga and tundra zones.

Materials and methods

Field sampling. — The study embraces eight key environments of pristine soils of the boreal (taiga) and polar (tundra) ecosystems of northeastern European Russia (Table 1). Soils types were identified according to WRB (FAO and IUSS 2015). The geographical setting of sampling sites is presented in Figure 1, whereas the climate description for the study regions is given in Table 2.

Soil sampling was conducted within different types of landscapes, from watersheds (automorphic well drained soils) to geochemically dependent landscapes (depressions, hydromorphic soils, peat soils). Short descriptions of the soils profiles and morphology are given in Table 3, basic characteristics

T a b l e 1

Study objects.

Study region	Vegetation type	Soil
South taiga	Sorrel-green-moss mixed birch-aspen-spruce woodland	Eutric Albic Retisol (Loamic, Ochric)
Middle taiga	Bilberry-green-moss birch-spruce woodland	Eutric Albic Retisol (Loamic)
	Haircap-sphagnum birch-spruce woodland	Eutric Albic Stagnic Histic Retisol (Loamic)
North taiga	Bilberry-green-moss spruce woodland	Eutric Albic Stagnic Retisol (Loamic)
	Sphagnum-haircap spruce woodland	Eutric Albic Stagnic Histic Retisol (Loamic)
South tundra	Willow-dwarf birch moss tundra	Stagnic Cambisol
	Moss and lichen tundra	Histic Gleysol
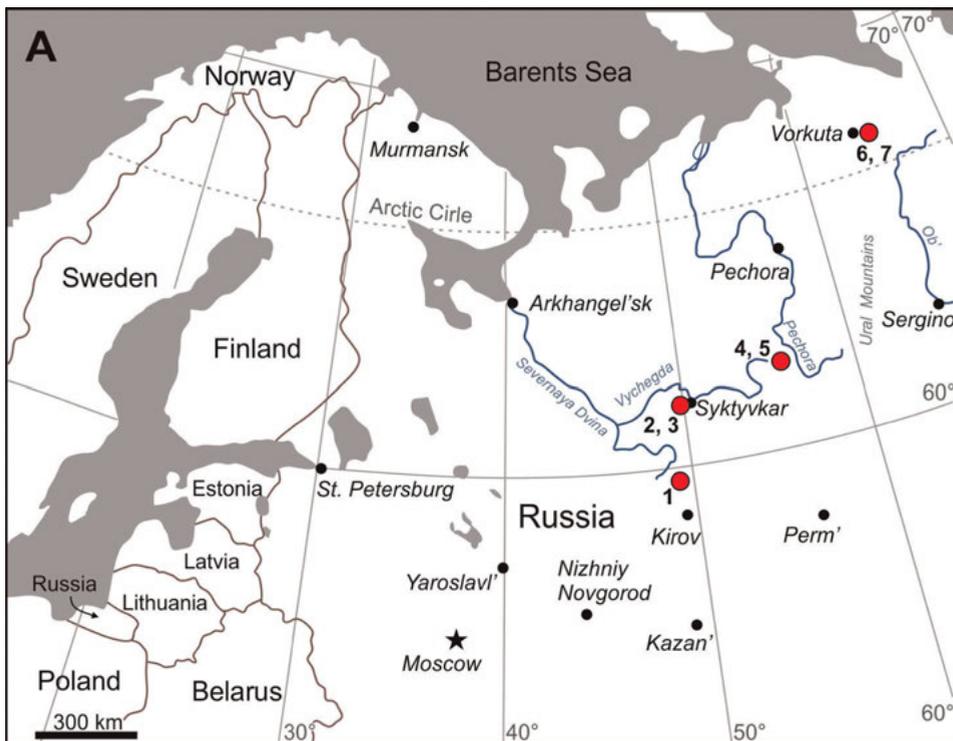
Fig. 1. Locations of sampling points. The south taiga: 1 – Eutric Albic Retisol (Loamic, Ochric); the middle taiga: 2 – Eutric Albic Retisol (Loamic), 3 – Eutric Albic Stagnic Histic Retisol (Loamic); the north taiga: 4 – Eutric Albic Stagnic Retisol (Loamic), 5 – Eutric Albic Stagnic Histic Retisol (Loamic); the south tundra: 6 – Stagnic Cambisol, 7 – Histic Gleysol.

Table 2

Climate parameters of the study regions.

Climate parameters	South taiga	Middle taiga	North taiga	South tundra
Mean annual air temperature, ºC	+1.3	+0.5	-1.1	-5.5
Mean air temperature, ºC: of the warmest month (July) of the coldest month (January)	+17.0 -14.3	+16.7 -15.3	+16.0 -17.8	+13.4 -20.1
Number of days with mean daily air temperature: > 0 ºC > 5 ºC > 10 ºC	202 153 110	187 145 102	175 135 92	125 90 43
Freezing depth, cm	43	89	101	139
Snow thickness, cm	61	57	80	60
Annual precipitation, mm	780	680	720	770

of soils are given in Table 4. The pictures from research site showing soil profiles were published in an earlier paper (Lodygin *et al.* 2017).

