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Molecular Composition of Humic Acids of Different Aged Fallow Lands and Soils of Different Types of Use in Northwest of Russia

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Abstract: Post-agrogenic transformation of fallow soils leads to changes in soil carbon content, the molecular composition of humic substances, and rates of organic matter stabilization, which can affect climate change on the planet. In this regard, we analyzed the molecular composition of humic acids isolated from natural and fallow soils in the southern Taiga zone of northwest Russia. Different-aged soils on fallow lands represent a model of soil transformation in time, and data on the transformation of soil humic acid molecular composition make a significant contribution to the understanding of soil organic matter stabilization aspect issues. In this case, the molecular structure of humic acids isolated from natural and fallow soils in northwest Russia was analyzed. To study the molecular composition of HAs, the elemental composition was analyzed, and ^{13}C (CP/MAS) NMR spectroscopy of HAs isolated from different aged abandoned soils and soils of different types of use was carried out. The obtained data showed that with the increasing age of soils in the fallow state, there is an increase in the carbon content of humic acids as well as a decrease in nitrogen content. As a result of the increasing age of soils in the fallow state, there are dynamics in the content of aromatic structural fragments in humic acids: 34% for 40 years old, 28% for 80 years old, and 31% for 120 years old. This is due to changes in the precursors of humification and the further transformation of plant residues in the soil. Re-involved fallow land soils lead to an increase in the content of aromatic structural fragments in the composition of HA in relation to HA extracted from mature soils. The lowest content of aromatic structural fragments was observed in the humic acids of 130-year-old agricultural soil, which is associated with the long-term application of organic fertilizers.

Keywords: ^{13}C NMR spectroscopy; boreal zone; fallow agrosoil; arable lands; Podzol

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1. Introduction

Worldwide, the problem of agricultural land conservation is a leading issue in the global fight against hunger [1,2]. Decisive steps are being taken to preserve, engage, and reclaim existing crop rotations on natural lands, as well as to monitor the water balance of these areas. According to the United Nations Food and Agriculture Organization (UN FAO), the area of agricultural land is about 4.8 billion hectares (37% of the world's landmass) [3]. As of 1 January 2023, the area of agricultural land in Russia amounted to 318 million hectares. About 44 million hectares of agricultural land are not used, of which about 20 million hectares are arable land [4]. Fallow lands in Russia are quite diverse in terms of soil types, vegetation succession, and post-agricultural dynamics [5]. According to Gagarina [6], the Northwest Federal Region includes the Leningrad, Novgorod, Pskov, and St. Petersburg regions. The history of arable land development began here at the end of the first millennium A.D., while counting-fire farming was known even earlier [7]. The cereal type of agriculture prevailed, the intensive development of animal husbandry is connected with the appearance of St. Petersburg, and a large amount of organic fertilizers, including manure, has appeared. In the vicinity of St. Petersburg, even the formation of thick superfertile soils—plaggen [8], which are now built up in residential areas—is known. The northwestern physico-geographical region is almost entirely located in the Valdai glaciation zone [6] and is a kind of "museum" of soil-forming rocks, which leads to the development of various scenarios of post-agrogenic soil evolution, including divergence and convergence of soil morphotypes and their chemical parameters. The least fertile soils are on the eluviae of granites and other massive crystalline rocks; the most fertile soils are characteristic of carbonate loams [9].

As a result of the large-scale transition of soils to a fallow state accompanied by landscape transformation, self-overgrowing, and waterlogging of territories, there was a significant accumulation of organic matter in soils, which is estimated to be in the range of 64 to 870 TgC [10]. Depending on the soil type and natural zone, they are able to sequester different amounts of SOM. The highest sequestration rates are observed in the boreal zone; this is due to the formation of natural vegetation and an increase in primary plant production [11]. The Chernozem zone is characterized by lower rates of plant residue accumulation, namely active carbon pools, because the degree of humification of plant residues here is significantly higher than in the boreal zone [12]. In the soils of the boreal zone, due to the formation of coniferous plant species, forest litter occurs, which contains the majority of SOM; this is characteristic of Podzols [13]. Retisols are characterized by the accumulation of occluded (passive pool) forms of carbon associated with clay particles; this accumulation mechanism is also characteristic of Chernozems [12].

The post-agrogenic dynamics of Retisols properties are well studied by classical soil-chemical and soil-microbiological methods for different parts of the boreal belt of Russia [13–15], with almost no data on the molecular organization of their organic matter. The qualitative composition of organic matter is an important indicator for assessing the level of carbon stabilization in an ecosystem [16]. The transition of soils to a fallow state leads to the formation of an active pool of carbon, which is more susceptible to biodegradation in relation to the passive pool [10], which is formed as a result of the formation of molecular complexes of "secondary nature," as well as occluded forms of carbon associated with the formation of organomineral complexes [17]. Nuclear magnetic resonance spectroscopy (NMR) methods are widely used in soil science, ecology, chemistry, and other natural sciences. With the help of these methods, the structural and functional organization of humic substances and organic matter in soils in general is determined. The advantages and limitations of this group of methods are described in detail in the review [18]. Previously, the most popular was the imaging of ^{13}C NMR spectra in extracted preparations of humic substances [19]. The use of solid-state spectra is more laborious but is also actively used in soil science [20]. The dominance of aromatic fragments in humic acids (HAs) and aliphatic fragments in fulvic acids (FAs) in cold boreal soils has been shown [21]. The reliable zonal dynamics of ^{13}C NMR spectra within the latitudinal range of soils in the Russian Plain were shown [22]. The possibilities of NMR spectroscopy in paleopedological reconstructions by types of lignin components have been revealed [23]. It has been established that soil contamination with petroleum hydrocarbons leads to the accumulation of aliphatic fragments and a relative decrease in aromatic components in the composition of soil organic matter [24]. Recently, heteronuclear NMR spectra (carbon, hydrogen, and nitrogen) have been increasingly used, which allows obtaining much more detailed information on the structure of organic matter [25]. Thus, spectroscopic methods of nuclear magnetic resonance have firmly entered the arsenal of modern soil chemistry and ecology. A huge number of works devoted to the content, stocks, and chemical characterization of organic matter in agrosoils are currently supplemented with information on the structure of humic substance molecules obtained by ^{13}C NMR [26,27]. In particular, it has been shown that different types of agricultural use are drivers that change the molecular composition of agrosoil humus [28]. The influence of erosion on the structural organization of organic matter in agrochernozems has been studied [29]. The role of agrosoil aggregation processes in the formation of the structural and functional organization of organic matter has also been studied [30]. The soils of the northwestern

Russian Plain, which were converted to fallow land after the collapse of the USSR, i.e., about 30 years ago, remain poorly studied. The hypothesis of this work is that the post-agrogenic transformation of soils leads to an increase in the content of aromatic structural fragments in the composition of HAs due to the stabilization of SOM. The novelty of the work consists of the fact that every year a significant amount of fallow land is transferred to the fallow state, which is subjected to post-agrogenic transformation and can make a significant contribution to climate change on the planet. Further research can be directed to the study of soil transformation as a result of the increasing age of fallow lands and organic matter accumulation rates. Therefore, the aim of this study was to investigate the structural and component composition of HAs of soils in the fallow lands. To achieve the goal, the following tasks were set: (1) investigate the morphological organization of soils of fallow lands as well as soils under different types of agricultural use; (2) determine the elemental composition of HAs extracted from soils of fallow lands and different types of use and intramolecular processes (hydrogenation/dehydrogenation and oxidation/reduction); and (3) evaluate the degree of stabilization of HAs based on ^{13}C NMR spectroscopy.

