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Changes in Molecular Structure of Humic Substances in Cambisols under Agricultural Use

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Abstract: Soil organic matter stability and transformation affected by agriculture is a global problem of great concern. This study aimed to reveal structural changes in humic molecules under conventional soil tillage in Cambisols of the Transbaikal area. Humic acids were isolated from the humus horizons of native and arable variants of Eutric Cambisol Cryic (gleyic, humic, loamic) from the forest steppe, and Haplic Cambisol (arenic, humic, protocalcic) from the steppe zone. The changes in the humic substances' quality were evaluated by means of an elemental analysis and ^{13}C nuclear magnetic resonance spectroscopy. The results indicate that different agronomic management affected the changes in the composition and molecular structure of humic acids. Soil tillage induced an increase in the carbon content, the proportion of aromatic compounds and carboxyl groups and the depletion in nitrogen. As measured by ^{13}C NMR spectroscopy, the intensity of these changes was determined by soil properties and the hydrothermal regime. Organic matter from Eutric Cambisol Cryic was suggested as providing significant environmental resistance to the agricultural impact due to the optimization of the water regime and a loamy texture. Prolonged ploughing of sandy Haplic Cambisol under the arid conditions promoted deep changes in the humic substances' composition and chemical structure.

Keywords: soil organic matter; arable soil; conventional tillage; elemental analyses; ^{13}C NMR spectroscopy; Transbaikal area

1. Introduction

Soil degradation affected by agriculture is a global problem and a current challenge concerning both the loss of soil fertility and elevated rates of CO_2 emissions to the atmosphere. As a complex multifunctional system, soil regulates carbon fluxes. Soil carbon produced as a result of respiration and organic substances' mineralization involves the carbon cycle and affects climate warming [1–3]. Positive carbon balance is mainly common for natural soils where the processes of C immobilizing in the form of relatively stable components of soil organic matter (SOM) prevail. Most thermodynamically stable compounds such as humic substances (HSs) are formed through the humification process [4,5]. Stable humic molecules are subjected to a lower rate of mineralization, and therefore, reduce CO_2 releasing from the soil [6].

Over the last few years, different concepts of the molecular organization of humic substances have been the subject of heated debates among scientists. According to the International Humic Substances Society (2022), HSs are mostly considered as a group of polydisperse and heterogeneous compounds formed during the decay and transformation of organic material in the diversity of the environments. Thus, SOM composition reflects its evolution and interaction with surroundings. The possibility of forming the HS specific groups under definite conditions of moisture, temperature and oxidation regime was shown in the experiment [5]. Humic acids (HAs) are the components of humic substances held together mainly by van der Waals and other interactions, hydrogen and metal-bridged intermolecular electrostatic bonds [6]. HAs stably exist in the soil in the form of organic-mineral

complexes and perform a multitude of ecological functions regulating physicochemical and biological soil properties due to a complex structure and a large number of different functional groups [7]. SOM quality dynamics should be taken into account for the estimation of organic matter stability [8,9]. More stable compounds are formed with the increase in the humification degree, which means the microbiological destruction of labile components and an increase in the rate of the aromatic part [4,10,11].

The agricultural use of soil affects soil properties and SOM quality and quantity due to tillage activity, stimulating aeration, oxidative biological processes and decomposition of SOM, different fertilizing additives for crop growing, the removal of nutrients with the harvest and soil compaction by heavy machinery [12–14]. Previously, a broad range of studies highlighted problems of soil agricultural use with the aspects of carbon and nitrogen losses evaluation, humus fractionation and degradation [15,16]. This important intermediary stage of SOM investigation has been gradually changed to the detailed study of the HSs' chemical structure transformation as affected by different agronomic practices with the development of modern research techniques and equipment. Thus, Lodygin E. and Abakumov E. demonstrated an essential increase in the proportion of aromatic components and depletion in the content of carboxyl and ester groups in the HA structure of arable soils [17]. Zhang et al. revealed the mineral fertilizers applied leads to form less aromatic HAs [18]. The importance of crop rotation and no tillage method for HSs' stability was confirmed [19].