The south taiga soil was studied at the Letka permanent monitoring station. Eutric Albic Retisol (Loamic, Ochric) pit was sampled at the distance of 1 km northwestwards from the Krutotyla village of the Priluzsky district. It is a west-oriented gentle hill slope in birch-aspen-spruce forest (59°38' N, 49°21' E; 180 m a.s.l.). Slope angles do not exceed 2°. Nano- and micro relief formations vary within 23 cm, on slope – within 20 cm. They insignificantly increase by size and depth in hollow due to stem hillocks and wind erosions. Cover vegetation is dominated by *Vaccinium myrtillus* L*., Rubus saxatilis* L., *Pyrola* L*.,* and *Oxalis acetosella* L. plants. Green mosses are few.

The middle taiga soils were studied at the Maximovsky research station of the Institute of Biology. Eutric Albic Retisol (Loamic) pit was dug at the distance of 8 km westwards from the city of Syktyvkar on the top of a watershed hill at the back of microdepression of 1.5 m low (61°39' N, 50°41' E; 160 m a.s.l.). Vegetation is bilberry-green moss birch-spruce forest with many fallen trees. Eutric Albic Stagnic Histic Retisol (Loamic) pit was dug at the distance of 74 m from the previous one (61°39' N, 50°41' E; 160 m a.s.l.). It is a microhollow between low and flat elevations. Vegetation is long moss-sphagnum birch-spruce forest. Near the pit, there is a sphagnum cover.

The north taiga soils were studied at the distance of 3 km westwards from the Troitsko-Pechorsk settlement. Eutric Albic Stagnic Retisol (Loamic) under green-moss spruce forests covers the periphery part of the top. Eutric Albic

T a b l e 3

Morphological descriptions of soils.

Soil	Horizon	Depth of sampling, cm	Soil horizon description
South taiga			
Eutric Albic Retisol (Loamic, Ochric)	AY	2–6	mull humus, friable, loamy
	EL	12–28	loamy, compacted, ortsteins
Middle taiga			
Eutric Albic Retisol (Loamic)	O	0–5	friable organic material without histic features
	OEL	5–7	silty loam, friable, contain many roots and humus cutans
Eutric Albic Stagnic Histic Retisol (Loamic)	T	0–12	undercomposed with fresh organic remnants
	ELg	12–15	loamy, friable, many nodules of iron oxides
North taiga			
Eutric Albic Stagnic Retisol (Loamic)	O	0–5	slightly decomposed histic material with coals and wood remnants
	ELg	5–10	with diffusion accumulation of iron oxides, loamy
Eutric Albic Stagnic Histic Retisol (Loamic)	T	0–12	weak decomposed forest floor
	ELg	12–15	loamy with features of gleiyfication
	ELg	15–20	with iron spots and coatings, iron nodules, loamy silt
	BELg	20–25	heterogeneous, loamy
South tundra			
Stagnic Cambisols	O	0–5	undercomposed litter
	Bg	5–10	loamy, contain roots
Histic Gleysol	T1	0–10	histic undercomposed material
	T2	10–26	histic slightly composed material

Stagnic Histic Retisol (Loamic) develops under long moss and sphagnum-long moss spruce forests near the centre of the hill. Eutric Albic Stagnic Retisol (Loamic) pit was dug on the top of a flat inter-stream hill (62°41' N, 56°08' E; 140 m a.s.l.). Vegetation is bilberry-green moss spruce forest. Floor vegetation is dominated by *Vaccinium myrtillus* L. and green mosses. Eutric Albic Stagnic Histic Retisol (Loamic) pit was dug at the distance of 60 m from the previous cut on a gentle slightly drained near top hill slope (62°41' N, 56°08' E; 140 m a.s.l.).

Table 4

Basic characteristics of soils.

Horizon / depth, cm	pH $_{H_2O}$	pH $_{KCl}$	TOC, %	Gravimetric concentrations, %	
				HAs	FAs
South taiga					
Eutric Albic Retisol (Loamic. Ochric)					
AY 2–6	4.5	3.6	3.6±0.5	2.04±0.10	0.105±0.005
EL 12–28	5.0	3.8	0.81±0.19	0.012±0.002	0.009±0.001
Middle taiga					
Eutric Albic Retisol (Loamic)					
O 0–5	5.3	4.5	35.4±1.2	0.56±0.03	1.82±0.09
OEL 5–7	4.1	3.3	1.8±0.4	0.60±0.03	0.550±0.028
Eutric Albic Stagnic Histic Retisol (Loamic)					
T 0–12	4.2	3.5	34.3±1.2	1.10±0.06	1.83±0.09
ELg 12–20	4.8	3.9	0.77±0.18	0.85±0.04	1.05±0.05
North taiga					
Eutric Albic Stagnic Retisol (Loamic)					
O 0–5	4.7	4.3	27.8±1.0	0.80±0.04	0.515±0.026
ELg 5–10	4.3	3.3	1.22±0.28	0.19±0.01	0.054±0.003
Eutric Albic Stagnic Histic Retisol (Loamic)					
T 0–12	4.0	2.9	42.3±1.5	1.71±0.09	0.81±0.04
ELg 12–15	4.1	3.3	2.4±0.4	0.79±0.04	0.374±0.019
ELg 15–20	5.0	3.8	0.35±0.08	0.32±0.02	0.082±0.004
BELg 20–25	5.3	3.8	0.29±0.07	0.38±0.02	0.153±0.008
South tundra					
Stagnic Cambisol					
O 0–5	4.6	3.7	18.4±1.8	1.67±0.08	1.37±0.07
Bg 5–10	4.3	3.0	0.46±0.11	0.43±0.02	0.180±0.009
Histic Gleysol					
T1 0–10	3.7	3.1	27.9±2.8	0.86±0.04	0.564±0.028
T2 10–26	3.8	2.6	31.3±1.1	1.35±0.07	0.59±0.03

Vegetation is sphagnum-long moss spruce forest. Vegetation cover is dominated by hypnum mosses, *Vaccinium vitis-idaea* L., *Equisetum* L., *Carex globularis* L., and *Rubus chamaemorus* L.