2. Materials and Methods

2.1. Study Area

The study areas (Figure 1) are located within the end moraine zone of the Valdai glaciation; however, due to the high variability of soil formation conditions, the composition of soil-forming rocks, local bioclimatic conditions, as well as long-term agricultural development, determine a high diversity of soils within the study area.

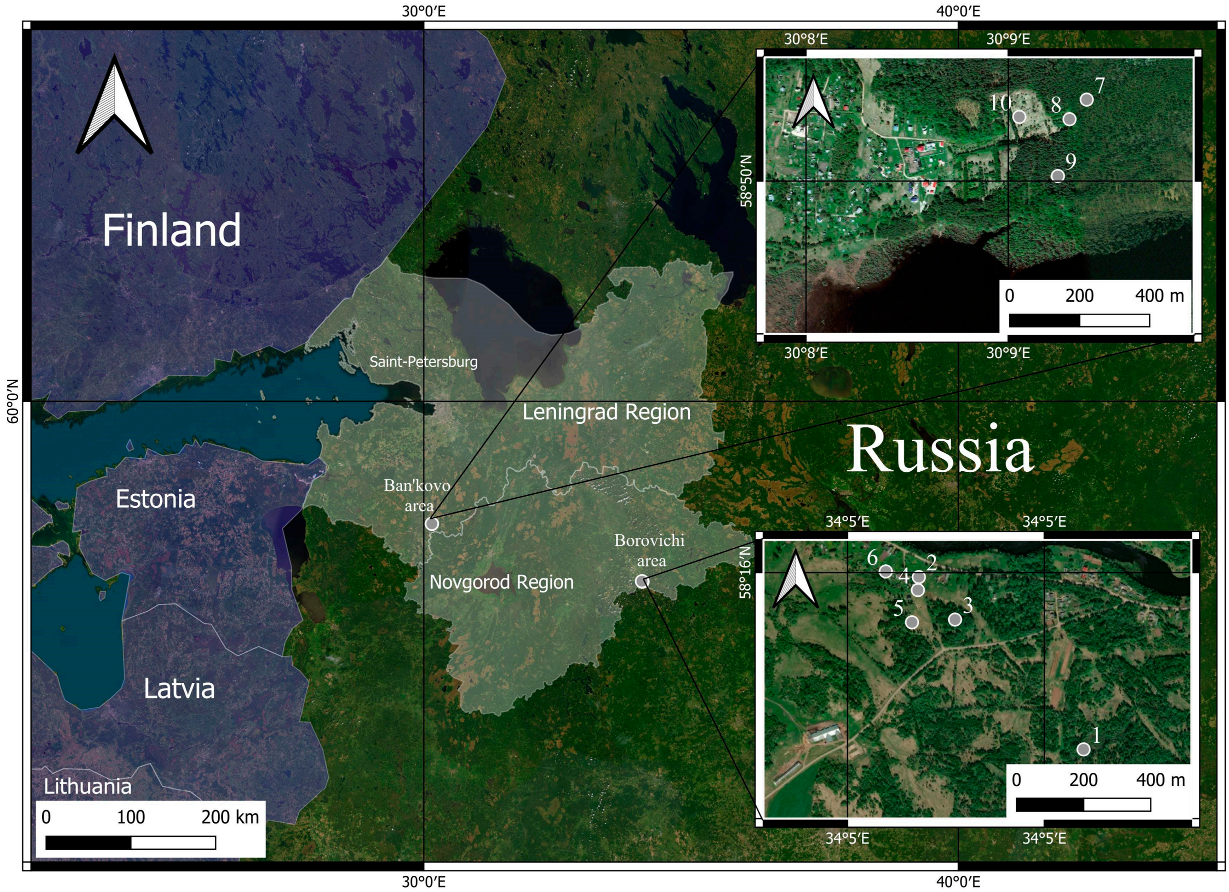

Figure 1. The study area of northwest Russia. Soil Id correspond to Table 1.

Table 1. Description of the studied soils used in the analysis of HAs molecular composition.

№	Horizon *	Depth, cm	Description of Soil Horizon	Soil Description and Type of Use	Coordinates	Soil Name **
			Novgorod region, Borovichi area			
1	Ah	0–30	Mineral horizon with accumulation of organic matter, roots, migration of iron oxide	Mature soil, not used in agriculture	N 58.265268 E 34.091997	Stagnic Retisol (loamic)
2	Ah	0–30	Post-agrogenic mineral horizon, sandy loam, illuvial accumulation of silicate clay	30-year-old fallow soil, used as a garden with shrubs and trees	N 58.269883 E 34.083628	Plaggic Retisol (loamic)
3	Ah	0–30	Post-agrogenic mineral horizon, sandy loam, illuvial accumulation of silicate clay	30-year-old fallow soil, occupied by secondary forest	N 58.268745 E 34.085468	Plaggic Retisol (loamic)
4	Ah	0–30	Post-agrogenic mineral horizon, sandy loam, illuvial accumulation of silicate clay	30-year-old fallow soil, used as pasture	N 58.269535 E 34.083580	Plaggic Retisol (loamic)
5	Ah	0–30	Post-agrogenic mineral horizon, sandy loam, illuvial accumulation of silicate clay	30-year-old fallow soil, used as hayfields	N 58.268671 E 34.083279	Plaggic Retisol (loamic)
6	Ah	0–30	Arable mineral horizon with an accumulation of organic matter, dense, dark	130-year-old Plaggic Retisol: soils formed as a result of long-term application of fertilizers in the form of litter manure	N 58.270034 E 34.081949	Plaggic Retisol (loamic)
			Leningrad region, Ban’kovo area			
7	Oe	0–4	Moss cover, poorly decomposed	Mature soil, not used in agriculture use	N 58.832090 E 30.153881	Stagnic Podzol (arenic)
	Ah	4–20	Mineral horizon, which is characterized by loss of silicate clay and migration of iron and aluminum oxides			
8	Ah	3–28	Post-agrogenic mineral horizon, sandy loam, with an accumulation of leached mineral grains	120-year-old fallow soil	N 58.831588 E 30.153031	Plaggic Podzol (arenic)
9	Ah	0–30	Post-agrogenic horizon, sandy loam, and accumulation of leached mineral grains	80-year-old fallow soil	N 58.830129 E 30.152454	Plaggic Podzol (arenic)
10	Ah	0–30	Post-agrogenic horizon, sandy loam, abundance of roots, accumulation of leached mineral grains	40-year-old fallow soil	N 58.831647 E 30.150548	Plaggic Podzol (arenic)

* Guidelines for soil description [31], ** world reference base FAO [32].