Advances in the technology of the nuclear magnetic resonance method provided detailed information not only about types of atomic groups and bonds, but also the quantity parameters and positions of molecular fragments without any treatment of the research object and molecule destruction [20,21]. For this reason, it is widely now used for the molecular characterization of individual soil components, such as humic substances, and for the assessment of chemical attributes and changes in the composition and structure under various impacts, both natural and anthropogenic [22,23].

Publications on the changes in the structure and element composition of HAs under different types of agricultural management in the soils of the Transbaikal area have not been found. This work aimed to assess the changes in the composition and molecular structure of HAs from Cambisols as affected by the tillage making use of ^{13}C NMR spectroscopy and elementary analyses. The data provided could reveal comprehension about organic matter stability in the soils of the region under an agricultural impact.

2. Materials and Methods

Two types of soils within the Transbaikal territory (Russian Federation) with different conditions of the hydrothermal regime were studied: Eutric Cambisol Cryic (Gleyic, Humic, Loamic) from the permafrost forest-steppe subzone, and Haplic Cambisol (Arenic, Humic, Protocalcic)–in steppe (Figure 1). Soils were classified according to [24]. Two variants of each soil type have been chosen for the study: those with no tillage (native soil) and arable ones. The development period was about 50 years. Most soils in the region had a weak developed profile and low thickness of the humus horizon due to severe natural and climatic conditions and the predominance of a physical weathering of rocks. It determined a small depth of plough for crop cultivation. Conventional tillage with the 20 cm deep moldboard has been used annually in the arable soils under the study.

The territory of the Transbaikal region is mountainous, of an extra-continental climate with a long, cold winter, and short, but warm, summer. The spatial heterogeneity of parent rocks and vegetation is marked in the region, as well as a variety of soil cover. The main nature zone is mountainous taiga. Steppe landscapes mainly occupy the southern, southeastern and southwestern slopes of low and middle mountainous areas, level parts of river valleys and drainless hollows.

Figure 1. Location of the sampling area: Plots 1 and 2-Eutric Cambisol Cryic (gleyic, humic, loamic); Plots 3 and 4-Haplic Cambisol (arenic, humic, protocalcic).

The plot of the Eutric Cambisol Cryic (gleyic, humic, loamic) native soil (Plot 1) is located at the southern part of the Vitim Plateau, on the territory of the field station of the Institute of General and Experimental Biology SB RAS (Republic of Buryatia, Eravninsky district, Sosnovo-Ozerskoe village (N52°30,894′, E111°32,738′, 963 m asl)). A plot of the arable soil (Plot 2) was laid at a distance of 200 m east from the native soil profile on the field with crops of oats. There is the southern border of the cryolithozone with a discontinuous type of permafrost distribution within this area. Currently, in the summer season, the permafrost table is fixed at a depth of 2.75–2.80 m [25]. Over the last decades, the degradation of the permafrost level was noted followed by an increase in the mean annual temperature [26]. The average sum of the positive temperature is from 1250 to 1550 °C, and the mean annual precipitation is 270–310 mm.

Eutric Cambisol Cryic (gleyic, humic, loamic) occurs on the lacustrine plain of Eravninskaya hollow under meadow steppe vegetation. Dominant plant species: *Potentilla acervata* Sojak, *Elytrigia repens* L. Nevski, *Vicia cracca* L., *Artemisia vulgaris* L., *Leontopodium campestre* (Ledeb.) Hand.-Mazz. The total projective cover is 60%. The soil is characterized by a loamy dark humus horizon of 0.25 m thickness, and a tongue border, with a fine subangular structure, high organic carbon content and neutral pH (Table 1). The carbonate horizon begins from a depth of 0.7 m, and it is of a light brown color, with a heavy loamy texture with an angular blocky structure. The soil moisture level increases down the soil profile as well as signs of gleying. The soil has cryogenic cracks and features of cryoturbations, and contains gravel and stones.