The tundra soils were studied in the Bolshezemelskaya tundra area in the Vorkuta district of the Komi Republic with massive-island permafrost distribution. The area is a gently undulating plain covered with silty loams being less than 10 m thick. Typical soil formation processes in the tundra zone are stagnic processes (gleyzation) and peat accumulation (Lodygin *et al.* 2014). Stagnic Cambisol pit was dug on a gentle slope of the Nerusovei-musyur moraine hill (67°31' N, 64°07' E; 220 m a.s.l.). It is a willow and dwarf birch mossy small-hummock tundra area. Vegetation is dominated by hypnum and some polytrichum mosses, *Vaccinium vitis-idaea* L., rare *Carex* L. and single *Betula nana* L. representatives. Histic Gleysol pit was dug in the centre of the south--western slope, with the slope angle of 3º, at the distance of 100 m southwards from the previous pit (67°35' N, 64° 09' E; 140 m a.s.l.).

The data on the soil basic properties (Table 4) showed that the total organic carbon (TOC) content in all Retisols was high in the superficial layers and low in mineral horizons. The excessive moisture significantly affected TOC and HS accumulation in hydromorphic soils (Eutric Albic Stagnic Histic Retisol (Loamic) and Histic Gleysol). This was indicative of the low decomposition and transformation degree of the precursor material and was comparable to the data on soils from the Yamal tundra (Ejarque and Abakumov 2016).

The acidic reaction of mineral soils was due to the high content of FAs which migrated from the ground litter. The lowest pH_{H2O} values were characteristic of top layers from the tundra soils which was associated with a high content of low-molecular-weight acids (Shamrikova *et al.* 2015).

Soil analysis. — Air-dried soil samples were homogenized and sieved through a 1 mm sieve. Carbon content was determined using an element analyzer EA-1110 (Carlo-Erba, Italy) in the Chromatography Common Use Center (Institute of Biology) and pH in water and in KCl suspensions using a pH meter pH-150 M.

Sample preparation. — The powders of HAs and FAs were extracted from the soil samples by double-extractions with 0.1 mol/dm^3 NaOH according to the IHSS recommendations (Swift 1996). The HAs were desalinated by dialysis. The FAs were purified on activated carbon AG-3 and desalinated on cationic KU-2 (H^+ form) (Lodygin and Beznosikov 2010). The powders of HAs and FAs were obtained by heating at 35°C in the baker.

^{13}C NMR measurements. — Measurements were taken with a JNM-ECA 400 (JEOL) NMR spectrometer (100.53 MHz for ^{13}C). Solid-phase samples were placed in a 4-mm zirconium oxide rotor and spun at the frequency of 6 kHz at the magic angle. The cross-polarization sequence of excitation pulses was used for the registration of ^{13}C spectra (CP/MAS). The contact time was 5 ms, the delay time was 5 s, and the number of scans was 13,000. Chemical shifts were

referenced to low field signal of adamantane at 38.48 ppm. The Fourier transform method with the subsequent adjustment of a base line was used for spectra treatment. Quantitative treatment was carried out via numerical integration by the areas corresponding to the positions of functional groups and molecular fragments using program Delta v. 5.0.2. (JEOL, Japan).

A ternary graph was used for the numerical description of the structural-group composition of humic and fulvic acids. The vertex of the triangle x(C,H--AL) corresponds to 100 % of the molar fraction of carbon atoms of C,H-substituted aliphatic fragments (the signals were summed for the regions of 0–47 ppm), the corner x(Ar) corresponds to 100 % of the molar fraction of carbon atoms of aromatic fragments (the signals were summed for the regions of 108–144, 144–164 and 183–190 ppm), and the corner x(O,N-AL) corresponds to 100 % of the molar fraction of carbon atoms of O,N-substituted aliphatic fragments (the signals were summed for the regions of 47–60, 60–108, 164–183 and 190–204 ppm). Any point on the surface of the triangle defines the composition of functional groups and molecular fragments of HAs and FAs. The graph was built with the use of Tri-plot for MS Excel (Graham and Midgley 2000).

Statistics. — Bivariate correlation analyses were conducted using the Pearson product-moment correlation coefficient (r) and its statistical significance was assessed via Neyman-Pearson's (NP) approach. Technically, the observed value of the coefficient (based on n pairs) was compared against the critical value (r_{cr}) for a two-tailed test and significance level (α_{NP}) of 0.05.

Results and discussion

Structural molecular composition of HSs (^{13}C NMR). — All ^{13}C NMR spectra of HA and FA samples obtained are characterized by broad absorption bands resulting from overlapping of several signals (Fig. 2). Due to intense peak overlap, signals in solid-state ^{13}C NMR spectra can be correlated to chemical shift ranges only, according to the position of resonance of atoms with a similar chemical environment.