In the Novgorod region, fallow soils in the vicinity of the Borovichi area were studied; the study sites represent a single agro-landscape of a former agroholding that ceased to exist after the collapse of the USSR. The site belongs to the southern Taiga bioclimatic zone, with a predominance of Podzols and Retisols in drained watersheds and Histosol and Gleysol in overwatered zones. The average annual precipitation is 587 mm, with evaporation of about 430 mm. The average annual temperature is 4.3 °C. Part of fallow land is revegetated by secondary forest; part of it is permanently used for haying; part of it is subjected to permanent pasture digression; and part of it is used for vegetable gardens. Arable soil was also studied, where agricultural practices have been carried out for more than 130 years. Here, thick Plagic Podzol has been developed. All studied soils were formed on one type of soil-forming rock: on sandy loams of water-glacial origin, underlain by red–brown moraine loams. The natural (reference) vegetation of the sampling sites is sagebrush and spruce forests. The area of plowed land in this territory exceeded 80% during the Soviet era, and even those spruce forests that appear to be primary have been subjected to very significant anthropogenic impact.

In the Leningrad region, different-aged fallow soils in the vicinity of the village of Ban'kovo were studied. The territory is characterized by favorable agroclimatic conditions—a rather mild and short winter, long, warm vegetation in the summer, and moderate precipitation. The site belongs to the southern Taiga bioclimatic zone. Retisols on carbonate rocks, as well as Podzols on the covered parent materials (sands subleyred by clays), prevail in the area. Vegetation cover is represented by mixed forests (spruce, birch, aspen, and oak). The investigated area is characterized by a long history of development, and we studied fallow soils with the ages of 120, 80, and 40 years, as well as natural soils. All the studied soils were formed on the same type of parent materials: sandy loam deposits of fluvioglacial origin, underlain at a depth of 70–80 cm by red–brown moraine loams.

The description of the studied soils is presented in Table 1.

2.2. Sampling Strategy

Fallow, natural, and agricultural soils were studied in the summer of 2023 in the Leningrad and Novgorod regions. In the Leningrad region, soils of different ages (120, 80, and 40 years old), as well as background soil (mature Podzol), were sampled. In the Novgorod region, soils of different types of use (garden, secondary forest, pasture, and hayfield), 130-year-old agricultural soil, and mature soil (Stagnic Retisol) were sampled. Ten soil samples were collected for the study of HAs; the main physicochemical parameters of soils were determined in three repetitions (pH, carbon content, and particle size distribution); the elemental composition of HAs was determined in three repetitions; and ^{13}C NMR spectroscopy was analyzed in one repetition. Samples for chemical analyses were stored at +4 °C to analyze the main soil parameters. The soil was grounded and passed through a 2 mm sieve to obtain a fine earth fraction.

2.3. Laboratory Analyses

2.3.1. Chemical Analysis

The pH values were determined using the pH analyzer by the potentiometric method [33]. The carbon content was determined on a CHN analyzer (EA3028-HT EuroVector, Pravia, PV, Italy) [34]. The particle size distribution of the samples was determined by the sedimentation method [35]. Precision Range: Carbon—±0.01 mg or ±0.5% RSD (relative standard deviation); Nitrogen—±0.02 mg or ±0.5% RSD.

2.3.2. Humic Acids Analysis

HAs were isolated from studied soils according to the method of the International Society for the Study of Humic Substances in Modification by Vasilevich [36]. The elemental composition of HAs is the percentage content of the elements C, H, N, and O. For the graphical analysis of the elemental composition, we used the van Krevelen diagram [37]. The elemental composition was corrected for weight, moisture, and ash content.

The elemental composition of HAs was determined on a CHN analyzer (EA3028-HT EuroVector, Pravia, PV, Italy). A van Krevelen diagram was constructed from the elemental analysis data. Oxygen content was calculated from the following equation:

$$O = 100 - (C + H + N) \tag{1}$$

where C, H, and N content are obtained by the CHN analyzer.

The degree of oxidation was calculated from the following equation:

$$\mathrm{w} = 2 \times ((O/16) - (H/1.01))/(C/12.01) \tag{2}$$

where C, H, and N content are obtained by the CHN analyzer.

Solid-state spectra of HAs were determined by CP/MAS ^{13}C-NMR spectroscopy on a Bruker Avance 500 NMR spectrometer in a 3.2 mm ZrO_2 rotor. Table 2 summarizes the observed structural fragments within the HAs.

Table 2. Description of structural fragment types depending on the ranges of chemical shifts in HAs based on ^{13}C NMR spectroscopy.

Chemical Shifts, ppm	Type of Molecular Fragments
0–46	Carbon of methyl groups (CH_3), carbon of methylene groups of long-chain alkyl chains (CH_2), and carbon of methylene groups of branched alkyl chains (CH, C)
46–60	Carbon of methoxy and ethoxy groups (O-CH_3), O,N—substituted aliphatic fragments
60–105	CH_2OH carbon of carbohydrate fragment groups, CHOH carbon of polysaccharide ring groups, and esters (CHOH)
105–144	Unsubstituted aromatic carbon (H-Arom) as well as allyl-substituted aromatic carbon (C-Arom)
144–164	O,N-substituted aromatic carbon (O,N-Arom)
164–183	Carbon of carboxyl groups, esters, and amides (COO-R)
183–190	Carbon of quinone fragments (Arom=O)
190–204	Carbon of fragments of aldehydes and ketones (C=O)

The aromatic fragments were determined by the sum of chemical shifts 108–164 and 183–190 ppm. The aliphatic fragments were determined by the sum of chemical shifts 0–105, 164–183, and 190–204 ppm. The degree of hydrophobicity (AL h,r + AR h,r) was calculated as a sum of 0–47 and 105–144 ppm. The degree of organic matter transformation (C,H-AL/O,N-AL) was determined by the ratio of integrals in the areas of 0–46/46–110 ppm.

2.3.3. Statistical Analysis

The soil chemical parameters and element composition of HAs were evaluated in three replicates. Principal component analysis was performed to summarize and visualize the spatial variation of the data. Statistical data analysis and visualization were performed in GraphPad Prism 9 (GraphPad Software LLC, Boston, MA, USA).

3. Results and Discussion

3.1. The Features of Post-Agrogenic Soil Formation in the Study Area

The investigated regions are characterized by significant differences in the structure of the soil profile, and this is due to differences in the soil-forming rocks and in the leading soil-forming processes.

Moraines, including local moraines and fluvioglacial sands, as well as ancient alluvial deposits of the Msta River overlain by thin, overwashed glacial sediments, are widespread in the Novgorod region. The natural (reference) vegetation of the sampling sites is sage-brush and spruce forests. Data on the chemical composition of soils are given in Table 3. The obtained data correspond to the results of Litvinovich et al. [13], which show that only after 25–30 years of a fallow state is a rapid change in soil acidity observed. In the initial thirty years, idle soils serve as a buffer against acidity, even when fertilizers are applied. This can prevent soil acidification for an extended period of time, although the opposite outcome is also possible [38]. All investigated soils belong to sandy soils, with a predominance of coarse sand fractions inherited mainly from the soil-forming rock. This is quite typical for Retisol [39]. In terms of carbon content, it can be noted that fallow soils are characterized by a relatively high carbon content in comparison with natural soil but a lower content in comparison with agricultural soil. This is caused by an increase in the input of plant residues into the soil at the initial stages of succession. As a result of succession, the composition of vegetation changes towards the natural ecosystem, which leads to a decrease in the input of plant residues into the soil and their longer transformation [12]. Thus, carbon is stored in the wood biomass and as forest litter.