The plots of Haplic Cambisol (arenic, humic, protocalcic) are located within the intermountain steppe hollows of the southeastern Transbaikalia (Zabaykalsky region, Mogoytuysky district, Tsagan-Ola (51°36′21″ N, 115°49′35″ E) (Plot 3—native soil, Plot 4—arable one). The distance between these variants is 50 m. Arable soil is cultivated with rye. The level of the mean annual precipitation in the area is 300–350 mm. The frostless period lasts 90–110 days, and the average sum of positive temperature values varies from 1750 to 2000 °C. Soils are long seasonally frozen. Weakly weathered sandy gravelly proluvial-deluvial strata are the main parent material.

Table 1. Soil's physical and chemical properties.

Horizon	Depth	pH_{H_2O}	Organic Carbon, %	Total Nitrogen, %	P_2O_5, mg/100 g	Texture Fractions, %		
						2–0.05	0.05–0.002 mm	<0.002
			Eutric Cambisol Cryic (Gleyic, Humic, Loamic)					
Ah	5–25	7.1 ± 0.2	3.4 ± 0.3	0.91 ± 0.18	95.0 ± 17.2	60.5 ± 12.1	35.5 ± 7.1	4.0 ± 0.8
AhBwtu	25–70	7.5 ± 0.2	1.5 ± 0.1	0.53 ± 0.11	55.0 ± 9.8	44.5 ± 8.9	37.3 ± 7.5	18.2 ± 3.6
Bccgl	70–90	8.3 ± 0.2	-	-	45.6 ± 7.2	44.1 ± 8.8	30.4 ± 6.1	25.5 ± 5.1
			Eutric Cambisol Cryic (Humic, Loamic) arable					
Ap	0–20	6.9	3.1 ± 0.3	0.62 ± 0.12	79.2 ± 12.2	55.0 ± 11.0	40.2 ± 8.0	4.8 ± 1.0
Bw	20–65	7.5	0.9 ± 0.1	0.21 ± 0.04	48.3 ± 8.1	46.4 ± 9.3	41.4 ± 8.3	12.2 ± 2.4
BCcc	65–95	7.9	-	-	37.5 ± 6.2	42.1 ± 8.4	36.5 ± 7.3	21.4 ± 4.3
			Haplic Cambisol (Arenic, Humic, Protocalcic)					
Ah	5–25	6.9 ± 0.3	2.8 ± 0.2	0.23 ± 0.04	38.0 ± 6.3	65.1 ± 13.2	33.3 ± 6.6	1.6 ± 0.3
Bw	25–65	7.6 ± 0.2	0.6 ± 0.05	0.06 ± 0.01	22.2 ± 3.7	54.5 ± 10.9	44.1 ± 8.8	1.4 ± 0.3
Bqc	65–97	8.2 ± 0.3	-	-	12.1 ± 2.0	56.5 ± 11.1	37.8 ± 7.6	5.7 ± 1.1
			Haplic Cambisol (Arenic, Humic, Protocalcic) arable					
Ap	0–20	7.1 ± 0.2	2.0 ± 0.2	0.12 ± 0.02	20.5 ± 4.2	68.2 ± 12.0	30.5 ± 7.7	1.3 ± 0.3
Bw	20–60	7.6 ± 0.2	0.5 ± 0.04	0.05 ± 0.01	12.7 ± 3.0	55.3 ± 11.1	43.1 ± 8.6	1.6 ± 0.3
Bqc	60–90	8.1 ± 0.2	-	-	7.2 ± 1.2	50.6 ± 10.1	44.5 ± 8.9	4.9 ± 1.0

Haplic Cambisol (Arenic, Humic, Protocalcic) (Plot 3) is located on the gentle slope (3°) of the eastern exposure of the hilly ridge, under virgin feather grass steppe with a predominance of *Stipa* capillata L., *Tanacetum vulgare* L., *Festuca lenensis* Drobow, *Astragalus membranaceus* (Fisch.) Bunge, *Artemisia scoparia* Waldst. and Kit and total projective cover of 65–70%, and the grass height is about 0.25 m. The soil has a sandy loamy light grey structureless humus horizon up to the 0.25 m depth, medium organic carbon content and pH 7.1 (Table 1). The morphologically pronounced light colored fawn whitish dense horizon with powdery carbonates and loamy texture lies at a depth of 65–100 cm.