The maximum intensity can be observed in the range of unsubstituted aliphatic fragments (Fig. 2). All ^{13}C NMR spectra have one relatively sharp peak in the 30 ppm region, which can be related to methylene atoms of carbon in positions α, β, δ, and ε from methyl end-groups (15 ppm) in alkyl chains (Simpson and Simpson 2009). These methylene carbon atoms, as suggested by some researchers, may result from accumulation of wax resins, lipids, and suberin plant structures (Lodygin and Beznosikov 2005; Alarcón-Gutiérrezet *et al.* 2009; Vasilevich *et al.* 2018). All spectra also show a signal in the range of 47–60 ppm, which some authors relate to methoxyl groups resulting from the presence of lignin fragments, *i.e.* syringil and guaiacyl propane units (Kovaleva
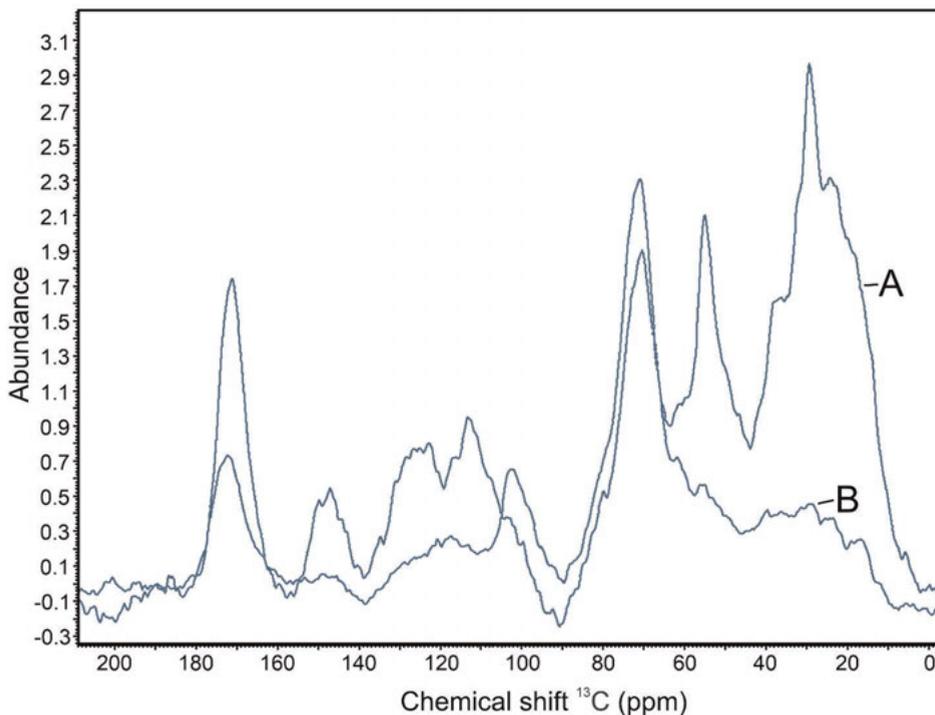
Fig. 2. CP/MAS ^{13}C NMR spectra of HAs (A) and FAs (B) isolated from the north taiga Eutric Albic Stagnic Retisol (Loamic).

and Kovalev 2015; Tadini *et al.* 2015). However, in this chemical shift region one can also observe signals from carbon atoms in the α position in polypeptides (–C(O)–**C***(R)H–NH–)$_n$ (Knicker *et al.* 2008). The reliably high correlation rate ($r = 0.51$, $n = 34$, $r_{cr} = 0.34$) between the signal intensity in the range of 47–60 ppm and mass fraction of nitrogen in the humic substances under study serves as an indirect evidence of the last-mentioned suggestion.

A very intense signal at 71 ppm in the spectra is characteristic of carbons in CH(OH) groups, such as those of the ring carbons of carbohydrates (Simpson and Simpson 2009). Resonance in this spectral region spreads from 64 to 90 ppm, which is likely to originate from the various CH(OH) groups of cellulose or other carbohydrate structures (Keeler *et al.* 2006). Besides this spectral signature, additional signals of carbohydrate-like structures were further identified in the majority of the HA and FA ^{13}C NMR spectra. A weak signal at about 62 ppm is typical of CH$_2$O hexose groups in polysaccharide fragments (Duarte *et al.* 2008). The presence of signal at 101 ppm, which is representative for anomeric (semiacetal) carbon atoms, also attests to the presence of carbohydrate fragments in HA and FA structures. The most intensive signals in the "carbohydrate" region are observed for FAs.

In the region of aromatic fragments, the signal at 108–144 ppm may be produced by unsubstituted and/or alkyl-substituted aromatic carbon atoms. The peak

at 147–149 ppm is typical of the spectra of lignin structural blocks, being referred to the oxygen-substituted carbon atoms of aromatic rings, *i.e.* syringil-and guaiacyl propane units (Keeler *et al.* 2006; Kovaleva and Kovalev 2015). ^{13}C NMR spectra of HAs show more intense signals in this area as compared to FAs (Fig. 2).

In the region 164–183 ppm with a maximum at 171–173 ppm, fixed atoms of carboxyl fragments occur (Lodygin and Beznosikov 2010), but they can also refer to the carbonyl-amide group (Keeler *et al.* 2006). The atoms of quinone fragments and the carbonyl groups of ketones and aldehydes provide weak signals in the ranges of 183–190 and 190–204 ppm, respectively. Detailed interpretations of the ^{13}C NMR spectra are given in Table 5.