Table 3. Main chemical parameters of the studied soils.

Soil ID	Soil Description and Type of Use	pH	C, %	Particle Size Distribution		
				Sand	Silt	Clay
	Novgorod region, Borovichi area					
1-Ah	Mature soil, not used in agriculture	5.73	0.83	88	5	7
2-Ah	30-year-old fallow soil, used as a garden with shrubs and trees	6.11	1.40	75	18	7
3-Ah	30-year-old fallow soil, occupied by secondary forest	6.67	2.55	77	16	7
4-Ah	30-year-old fallow soil, used as pasture	6.37	2.42	86	11	3
5-Ah	30-year-old fallow soil, used as hayfields	6.23	2.21	85	10	5
6-Ah	130-year-old agriculture soil	6.87	3.77	83	11	6
	Leningrad region, Ban'kovo area					
7-Oe	Mature soil, not used in agriculture	4.22	43.67		-	
7-Ah		4.98	1.04	92	4	4
8-Ah	120-year-old fallow soil	5.64	1.39	88	7	5
9-Ah	80-year-old fallow soil	5.68	0.62	93	4	3
10-Ah	40-year-old fallow soil	5.89	1.44	89	7	4

The territory of the Leningrad Region is also located at the end of the moraine zone of the Valdai glaciation; the soil-forming rocks are red–brown loams of moraine plains, where the moraine is in some places covered by limnoglacial loam [6]. The studied soils of fallow lands are characterized by weak transformation processes of old arable horizons, which are probably related to the quality of soil-forming rocks. The morphological structure of different-aged soils on fallow lands has weak differences, which are expressed in the color of the soils and the development of stagnant processes. Soils are characterized by acid reactions; a strongly acidic reaction was possessed by the upper soil horizons of mature Stagnic Podzol (arenic). The presence of an acidic pH reaction leads to the destruction of primary soil minerals and the migration of secondary soil minerals through the profile and accumulation in the Bs horizon. In fallow soils, the upper old arable horizon, in most cases, is weakly expressed by moss cover. The carbon content in the 80-year-old soils is the lowest among the studied fallow soils. Along with the occurrence of a stagnant process, the formation of soils in well-drained positions leads to the active transformation of soil organic matter at the late stages of ecogenesis. After 120 years, as a result of reforestation, there is an active accumulation of carbon as forest litter, and its active transformation is observed. All investigated soils belong to sandy loam, with a predominance of medium-sized sand fractions inherited from the soil-forming rock.

3.2. Elemental Composition of HAs Isolated from Studied Soils

The elemental composition of HAs plays a crucial role in assessing the advancement of humification, oxidation, and the level of condensation within them. The data on the elemental composition content of the studied HAs are presented in Table 4.

As a result of the analysis of the obtained data, it was revealed that the highest carbon content in the composition of HAs isolated from soils in the Novgorod region was observed in the mature soil (50.56%), and the lowest content was recorded in the soil used as pasture. The highest carbon content in the mature soil (57.32–59.79%) is also characteristic of the HAs isolated from soils in the Leningrad region. This may be due to the long-term formation of humus-accumulative horizons and humic substances. The lowest carbon content in the soils of the Leningrad region was observed in 80-year-old fallow soil (49.91%); this may be due to the processes of organic matter transformation as a result of the change of humification precursors. A relatively high level of nitrogen content is also noted in the HA composition of these soils, which may also be due to the change in plant communities. A relatively high level of nitrogen content is noted among the HAs of soils in the Novgorod region, namely in re-involved fallow lands (3.98–4.87%), which may be due to the application of biogenic elements into the soil. The lowest nitrogen content is observed in the HAs of mature soil (3.39%). A characteristic feature of fallow soils is their relatively high nitrogen content in comparison with natural soils. This may indicate more active processes of nitrogen fixation from the atmosphere and the formation of nitrogen-containing structural fragments. The highest hydrogen content in HAs isolated from soils in the Novgorod region was noted in 30-year-old fallow soil occupied by secondary forest (5.31%); the lowest content was noted in mature soil (1-Ah) and 30-year-old fallow soil used as pasture (4.35%). Among the HAs isolated from soils in the Leningrad region, the highest hydrogen content was found in mature soil (7-Oe, 7-Ah), and the lowest content was found in 120-year-old fallow soil (4.63%). Relatively high hydrogen content may indicate the formation of a branched aliphatic periphery in the HA structure. Oxygen content changed to a lesser degree among the studied HA preparations. The increase in oxygen content in HAs is associated with the better solubility of oxygen-enriched hydrophilic HA molecules and their migration ability. In terms of statistical analysis of the level of differences between the studied objects in terms of carbon content in HA composition, an ordinary one-way ANOVA analysis was performed, which showed a high level of significant differences (p-value < 0.0001, R^2 = 0.99) between the studied objects. Non-significant differences were observed between HAs of mature soil (1-Ah) and 80-year-old fallow soil in the Leningrad region (9-Ah), as well as between 30-year-old fallow soil occupied by secondary forest (3-Ah) and 30-year-old fallow soil used as hayfields (5-Ah). This may be due to a similar mechanism of HA formation.

Table 4. Elemental composition of the studied HAs. Soil ID corresponds to Table 1.

Soil ID	Soil Description and Type of Use	Mass Fraction, %				Molar Ratios			Degree of Oxidation
		N, %	C, %	H, %	O, %	C/N	H/C	O/C	w
	Novgorod region, Borovichi area								
1-Ah	Mature soil, not used in agriculture	3.39	50.56	4.35	41.70	17.38	1.02	0.62	0.22
2-Ah	30-year-old fallow soil, used as a garden with shrubs and trees	4.15	45.19	4.90	45.76	12.71	1.29	0.76	0.23
3-Ah	30-year-old fallow soil, occupied by secondary forest	4.87	46.98	5.23	42.92	11.26	1.32	0.69	0.05
4-Ah	30-year-old fallow soil, used as pasture	3.98	42.91	4.35	48.76	12.58	1.21	0.85	0.50
5-Ah	30-year-old fallow soil, used as hayfields	4.70	46.81	5.16	43.33	11.63	1.31	0.70	0.08
6-Ah	130-year-old agricultural soil	4.64	48.55	5.31	41.50	12.21	1.30	0.64	−0.02
	Leningrad region, Ban'kovo area								
7-Oe	Mature soil, not used in agriculture	3.21	59.79	6.17	30.83	21.72	1.23	0.39	−0.45
7-Ah		3.28	57.32	5.28	34.12	20.37	1.10	0.45	−0.20
8-Ah	120-year-old fallow soil	3.73	54.36	4.63	37.28	17.01	1.01	0.52	0.02
9-Ah	80-year-old fallow soil	4.19	49.91	4.88	41.02	13.89	1.16	0.62	0.07
10-Ah	40-year-old fallow soil	3.78	52.79	4.66	38.77	16.31	1.05	0.55	0.05

The H/C index can serve as an indirect index indicating the resistance of HAs molecules to biodegradation; thus, the lower the H/C index, the higher the resistance. For the two studied regions, uneven data are noted; the Novgorod region is characterized by condensation of monomers for HAs from natural soil, while no significant differences are noted between HAs from fallow soils of different types of use. The opposite situation is observed in the Leningrad region; monomer condensation occurs in HAs from different-aged fallow soils, while this process is less developed in HAs from natural soil. According to the W index, it was noted that most of the studied HAs are in the oxidized state, except HAs from natural soils of the Leningrad region and 130-year-old agriculture soil. Oxidizing conditions indicate active processes of organic matter humification, while reduction conditions cause a low degree of monomer condensation.