The soil samples for analysis were collected from top organic horizons: a depth of 5–15 cm in native soil and 0–20 cm in the arable soil variant. At each site, five soil cores (5 cm diameter) were randomly taken from a 100×100 cm area and then combined to a single sample for each variant. Soil samples were air-dried, ground and passed through a 2 mm sieve for the analysis of soil organic carbon (SOC) content and humic substances' extraction [27].

The soil organic carbon (SOC) content was determined on the SNC-100 Auto-Analyzer (Scalar, Tokyo, Japan) [28].

Four HS preparations were isolated from the mixed samples of soil humus horizons (5–15 cm depth of native soils and 0–20 cm depth of arable ones) from each soil type in the study and followed the recommendations of the International Humic Substances Society (IHHS) without nitrogen purging. In the first stage, soil samples were treated with 1 M HCl until the pH declined to 1–2. Then, 0.01 M HCl was added to reach the water-to-soil ratio 10 mL: 1 g, and the suspension was shaken for 4 h and centrifuged. Humic substances were extracted from the residue with 1 M NaOH treatment to pH 7, and next there was the 0.01 M NaOH addition to the same ratio of the liquid and solid phase, followed by 4 h shaking and after night centrifugation. The humic acid fraction was isolated from the fulvic acid by acidifying the alkaline supernatants to pH 1.0 with the treatment of 6 M HCl, followed by centrifugation. Then, the obtained humic preparations were redissolved in 0.1 M KOH, then 20% Na_2SO_4 solution was added, and centrifuged at a high speed to remove the fine suspended particles. HAs were isolated again, and the precipitate was treated with the 0.1 M HCl and 0.3 M HF, then taken to dialysis and dried in the vacuum desiccator at 40 °C.

The humic materials obtained were characterized by an elementary analysis and ^{13}C NMR spectroscopy. C, H and N contents were determined in the CHNS/O Elementary Analyzer Series II (PerkinElmer Inc., Waltham, MA, USA) at the Institute of General and

Experimental Biology, Siberian Branch, Russian Academy of Sciences. The ash content was defined by the weight difference before and after combustion in a quartz tube at 750 °C. Data obtained and all calculations for the elementary analysis were corrected to the ash-free basis. The oxygen amount was calculated as the difference between the weight of the absolutely dry HS portion and the total content of C, H and N.

Differences in the molecular structure of HSs were determined by means of ^{13}C NMR spectroscopy. The ^{13}C NMR spectra were recorded on a DRX-500 spectrometer (Bruker, Fällanden, Switzerland) at 150.95 MHz in the liquid phase (0.5 M NaOH, D_2O) in the N.N. Vorozhtsov's Novosibirsk Institute of Organic Chemistry, Siberian Branch of the Russian Academy of Sciences. The spectra were recorded in the IG mode without NOE. The number of accumulations was 60–100 thousand scans. Chemical shift values were externally referenced to the methylene resonance of the adamantane standard at 38.4 ppm. Quantification was performed by integration of the corresponding absorption lines [29,30].

Microsoft Excel and Statistica 12 software were used for statistical data processing.

3. Results and Discussion

3.1. SOC Content

As a result of the study, average soil organic carbon concentrations were composed of 3.06% ($n = 30$) in the organic horizon of Haplic Cambisol (arenic, humic, protocalcic), and 3.26% ($n = 25$) in Eutric Cambisol Cryic (gleyic, humic, loamic). The differences in this parameter among native soil variants were not obvious and amounted to 6%. In permafrost soil, the SOC content decreased down the profile more gradually due to the translocation of humus material along frost cracks and cryoturbations. The reduction in C content in the arable soil variants was noted as a result of soil tillage. Arable soils have been ploughed annually, which caused the oxidation of SOM [1,31]. The marked SOC losses were more significant in the Haplic Cambisol (arenic, humic, protocalcic) arable soil (Plot 4) and amounted to 28%. This could be explained by the more intensive organic matter decomposition under the agricultural use of soils with a light texture and more arid environment [8,15]. Eutric Cambisol Cryic (gleyic, humic, loamic) had the highest SOM storage capacity due to the loamy texture and permafrost impact, which slowed down the oxidation processes. Being the source of available moisture in a summer season and temperature regulator inside the profile, it controlled the level of biochemical activity in soil [32,33].