The comparative study of the molecular composition of HA and FA samples (Table 5) showed that all mineral soil FA samples are enriched by oxygen-containing fragments, which ensures their solubility in water and high migration rate within the soil profile. High concentration of carboxyl groups (up to 27.1%) within the FA structure provides for their acid "aggression" against soil minerals and the ability to generate complex compounds with iron, aluminum and other metal cations converting them in soluble forms.

High portion of carbohydrate fragments (up to 54.1% in mineral soils and up to 80.1% in peat soils) in the FA structure makes FAs a more preferable substance for soil microbiota than HAs, which contain over fifty percent of aromatic fragments and aliphatic chains and are energetically more difficult to be oxidized. This fact is supported by a reliably lower concentration of carbohydrate fragments in the molecular structure of HAs and FAs from mineral horizons than the ones from HAs of organic horizons.

Humic acids of all soils under study are characterized by a higher content of unsubstituted molecular fragments, both aliphatic and aromatic ones, than FAs, which is clearly observed through isolated positions of these SOM classes at their structural and functional composition diagram (Fig. 3).

The assessment of composition of HAs main functional groups and molecular fragments proves a significant variation of their molecular composition in going from organic horizons to mineral ones within all profiles of the soils under study (Table 5). Thus, HAs from organic horizons are enriched by carbohydrate, amino and methoxyl groups. Their share decreases down along the profile, seemingly as a result of microbial destruction and oxidation processes (Lodygin and Beznosikov 2010; Vasilevich and Beznosikov 2017). At the same time, there is an increased portion of paraffin chains in HSs. Since oxidation of such structures is less beneficial thermodynamically, if compared to O,N-alkyls, they tend to accumulate in HAs and FAs. The profile distribution of the content of HAs molecular fragments from peat soils (Vasilevich *et al.* 2018) has some peculiarities, as opposed to the ones from soils: the portion of unsubstituted aliphatic components decreases significantly, while the reduction of carbohydrate fragments is less intense. The profile distribution of unoxidized aliphatic fragments of peat soil HAs demonstrates a statistically significant negative correlation with the portion of HA

Table 5

Percentage of carbon in the main structural fragments of HAs and FAs (according to ^{13}C NMR).

Horizon/ depth, cm	Chemical shift, ppm							
	0–47	47–60	60–108	108–144	144–164	164–183	183–190	190–204
	C,H-Alkyl	O,N-Alkyl		Aromatic		Carboxyl, ester, amide	Quinone	Carbonyl
South taiga								
Eutric Albic Retisol (Loamic, Ochric)								
AY 2–6	26.8¹ / 13.5	8.6 / 9.1	16.9 / 54.1	25.6 / 9.8	6.5 / 1.0	12.9 / 11.7	1.7 / 0.3	1.0 / 0.5
EL 12–28	44.3 / 18.1	8.7 / 6.1	11.6 / 49.7	17.1 / 11.7	0.2 / 0.9	14.2 / 12.3	2.1 / 0.7	1.9 / 0.5
Middle taiga								
Eutric Albic Retisol (Loamic)								
O 0–5	22.4 / 23.3	8.1 / 6.6	12.8 / 23.2	33.5 / 18.6	10.0 / 7.4	11.3 / 19.6	0.5 / 0.6	1.4 / 0.7
OEL 5–7	40.7 / 25.1	8.6 / 6.4	8.9 / 18.6	18.8 / 10.3	7.1 / 4.2	12.1 / 25.0	1.7 / 3.2	2.1 / 7.2
Eutric Albic Stagnic Histic Retisol (Loamic)								
T 0–12	23.2 / 19.5	6.0 / 2.4	11.8 / 20.2	22.4 / 12.9	8.8 / 4.2	17.0 / 26.3	4.3 / 5.1	6.5 / 9.4
ELg 12–20	50.5 / 31.2	2.8 / 2.7	7.3 / 17.9	16.9 / 8.7	4.7 / 3.3	12.8 / 27.1	2.7 / 2.5	2.3 / 6.6
North taiga								
Eutric Albic Stagnic Retisol (Loamic)								
O 0–5	42.5 / 17.2	12.6 / 9.6	18.4 / 53.4	14.2 / 8.0	2.8 / 1.3	9.3 / 10.5	0.0 / 0.0	0.0 / 0.1