The van Krevelen diagram was used to graphically represent the elemental composition and the contribution of oxidation/reduction and hydrogenation/dehydrogenation processes in the studied HAs (Figure 2).

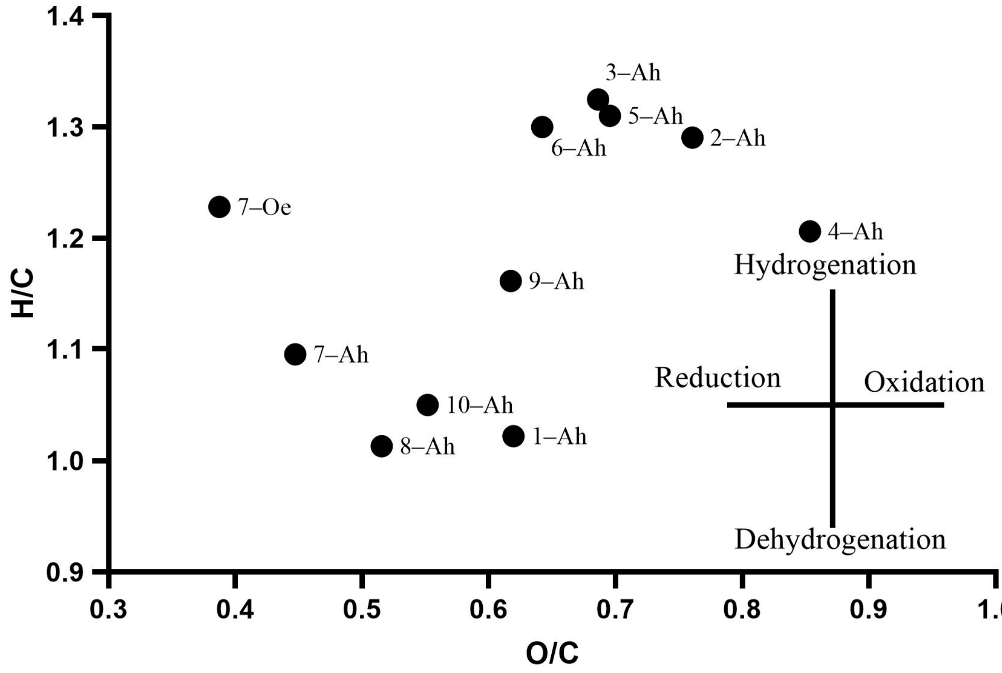

Figure 2. Van Krevelen diagram of HAs. Soil ID corresponds to Table 1.

Based on the obtained diagram, fallow soils of different types are characterized by the process of hydrogenation, which may indicate the presence of a branched net consisting of aliphatic structural fragments. At the same time, the soils of the Leningrad region, which are in the process of self-restoration, are characterized by the process of dehydrogenation, during which the initial molecular composition is changed and HA stability is acquired. In the work of Shpyinova et al. [40], it is noted that sapropels formed in the Taiga zone of the Khanty-Mansiysk Autonomous Okrug confirm the data obtained by us, indicating that with the increase in the H/C index, an increase in the content of aliphatic structural fragments is observed. Soils and HAs respond differently to agricultural development, according to the data of Stekolnikov et al. [41], which showed that the application of fertilizers to chernozems leads to an increase in the carbon content of HAs but results in an increase in the H/C ratio. This is also confirmed by Zavyalova and Vasbieva [42]. When high doses of fertilizers are applied, there is an increase in carbon content in the composition of HAs, while the content of hydrogen and nitrogen decreases. The increase in carbon content and decrease in nitrogen content may indicate a relatively high content of aromatic structural fragments in the composition of HAs. The work of Gorbov et al. [43] noted that in urbanized soils (park zone, city center) of the chernozem zone, there is a decrease in the carbon content in the composition of HAs in comparison with natural soils.

3.3. ^{13}C NMR Spectra of Studied Humic Acids

Numerous molecular fragments were identified by ^{13}C (CP/MAS) NMR spectroscopy, which indicates the great complexity of the structure of HAs and polyfunctional properties [21] (Figure 3).

According to the obtained data, it was found that the main groups of structural fragments in the molecular composition of HAs are C,H—Alkyl. This group of structural fragments dominates in all obtained HAs, C,H—Arom, and a group of carbohydrate fragments, CH_2ON. The molecular composition of HAs is presented in Table 5.