3.2. Elemental Analysis

The element composition of humic substances is an important characteristic, which indicates the humification level, oxidation and condensation degree of humic acids. Data on the elemental composition of HAs isolated from the soils under study are presented in Table 2. It was revealed that the HA consisted of 36–40 mol % of C, 36–38% of H, 2% of N and 21–24% of O. The HAs of different soil variants were mainly different in the C content. The lowest C concentrations were observed in the HAs of Haplic Cambisol (arenic, humic, protocalcic) native soil, whereas this value significantly increased in the arable soil variant to a maximal value. The humic substances of both variants of permafrost-affected soils differed slightly in the C amount. A regular decrease in N content was noted in the HAs of arable soils. The increased N consumption and leaching were caused by nitrifiers' enhancing activity in more aerated soils under the tillage. The nitrogen content in the HA may be used as an indicator of agrogenic soil transformation using a long-term perspective [17].

Table 2. Elementary composition of HAs in virgin and arable soils.

Soil	The Content, Weight % Molar %				Atomic Ratios			H/C_{cor} [1]	Oxidation Rate (ω) [2]
	C	H	N	O	H/C	O/C	C/N		
EC [3]	$\frac{52.5 \pm 0.5}{39.0 \pm 0.4}$	$\frac{4.2 \pm 0.2}{37.0 \pm 0.7}$	$\frac{3.6 \pm 0.1}{2.0 \pm 0.01}$	$\frac{39.7 \pm 0.4}{22.0 \pm 0.2}$	0.95	0.54	19.5	1.67	+0.30
ECa	$\frac{51.5 \pm 0.5}{38.5 \pm 0.4}$	$\frac{4.1 \pm 0.2}{36.0 \pm 0.6}$	$\frac{2.8 \pm 0.1}{2.0 \pm 0.01}$	$\frac{41.6 \pm 0.4}{23.5 \pm 0.2}$	0.94	0.61	19.0	1.77	+0.44
HC	$\frac{48.4 \pm 0.5}{36.0 \pm 0.3}$	$\frac{4.4 \pm 0.2}{38.0 \pm 0.8}$	$\frac{3.3 \pm 0.1}{2.0 \pm 0.01}$	$\frac{43.9 \pm 0.4}{24.0 \pm 0.2}$	1.06	0.67	18.0	1.95	+0.44
HCa	$\frac{55.1 \pm 0.6}{40.0 \pm 0.4}$	$\frac{4.3 \pm 0.2}{37.0 \pm 0.7}$	$\frac{2.7 \pm 0.1}{2.0 \pm 0.01}$	$\frac{37.9 \pm 0.4}{21.0 \pm 0.2}$	0.93	0.53	20.0	1.64	+0.28

[1] (H/C) cor $= (H/C) + 2(O/C) \times 0.67$. [2] $\omega = (2 \times (O) + 3 \times (N) - \times(H)) : \times(C)$ [4]. [3] EC—Eutric Cambisol Cryic (gleyic, humic, loamic) native soil; ECa—Eutric Cambisol Cryic (humic, loamic) arable soil; HC—Haplic Cambisol (arenic, humic, protocalcic) native soil; HCa—Haplic Cambisol (arenic, humic, protocalcic) arable soil.

An assessment of atomic ratios and the degree of HAs oxidation made it possible to indirectly evaluate the level of SOM stabilization [34]. The x(H):x(C) atomic ratio of HAs was calculated as indicators of the condensation degree, related to climatic conditions at the period of their formation [35,36]. The values of x(H):x(C) in the HSs studied varied from 0.93 to 1.06. The maximal value was observed in humic acids of the Haplic Cambisol (arenic, humic, protocalcic) virgin soil that reflected an increased share of the aliphatic components. Lower, very similar values of the saturation degree were noted for other preparations, including both variants of HAs, isolated from Eutric Cambisol Cryic (gleyic, humic, loamic) soil. A reduction in this index was noted at Plot 4, which indicated significantly aromatic carbon (Car) increasing into the HS structure, and the C/N ratio was the highest for this sample depleted in nitrogen.