Table 5 continued

Horizon/ depth, cm	Chemical shift, ppm							
	0–47	47–60	60–108	108–144	144–164	164–183	183–190	190–204
	C,H-Alkyl	O,N-Alkyl		Aromatic		Carboxyl, ester, amide	Quinone	Carbonyl
ELg 5–10	47.6 / 17.8	8.6 / 7.5	11.5 / 43.8	14.3 / 14.3	3.7 / 3.5	13.4 / 11.7	0.5 / 0.4	0.3 / 1.0
Eutric Albic Stagnic Histic Retisol (Loamic)								
T 0–12	40.1 / 18.3	6.6 / 7.4	16.0 / 45.0	20.9 / 12.0	4.2 / 3.4	10.4 / 13.9	0.8 / 0.0	1.0 / 0.1
ELg 12–15	33.4 / 17.2	6.1 / 6.5	15.6 / 38.9	25.3 / 12.3	5.2 / 2.3	12.1 / 22.3	0.9 / 0.3	1.4 / 0.2
ELg 15–20	58.3 / 21.4	4.8 / 7.1	6.5 / 23.5	17.3 / 20.0	1.9 / 4.6	10.6 / 21.7	0.2 / 0.3	0.4 / 1.4
BELg 20–25	51.9 / 21.0	4.2 / 5.8	7.8 / 16.6	20.5 / 26.7	2.0 / 6.5	12.0 / 20.8	1.3 / 1.2	0.5 / 1.4
South tundra								
Stagnic Cambisol								
O 0–5	28.8 / 18.5	10.5 / 7.0	26.5 / 34.2	15.9 / 17.0	5.7 / 5.7	12.5 / 15.7	0.0 / 0.3	0.1 / 1.6
Bg 5–10	50.4 / 13.6	7.9 / 6.5	10.1 / 33.7	15.4 / 20.3	3.8 / 5.9	12.4 / 18.3	0.0 / 0.2	0.2 / 1.4
Histic Gleysol								
T1 0–10	28.3 / 18.1	10.5 / 8.2	26.9 / 42.0	16.2 / 11.2	6.1 / 2.1	11.8 / 16.9	0.0 / 0.7	0.2 / 0.8
T2 10–26	27.4 / 19.6	10.0 / 7.7	25.0 / 23.2	18.0 / 24.6	6.9 / 9.4	11.7 / 13.7	0.3 / 0.3	0.7 / 1.4

[1] Data for HAs (underlined) and FAs.
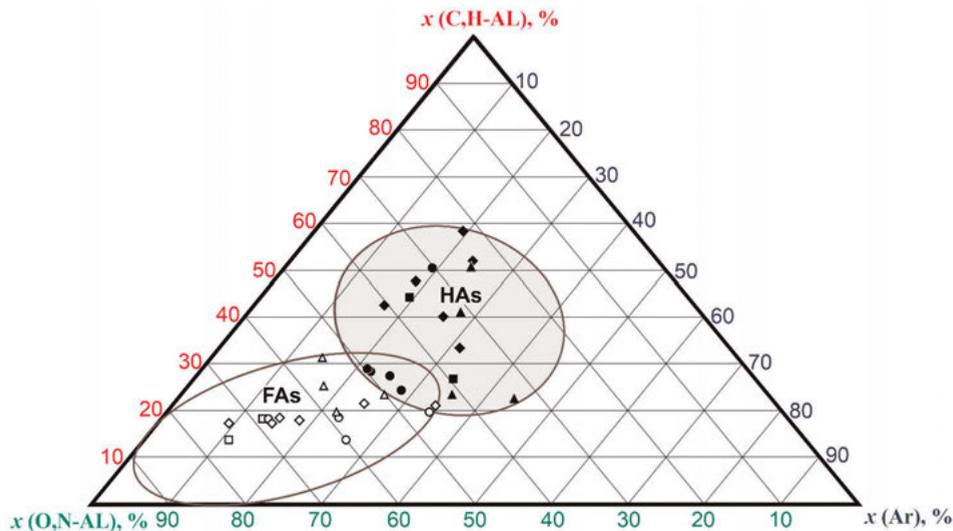
Fig. 3. Diagram of structural and functional composition of HSs from soils. The south taiga: HAs – black squares, FAs – white squares; the middle taiga: HAs – black triangles, FAs – white triangles; the north taiga: HAs – black diamonds, FAs – white diamonds; the south tundra: HAs – black circles, FAs – white circles.

aromatic fragments (r = -0.95, n = 18, r_{cr} = 0.47), which may indicate the cyclization of aliphatic chains with subsequent dehydrogenation and the formation of aromatic fragments (Vasilevich *et al.* 2018).

The portion of carboxylic groups in HAs of automorphic soils increases from organic horizons to mineral ones, while semihydromorphic soils show an opposite relation (Table 5), which, in our opinion, can be explained by anaerobic processes and shortage of oxygen under long exposure to excessive moistening, leading to a changed composition of carboxylic groups.

Changes in soil FAs composition are better expressed further away from organic horizons, and closer to mineral ones, than in HAs (Table 5). Here, we still observe the trend for lowering of portion of carbohydrate fragments down the profile. The content of carboxylic groups grows in FAs from mineral horizons of both automorphic and semihydromorphic soils, which results from good FA solubility in water, in contrast to HAs, and higher migration capacity of FAs in case of a larger portion of carboxylic groups in their structure.

A statistically significant negative correlation ($r_{\text{HA-COOH and pH}}$ = -0.62, $r_{\text{FA-COOH and pH}}$ = -0.66, n = 14, r_{cr} = 0.53) was noted between the content of carboxylic groups in the structure of HAs and FAs, respectively, and integral parameter of soil acidity (pH_{H2O}) for southern and middle taiga soils, which indicates a significant contribution of specific organic compounds in actual acidity of soils. The absence of this relation in northern taiga and southern tundra soils is possibly related to substantial accumulation of low-molecular-weight organic acids building acidity (Shamrikova *et al.* 2018).

When comparing the molecular structure of HSs in soils of different degrees of hydromorphism, it should be noted that HAs and FAs extracted from wetter soils (Eutric Albic Stagnic Histic Retisol (Loamic)) are richer in alkyl fragments than HAs and FAs from automorphic soils (Eutric Albic Retisol (Loamic)) (Table 5). This can be explained by anaerobic conditions of humification, but also by a higher share of mosses and lichens in the dead soil cover that have a higher content of aliphatic compounds than tree vegetation (Orlov 1995). In addition, excess humidity reduces microbiological activity (Khabibullina *et al.* 2014) in Eutric Albic Stagnic Histic Retisol (Loamic), thus slowing down soil organic matter transformation and contributing to accumulation of paraffin fragments in the structure of humic substances.