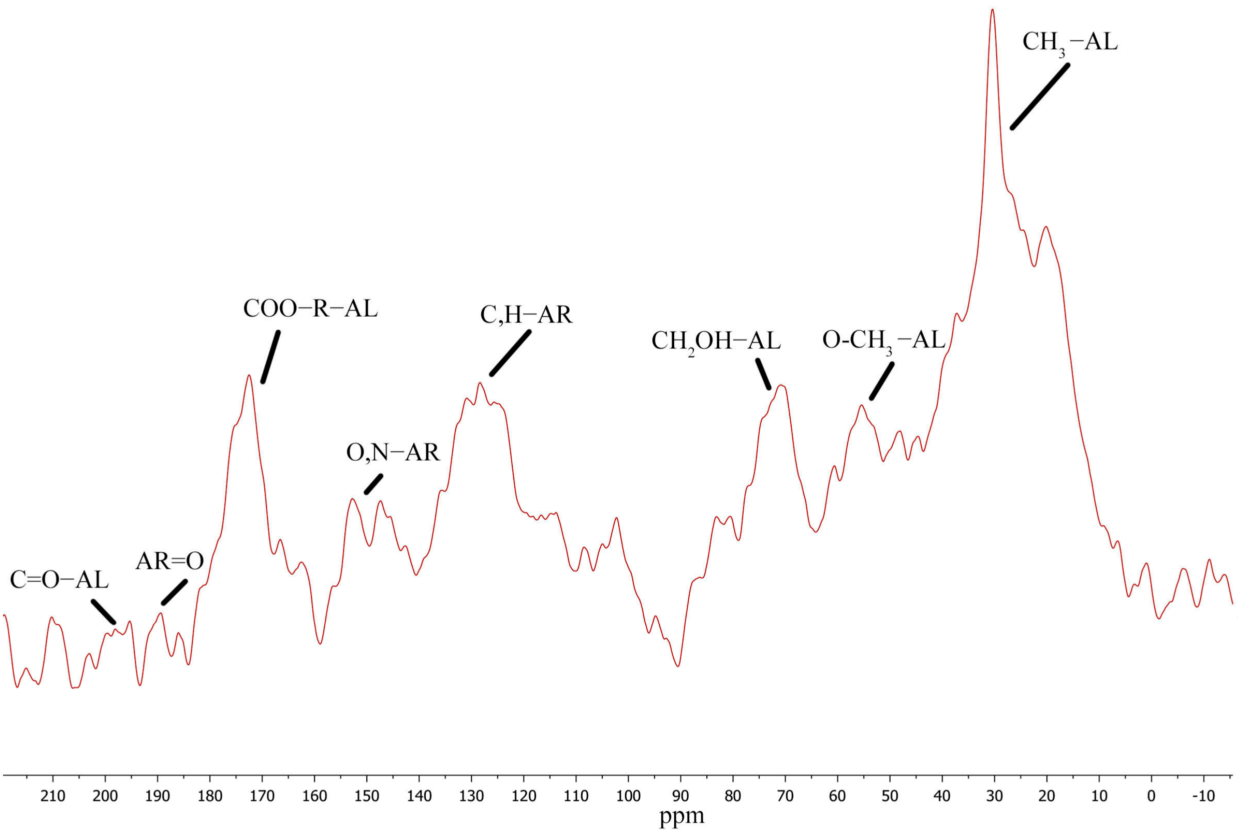

Figure 3. Characteristic ^{13}C NMR spectrum of HA extracted from mature soil (Stagnic Podzol (arenic)) near Bankovo village.

Aliphatic structural fragments of HAs (66–76%) dominate in the studied soils, which indicates weak transformation of initial precursors of humification. Fertilizer application causes a relatively high level of aliphatic structural fragment content because it is necessary to quickly incorporate biogenic elements into agricultural turnover. We can note that the transition of soils to a fallow state rather actively affects the molecular composition of HAs. The prevalence of aliphatic structural fragments ranging from 66 to 76% is characteristic of HAs from the natural and fallow soils of Ban'kovo village. Nevertheless, we can note the fact that fallow soils are characterized by a relatively high content of aromatic structural fragments in relation to the mature soil, which indicates the formation of relatively stable molecular complexes in the HAs composition. The highest content of aromatic structural fragments was observed in the HAs of 40-year-old fallow soil; the content of aromatic structural fragments amounted to 34%. This may be due to the active transformation of plant and animal residues in the soil, which has relatively recently passed into a fallow state. Active transformation of labile forms of carbon and their further condensation leads to the formation of environmentally stable compounds [28]. With the increasing age of soil transitioning to a fallow state (80 years old), an increase in the content of aliphatic structural fragments can be noted; this is associated with the change of plant communities and the quality of humification precursors. The molecular composition of HAs in fallow soil with an age of 140 years is characterized by an intermediate position between the youngest fallow soil and fallow soil with an age of 80 years. The increase in the content of aromatic structural fragments with age is a characteristic stage in the dynamics of soil carbon evolution [17,44]. Organic matter acquires greater stability, which is associated with a long stage of carbon transformation in the old arable soil horizon.

Table 5. Molecular composition of studied HAs isolated from soils.

Soil ID	Soil Description and Type of Use	0–46	46–60	60–105	105–144	144–164	164–183	183–190	190–204	AR	AL	AR/AL	AL h,r + AR h,r	C,H—AL/O,N—AL
Novgorod region, Borovichi area														
1-Ah	Mature soil, not used in agriculture	45	8	9	12	4	10	5	7	21	79	0.27	57	2.65
2-Ah	30-year-old fallow soil, used as a garden with shrubs and trees	38	6	17	18	9	10	1	1	28	72	0.39	56	1.65
3-Ah	30-year-old fallow soil, occupied by secondary forest	35	11	15	19	6	12	1	1	26	74	0.35	54	1.34
4-Ah	30-year-old fallow soil, used as pasture	50	3	12	21	4	8	1	1	26	74	0.35	71	3.33
5-Ah	30-year-old fallow soil, used as hayfields	36	9	18	19	4	12	1	1	24	76	0.32	55	1.33
6-Ah	130-year-old agricultural soil	42	11	23	11	1	10	1	1	13	87	0.15	53	1.23
Leningrad region, Ban'kovo area														
7-Oe	Mature soil, not used	42	10	15	18	5	8	1	1	24	76	0.32	60	1.68
7-Ah	in agriculture	47	7	11	20	4	9	1	1	25	75	0.33	67	2.61
8-Ah	120-year-old fallow soil	35	7	16	24	6	10	1	1	31	69	0.45	59	1.52
9-Ah	80-year-old fallow soil	35	10	15	19	8	11	1	1	28	72	0.39	54	1.40
10-Ah	40-year-old fallow soil	33	7	16	28	5	9	1	1	34	66	0.52	61	1.43

The molecular composition of HAs from soils in the Novgorod region revealed that fallow and 30-year-old soils have quite similar molecular compositions of HAs, which is explained by the one-directional process of SOM transformation during the transition of soils to fallow states. This process is determined by the one-time transition of soils to a fallow state (30 years) and the formation of relatively stable forms of carbon in the soil. This process is less pronounced than in soils under self-restoration, i.e., these soils are under the influence of agricultural development. HAs are characterized by a relatively high content of aliphatic structural fragments; their content varies from 72 to 87%, which is due to a relatively low degree of SOM stabilization. The mature soil is characterized by a lower content of aromatic structural fragments in comparison with the HAs of fallow soils 30 years old. In the same situation we could observe in the soils of Bankovo village, fallow soils are characterized by a higher content of aromatic structural fragments in relation to natural soil. However, among the studied soils of the Novgorod region, we can note the HAs extracted from 130-year-old agriculture soil; here, the lowest content of aromatic structural fragments is observed. This is due to the long-term application of organic forms of fertilizer in the soil; these approaches are aimed at the formation of a labile pool of carbon in the soil because it can be rapidly transformed. As a result of the transformation of the labile carbon pool, there is a release of biogenic elements into the soil, thus maintaining soil fertility.

The following parameters were used to standardize the quantitative characteristics of HAs molecules: degree of organic matter degradation and integral index of HAs hydrophobicity (Figure 4).