The maximal level of polarity (x(O):x(C)) in the molecular composition of HSs of Haplic Cambisol (arenic, humic, protocalcic) native soil was determined by high contributions of oxygen-rich aliphatic polysaccharide structures. A sharp decrease in this ratio in the HAs of the arable soil variant accompanied in the x(H):x(C) ratio reduction indicated the deep degradation processes such as dehydrogenation and decarboxylation under agricultural use. This data in accordance with the previous research testified that soil cultivation intensified the microbial activity and destruction of alkyl constituents, N consuming and HS polarity increase [10,37]. In general, the elemental composition of the HAs under the study was in agreement with prior data [4,13,23].

It should be noted that different techniques of HS extraction may affect the amount of preparation obtained and its chemical characteristics. For example, it was suggested that high performance size-exclusive chromatography and ultrafiltration were two of the state-of-art analytical methods in fractionating and characterizing humic substances in natural soil [38]. Moreover, a novel fractionation procedure, called Humeomics, that enables a detailed characterization of the structure of humic molecules in soil, has been performed [39]. "Humeomics produces homogenous fractions by progressively braking esters and ester C–O bonds, but not C–C bonds". Traditional alkali extraction followed recommendations of the IHHS used under the study and was the most common procedure and provided the possibility to match the data with other investigations. In addition, it was presented that in soils with a high degree of oxidative transformation of SOM, further oxidation during alkali extraction without inert gas purging was insignificant [40]. Other findings proved that a single extraction was sufficient for obtaining a representative HA sample as compared to sequential alkaline extraction [41].

3.3. Humic Acids' Molecular Structure (by Means of ^{13}C NMR Spectroscopy)

All of the obtained ^{13}C NMR spectra were typical for humic substances and featured multiple signal overlays in a broad range. The ^{13}C NMR spectra (Figure 2) were identified

as alkyl C, O-alkyl C (methoxyl C and carbohydrate C), aromatic C (Car) (aryl C and phenolic C), carboxyl C and ester groups, C atoms of quinone and ketone groups (Table 3).
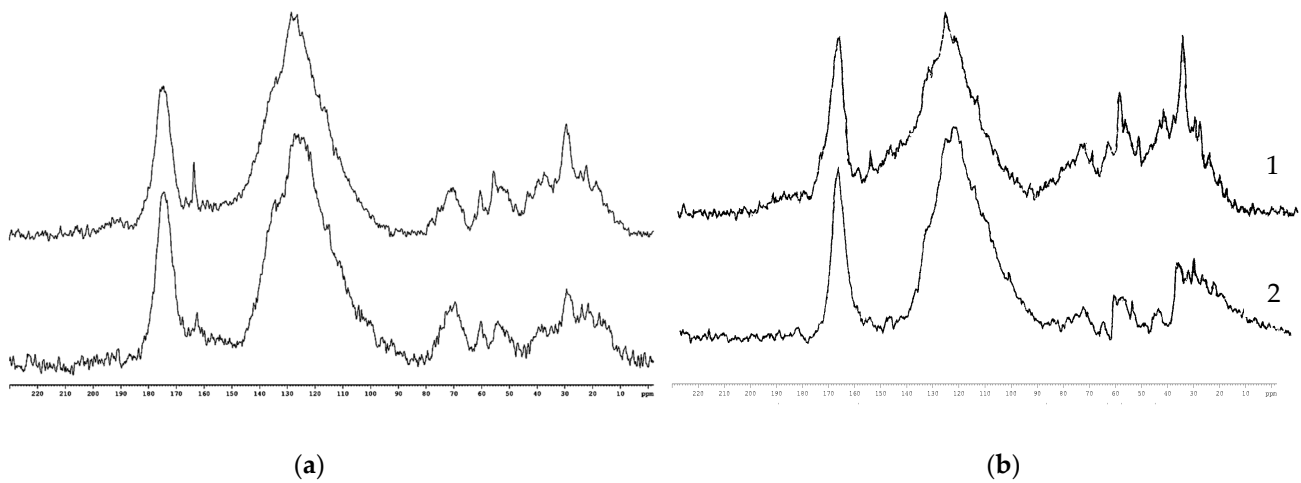

(**a**)   (**b**)

Figure 2. ^{13}C NMR spectra of HAs from native (1) and arable soils (2): (**a**)—Eutric Cambisol Cryic (gleyic, humic, loamic), (**b**)—Haplic Cambisol (arenic, humic, protocalcic).