To standardize the qualitative characteristics of HS macromolecules the following parameters were used: the ratio of aromatic to aliphatic carbon – Ar/AL (Lorenz *et al.* 2006), the percentage of aromaticity – Ar/(Ar+AL) (Liang *et al.* 1996) and the degree of organic matter decomposition (C,H-alkyl/O,N--alkyl) (Pedersen *et al.* 2011). Moreover, an integral index of molecule hydrophobicity was used for humic substances ($AL_{H,R}+Ar_{H,R}$), which calculated the sum of unoxidized atoms of carbon, *i.e.* the ones substituted by hydrogen atoms or other aliphatic fragments (Table 6), providing information about the amphiphilic properties of HSs (Lodygin *et al.* 2014).

The analysis of integral indices of molecular composition of humic substances revealed that HAs are generally more resistant to oxidation (including the microbial one) than FAs (Table 6). The total share of unoxidized atoms of carbon in HAs falls in the range of 44.5–75.6 %, while for FAs this index is much lower – 9.4–47.7 %. In the taiga zone, HAs of mineral horizons Eutric Albic Stagnic Histic Retisol (Loamic) are the most hydrophobic, while in tundra – these are HAs from Hemic Folic Cryic Histosol (Vasilevich *et al.* 2018), which is preconditioned by excess humidification and anaerobic processes of such soils. Notably, for HAs the degree of hydrophobicity is typically decreasing from organic to mineral horizons, while for FAs the trend is in the opposite direction. This feature is preconditioned by water solubility of FAs and the ability of most oxidized and hydrophilic molecules of FAs to migrate down the soil profile.

The C,H-alkyl/O,N-alkyl ratio reflecting the degree of organic matter decomposition is at its highest for HA samples and falls within the range from 0.7 to 5.2. For FAs, the values are lower and lie in the range of 0.1–1.5. This indicator increases sharply in the shift from organic to mineral horizons for both HA and FA samples, which proves a high degree of humification of humic substances in mineral horizons. HS samples from tundra soils have a slightly lower ratio than the one of HAs and FAs of taiga soils (Table 6), which serves as the evidence of low degree of organic matter decomposition in tundra soils.

The study of the molecular composition of humic acids of tundra soils demonstrated that the tundra HA samples had lower content of aromatic structures and contained a high portion of aliphatic fragments, which makes them

Table 6

Integrated indicators of HS macromolecules.

Horizon /depth, cm	Ar / AL[1]	Aromaticity, %	$AL_{H,R}+Ar_{H,R}$[2], %	C,H-Alkyl / O, N-Alkyl
South taiga				
Eutric Albic Retisol (Loamic, Ochric)				
AY 2–6	0.51[3] / 0.13	33.7 / 11.1	52.4 / 23.4	1.0 / 0.2
EL 12–28	0.24 / 0.15	19.3 / 13.3	61.3 / 29.9	2.2 / 0.3
Middle taiga				
Eutric Albic Retisol (Loamic)				
O 0–5	0.79 / 0.36	44.0 / 26.6	55.9 / 41.9	1.1 / 0.8
OEL 5–7	0.38 / 0.22	27.6 / 17.7	59.5 / 35.4	2.3 / 1.0
Eutric Albic Stagnic Histic Retisol (Loamic)				
T 0–12	0.55 / 0.29	35.5 / 22.2	45.6 / 32.4	1.3 / 0.9
ELg 12–20	0.32 / 0.17	24.3 / 14.5	67.4 / 39.9	5.0 / 1.5
North taiga				
Eutric Albic Stagnic Retisol (Loamic)				
O 0–5	0.21 / 0.10	17.1 / 9.3	56.8 / 25.2	1.4 / 0.3
ELg 5–10	0.23 / 0.22	18.6 / 18.2	61.9 / 32.1	2.4 / 0.3
Eutric Albic Stagnic Histic Retisol (Loamic)				
T 0–12	0.35 / 0.18	25.9 / 15.4	61.1 / 30.3	1.8 / 0.3
ELg 12–15	0.46 / 0.18	31.4 / 15.0	58.7 / 29.5	1.5 / 0.4
ELg 15–20	0.24 / 0.33	19.3 / 24.9	75.6 / 41.4	5.2 / 0.7
BELg 20–25	0.31 / 0.52	23.7 / 34.3	72.4 / 47.7	4.3 / 0.9

Table 6 continued

Horizon /depth, cm	Ar / AL[1]	Aromaticity, %	$AL_{H,R}+Ar_{H,R}{}^{2}$, %	C,H-Alkyl / O, N-Alkyl
South tundra				
Stagnic Cambisol				
O 0–5	0.27 / 0.30	21.5 / 23.0	44.7 / 35.4	0.8 / 0.4
Bg 5–10	0.24 / 0.36	19.2 / 26.4	65.7 / 34.0	2.8 / 0.3
Histic Gleysol				
T1 0–10	0.29 / 0.16	22.3 / 13.9	44.5 / 29.3	0.8 / 0.4
T2 10–26	0.34 / 0.52	25.2 / 34.3	45.4 / 44.2	0.8 / 0.6

[1] The signals from the aromatic structures (Ar) were summed for the regions of 108–164 and 183–190 ppm; those from the aliphatic structures (AL) were summed for the regions of 0–108, 164–183, and 190–204 ppm.
[2] The signals were summed for the regions of 0–47 and 108–144 ppm.
[3] Data for HAs (underlined) and FAs.

similar to FAs of taiga soils in terms of their relative composition. The analytical data for tundra soils obtained demonstrate that excess humidification leads not only to HS accumulation, but also preconditions the accumulation of HS rich in aliphatic structures in hydromorphic soils (Fig. 3).