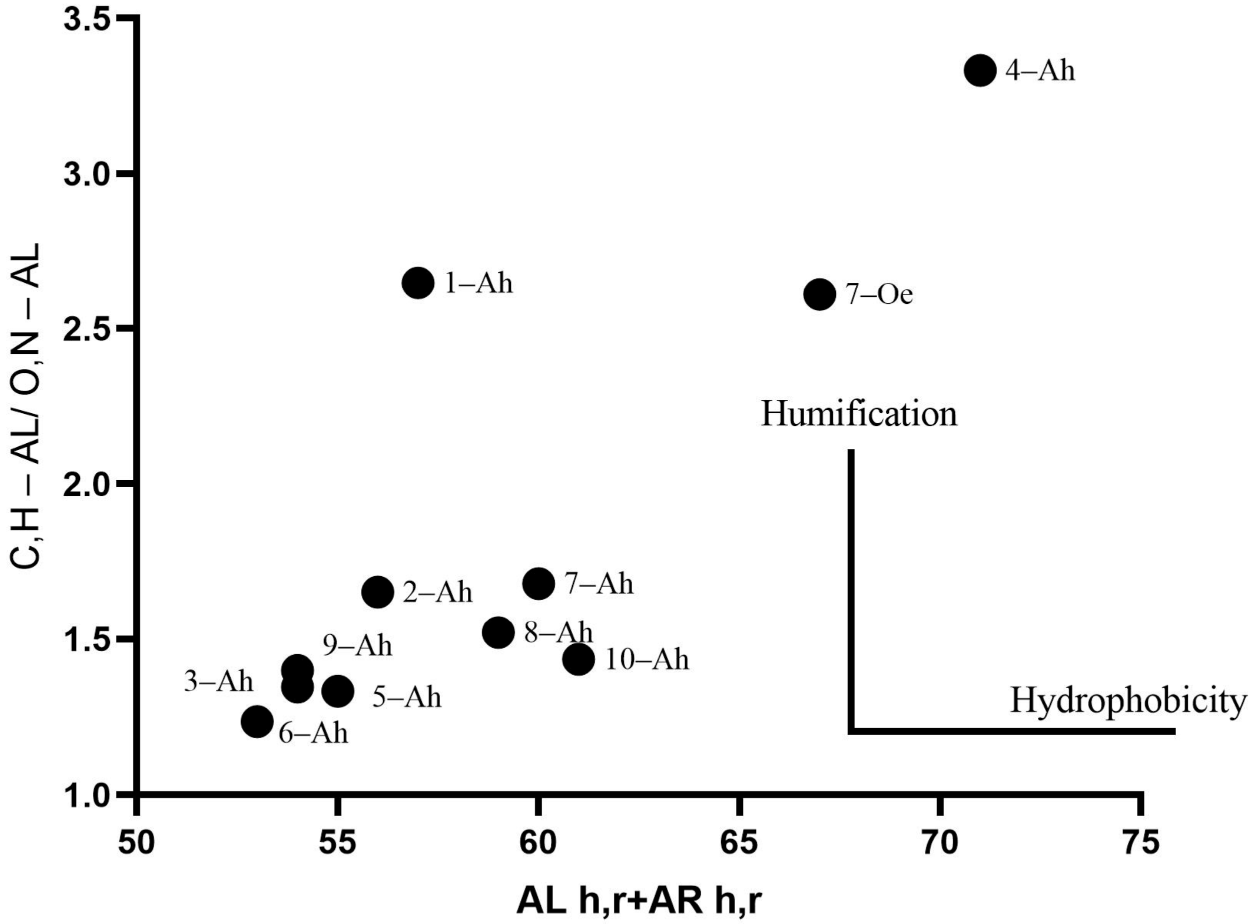

Figure 4. Diagram of integral indices of molecular composition of HAs extracted from fallow and natural soils in northwest Russia.

Based on the obtained diagram, it was found that the most active rates of humification are observed in mature soils and relatively young deposits used as pasture. This indicates that there are labile forms of carbon that can be relatively quickly transformed. At the same time, most of the HAs in the soils of fallow lands have quite similar values, indicating similar conditions of SOM formation and their relative resistance to biodegradation. An increase in the duration of soil presence in the fallow state leads to the stabilization of organic matter and the formation of thermodynamically stable molecular compounds in the composition of HAs.

The principal component method was used to statistically analyze the molecular composition of HAs (Figure 5).

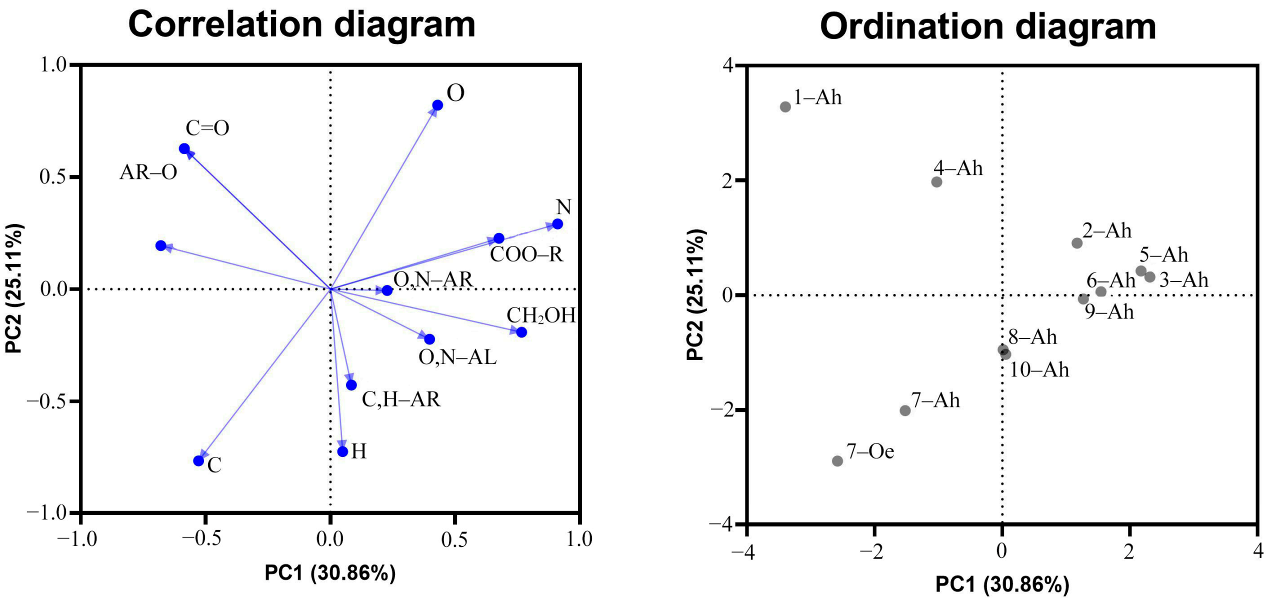

Figure 5. Results of principal component analysis for structural fragments and elemental composition of HAs. Sample numbers correspond to the numbers in Table 1.

Based on the presented diagrams, we can note that the distribution of the study points along the principal component (PC1) is associated with variation in the content of nitrogen as well as the carboxyl group of structural fragments in HAs along PC2—aliphatic structural fragments (C, H—Alkyl), fragments of aldehydes and ketones (C=O), and aromatic structural fragments (Arom=O). Analysis of the ordination of structural fragments and elemental composition of HAs in the space of two principal components did not reveal a clear relationship between the studied objects, indicating heterogeneous conditions of humification and maturation of HAs.

According to the obtained data, we can conclude that the greatest potential for the accumulation of stable carbon pools is in fallow soils at the early stages (40 years), where molecular complexes relatively resistant to biodegradation are formed and the content of aromatic structural fragments is up to 34%, as well as at the later stages (120 years), where the content of aromatic structural fragments is 31%. The greatest potential for humification of plant residues is fallow soils used as pastures, which indicates that re-involving fallow soils has a positive effect on the processes of organic residue transformation, while long-term use of soils in agriculture leads to a significant decrease in aromatic structural fragments up to 13% and the formation of an active carbon pool.