Table 3. ^{13}C NMR chemical shifts of HAs' molecular groups.

Chemical Shift, ppm	Structure Group
0–47	Alkyl group C (–CH, –CH$_2$, –CH$_3$)
47–60	Amino group C and –O–CH$_3$ structures
60–108	Carbohydrate, alcohol and ether –C–O groups
108–164	Aromatic Car, phenol CAr –O
164–183	Carboxyl C
183–204	Quinone CAr=O, aldehyde and ketone –C=O groups

Analyses of the ^{13}C NMR spectra revealed the high intensity of signals in the range of 108–145 ppm related to the carbon of aromatic groups in the majority of HSs. Intense and precise signals were also indicated in the range corresponding to the carbon of alkyl, O-alkyl and carboxyl groups.

The relative content of carbon atoms of the main functional groups and molecular fragments of HAs as indicated by ^{13}C-NMR spectroscopy data is presented in Table 4.

Table 4. Structural composition of HAs investigated as measured by ^{13}C NMR spectroscopy.

Plot N	Chemical Shift, ppm						$\Sigma C_{ar}/$ ΣC_{al}	Aromaticity, %
	0–47	47–60	60–108	108–164	164–183	183–204		
1	17.0	6.0	7.0	55.0	13.0	2.0	1.3	55.0
2	15.0	7.0	5.0	57.0	15.0	1.0	1.4	57.0
3	19.0	9.0	13.0	45.0	12.0	2.0	0.9	45.0
4	14.0	4.0	7.0	60.0	13.0	1.0	1.6	60.0

The results indicated that the proportion of Car prevailed (45–60%) in HAs of all soil variants followed by alkyl C (14–19%) and O-alkyl C (11–22%), and carboxyl C (12–15%). Furthermore, the highest content of aromatic carbon occurred in the HSs of soils under the tillage [42]. While comparing the native soils, the HAs from Eutric Cambisol Cryic (gleyic, humic, loamic) were more reach in Car. This is consistent with the data on elemental analyses for the same samples in the study. According to Spaccini R. and Piccolo A. [43], an increased amount of aromatic molecules, together with a concomitant depleting of O-alkyl components may indicate a larger stability of HA due to a greater protection of SOM from

microbial degradation. This suggestion was proved by our results obtained for the HAs of Eutric Cambisol Cryic (gleyic, humic, loamic) arable soil, where the changes in the HAs' molecular structure under soil tillage were less expressed as compared to Haplic Cambisol (arenic, humic, protocalcic) soil.

The ratio of aromatic carbon to aliphatic carbon (Cal)-Car/Cal was used to assess the quantitative changes in the molecular parameters of HSs [44]. The content of aromatic fragments, as well as their ratio to the proportion of aliphatic components in HA, reflected the degree of humification and climatic conditions [45]. Signals of Car were taken into account in the range of 108–164 and 183–204 ppm, Cal–0–108 and 164–183 ppm. It has been noted that the Car/Cal values were higher in the humic substances of arable soils due to a reduction in the number of aliphatic chains and relative increase in the Car content [46]. These changes were mostly pronounced in Haplic Cambisol (arenic, humic, protocalcic) HSs: Car increased by 33%, and the Car/Cal ratio almost doubled. Such a result was also supported by the significantly larger alkyl-C to O-alkyl-C ratio in the HA of this soil variant. This ratio was commonly used as an index for the SOM degradation degree [47].