The specific character of tundra humification lies in levelling-off of structural and functional parameters of major classes of specific organic compounds of soils – HAs and FAs (Fig. 3), which proves Orlov's (1995) theory on lower zonal differentiation of humic substances in going towards the Arctic. The results obtained with regard to the structural and functional composition of humic substances of tundra soils, correspond to the literature data and are similar with the ones related to HSs of Alaska tundra soils (Dai *et al.* 2001), the European Arctic (Polyakov *et al.* 2019) and the Antarctic (Chukov *et al.* 2015; Nadporozhskaya *et al.* 2017).

When going north from southern taiga to southern tundra, a significant transformation of the molecular composition of HAs can be observed, which is expressed through the statistically significant increased portion of labile carbohydrate fragments (Fig. 4). Lower temperatures in tundra soils lead to reduced microbiological activity (Khabibullina *et al.* 2014), thus leading to a slower organic matter humification process and promoting accumulation of thermodynamically unstable carbohydrate structures in humic substances. The comparative study of the data on HS samples of taiga and tundra soils under
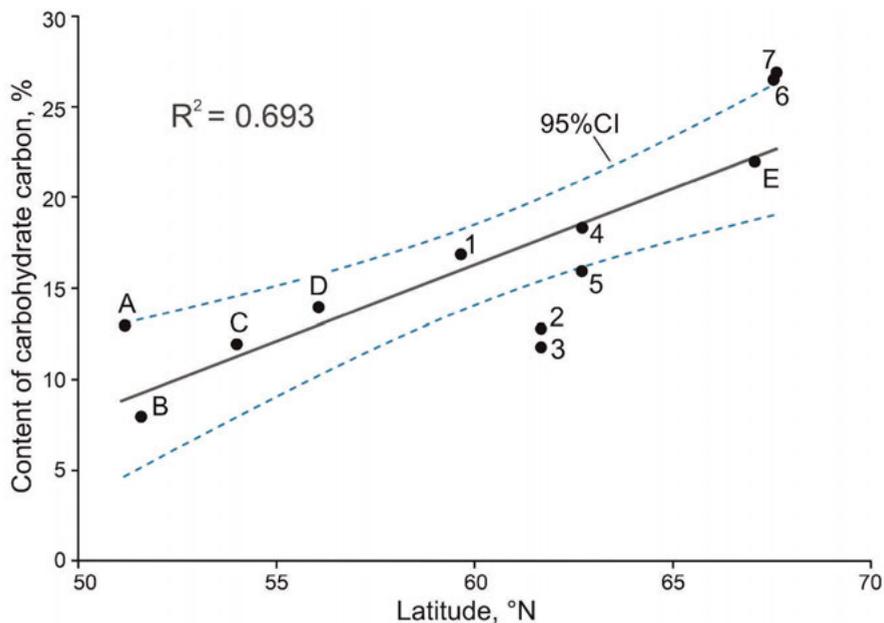
Fig. 4. Content of HA carbohydrate fragments from soil organic horizons. The steppe: A, B – Vermic Chernozems; the forest-steppe: C – Albic Luvisol; the south taiga: D, 1 – Eutric Albic Retisol (Loamic, Ochric); the middle taiga: 2 – Eutric Albic Retisol (Loamic), 3 – Eutric Albic Stagnic Histic Retisol (Loamic); the north taiga: 4 – Eutric Albic Stagnic Retisol (Loamic), 5 – Eutric Albic Stagnic Histic Retisol (Loamic); the south tundra: 6 – Stagnic Cambisol, 7 – Histic Gleysol. A, B, C, D – data after Kholodov *et al.* (2011), E – Hemic Folic Cryic Histosol (Vasilevich *et al.* 2018).

study, and the results on the structural composition of humic acids of soils from other climatic zones demonstrated that the HAs are more enriched by carbohydrate structures than HSs of soils of Central and Southern Europe, such as Albic Luvisols and Vermic Chernozems (Kholodov *et al.* 2011).

Conclusions

The study focused on the effect of environmental and geographic factors (latitude, temperature and moisture degree) on molecular composition of HAs and FAs from taiga and tundra soils of the northeastern European Russia. It was shown that excess humidification affects HS accumulation in a statistically significant manner and influences their molecular structure: hydromorphic taiga soils accumulate HSs enriched by unoxidized aliphatic fragments, tundra soils – the ones enriched by carbohydrate fragments. The study also revealed the difference in the profile transformation of HSs of mineral and peat soils related to accumulation of paraffin chains in mineral soil HSs and aromatic carbon atoms in peat soil HSs. Various conditions of soil genesis predefine the specific character

of structural and functional parameters of HSs in southern taiga to southern tundra soils, as expressed through the increased portion of labile carbohydrate and amino acid fragments, and methoxyl groups within the structure of HSs of tundra soils. The specific character of tundra humification lies in levelling-off of structural and functional parameters of major classes of specific organic compounds of soils – HAs and FAs.

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