The aeration and temperature regime in Eutric Cambisol Cryic (gleyic, humic, loamic) became more favorable in the point of view of biochemical activity as a result of the soil plough. The oxidation of aliphatic chains increased, but their complete elimination, as compared to Haplic Cambisol (arenic, humic, protocalcic) HSs, did not occur. Thus, the SOM of Eutric Cambisol Cryic (gleyic, humic, loamic) was more resistant to soil tillage. According to the ^{13}C NMR spectra, the high content of carboxyl group C was detected in the humic preparations obtained (Figure 3) that were determined by the oxidative conditions of soil humus horizons in the arid summer period. The proportion of these groups tended to increase in the HAs of arable soils, due to the intensifying of bioactivity and oxidation. This was in agreement with prior data [48]. The content of carbonyl groups of aldehydes and ketones declined in these samples, confirmed by a decrease in the integral intensities of the carbon signals of the relevant groups. These groups were most likely to oxidize to carboxyl groups under soil tillage.
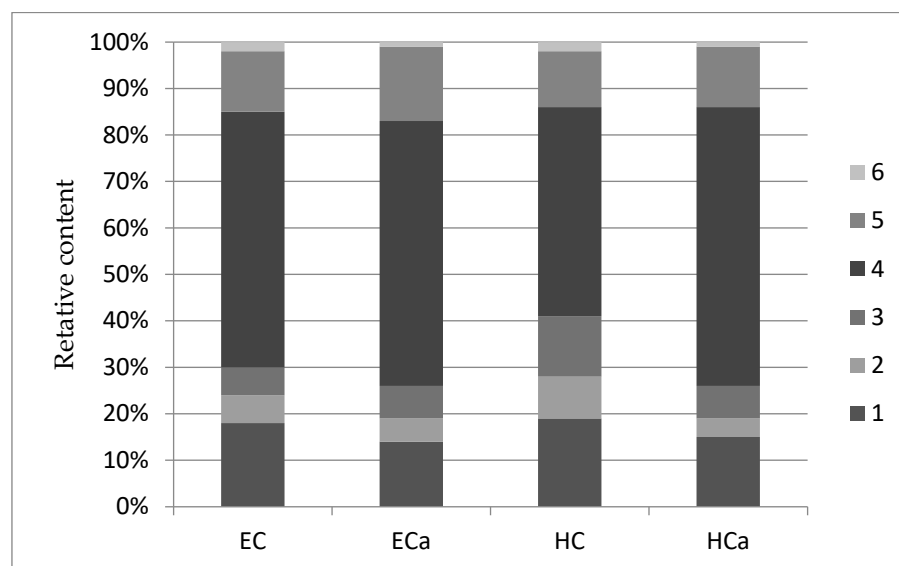

Figure 3. Percentage of carbon in the main structural fragments of HAs: 1—alkyl-group C; 2—amino-group C and –O–CH$_3$ structures; 3—carbohydrate, alcohol and ether –C–O groups C; 4—aromatic Car, Phenol CAr–O; 5—carboxyl C; 6—quinone CAr=O, aldehyde and ketone –C=O groups.

Our results are in accordance with other findings that showed that different cropping systems in long-term field experiments changed the SOM molecular dynamics and stability by primarily altering the hydrophobic components with a decrease in alkyl and aliphatic compounds, and Maize mono-cultivation led to a degradation of SOM quality. The authors

revealed the decrease in SOM hydrophobic protection with prolonged tillage and a potential loss of small nitrogen-rich compounds [49].

4. Conclusions

Our study has demonstrated that different agronomic management affected the changes in composition and molecular structure of humic acids. Conventional soil tillage induced an increase in the carbon content, proportion of aromatic compounds and carboxyl groups and depletion in nitrogen as compared to soil with no tillage. As measured by ^{13}C NMR spectroscopy, the intensity of these changes was determined by soil properties and the hydrothermal regime. Organic matter from Eutric Cambisol Cryic was suggested as having significant environmental resistance to the agricultural impact due to the optimization of the water regime and a loamy texture. The prolonged ploughing of sandy Haplic Cambisol under the arid conditions promoted deep changes in humic substances' composition and chemical structure.

EC—Eutric Cambisol Cryic (gleyic, humic, loamic) native soil; ECa—Eutric Cambisol Cryic (humic, loamic) arable soil; HC—Haplic Cambisol (arenic, humic, protocalcic) native soil; HCa—Haplic Cambisol (arenic, humic, protocalcic) arable soil; (according to ^{13}C NMR spectroscopy data).

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