The obtained data are confirmed by Mohammed et al. [45], where agricultural development leads to an increase in the content of aliphatic structural fragments since fertilizer application actively affects microbial biomass synthesis and C-Alkyl group formation. According to Zavyalova and Vasbieva [42], long-term application of high doses of fertilizers $N_{150}P_{150}K_{150}$ leads to an increase in the content of aromatic C=O structural fragments due to the mineralization of organic matter; we note this process in HAs of 130-year agriculture soil; here, among the studied HAs, the highest content of Arom=O structural fragments is observed. Our data correlate with the data of Beznosikov et al. [44], who studied in the Taiga zone Umbric Retisol. The results showed that in Retisols, the AR/AL ratio in HAs is 0.31. With the increase in biological activity, there is a rapid destruction of non-specific organic compounds and simple humus substances with the formation of organic compounds of "secondary nature", which are more stable in the environment. The type of soil utilization introduces significant changes in the molecular composition of HAs. A study of HAs conducted in agricultural soils in Slovakia showed that the properties of HAs depend on the type of soil used in agriculture as well as the content of physical clay. Clay content was positively correlated with carbon content and HAs "maturity" [16]. Data from Kukuļs et al. [46] show that the transformation of labile forms of carbon leads to the formation of stable high molecular weight associations within HAs. According to our data, we also observe a tendency to increase aromatic structural fragments with the increasing age of fallow soils.

4. Conclusions

As a result of this work, we analyzed the features of soil morphological structure as well as the elemental and molecular composition of HAs isolated from different-aged and re-involved soils of fallow lands. According to the analysis of the morphological structure of soils, it was revealed that different-aged fallow soils are characterized by the weak degree of transformation of arable horizons, which is caused by the insufficient time of transformation and development of Podzol formation on red–brown moraine loams. With the increase in time of soils being in the fallow state, a dynamic in soil carbon content for 40-year-old soil—1.44%, for 80-year-old soil—0.62%, and for 120-year-old soil—1.39% was observed; it is caused by the transformation of SOM as a result of the transition of soils to the fallow state and the formation of more stable molecular complexes. According to the data on the elemental composition of HAs, it was found that the transformation of HA composition is directed towards HAs formed in mature soils. As a result of studying the molecular composition of HAs in natural and fallow soils, we can note a general tendency in the prevalence of aromatic structural fragments in the composition of HAs in fallow soils in relation to natural soils, with the exception of fallow agrozem, to which organic forms of fertilizers are currently applied. It was found that a passive pool of carbon is formed in fallow soils where 31–34% of aromatic structural fragments are found in the structure of HAs, but the greatest degree of humification of organic matter is observed in fallow soils used as pasture. The increase in the content of aromatic structural fragments is due to the stabilization of SOM in fallow soils, which is a characteristic stage of carbon dynamics.

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References

1. FAO. *The State of Food and Agriculture 2020: Overcoming Water Challenges in Agriculture*; FAO: Rome, Italy, 2020; p. 210.
2. FAO. *The State of the World's Land and Water Resources for Food and Agriculture—Systems at Breaking Point*; FAO: Rome, Italy, 2021; p. 82.
3. FAO. *Measuring and Modelling Soil Carbon Stocks and Stock Changes in Livestock Production Systems—A Scoping Analysis for the LEAP Work Stream on Soil Carbon Stock Changes*; FAO: Rome, Italy, 2019; p. 84.
4. Federal Service for State Registration. *State (National) Report on the Condition and Use of Land in the Russian Federation in 2021*; Federal Service for State Registration, Cadastre and Cartography Registration, Cadastre and Cartography: Moscow, Russia, 2022; p. 206.
5. Lyuri, D.I.; Goryachkin, S.V.; Karavaeva, N.A.; Denisenko, E.A.; Nefedova, T.G. *Dynamics of Agricultural Lands of Russia in XX Century and Postagrogenic Restoration of Vegetation and Soils*; GEOS: Moscow, Russia, 2010; p. 420.
6. Gagarina, E.I. *Lithological Factor of Soil Formation (by the Example of the North-West Russian Plain)*; Saint-Petersburg State University: Saint-Pestersburg, Russia, 2004; p. 260.
7. Isachenko, G.A. *"Window to Europe": History and Landscapes*; Saint-Petersburg State University: Saint-Petersburg, Russia, 1998; p. 476.
8. Kalinina, O.; Goryachkin, S.V.; Karavaeva, N.A.; Lyuri, D.I.; Najdenko, L.; Giani, L. Self-restoration of post-agrogenic sandy soils in the southern Taiga of Russia: Soil development, nutrient status, and carbon dynamics. *Geoderma* **2009**, *152*, 35–42. [CrossRef]
9. Abakumov, E. Rendzinas of the Russian Northwest: Diversity, Genesis, and Ecosystem Functions: A Review. *Geosciences* **2023**, *13*, 216. [CrossRef]
10. Kalinina, O.; Cherkinsky, A.; Chertov, O.; Goryachkin, S.; Kurganova, I.; Lopes de Gerenyu, V.; Lyuri, D.; Kuzyakov, Y.; Giani, L. Post-agricultural restoration: Implications for dynamics of soil organic matter pools. *Catena* **2019**, *181*, 104096. [CrossRef]
11. Susyan, E.A.; Wirth, S.; Ananyeva, N.D.; Stolnikova, E.V. Forest succession on abandoned arable soils in European Russia—Impacts on microbial biomass, fungal-bacterial ratio, and basal CO_2 respiration activity. *Eur. J. Soil Biol.* **2011**, *47*, 169–174. [CrossRef]
12. Kalinina, O.; Goryachkin, S.V.; Lyuri, D.I.; Giani, L. Post-agrogenic development of vegetation, soils, and carbon stocks under self-restoration in different climatic zones of European Russia. *Catena* **2015**, *129*, 18–29. [CrossRef]
13. Litvinovich, A.V. Postagrogenic Evolution of Well Cultivated Soddy-Podzolic Soils in the Northwestern Nonchernozemic Zone. *Agronomy* **2009**, *7*, 85–93.
14. Telesnina, V.M.; Vaganov, I.E.; Karlsen, A.A.; Ivanova, A.E.; Zhukov, M.A.; Lebedev, S.M. Specific features of the morphology and chemical properties of coarse-textured postagrogenic soils of the southern taiga, Kostroma oblast. *Eurasian Soil Sci.* **2016**, *49*, 102–115. [CrossRef]
15. Kurganova, I.N.; Telesnina, V.M.; Lopes de Gerenyu, V.O.; Lichko, V.I.; Karavanova, E.I. The Dynamics of Carbon Pools and Biological Activity of Retic Albic Podzols in Southern Taiga during the Postagrogenic Evolution. *Eurasian Soil Sci.* **2021**, *54*, 337–351. [CrossRef]
16. Banach-Szott, M.; Debska, B.; Tobiasova, E. Properties of humic acids depending on the land use in different parts of Slovakia. *Environ. Sci. Pollut. Res.* **2021**, *28*, 58068–58080. [CrossRef]
17. Semenov, V.M.; Ivannikov, L.A.; Tulina, A.S. Stabilization of organic matter in the soil. *Agrochimia* **2009**, *10*, 77–96.
18. Chukov, S.N.; Lodygin, E.D.; Abakumov, E.V. Application of 13C NMR Spectroscopy to the Study of Soil Organic Matter: A Review of Publications. *Eurasian Soil Sci.* **2018**, *51*, 889–900. [CrossRef]
19. Kholodov, V.A.; Konstantinov, A.I.; Perminova, I.V. The carbon distribution among the functional groups of humic acids isolated by sequential alkaline extraction from gray forest soil. *Eurasian Soil Sci.* **2009**, *42*, 1229–1233. [CrossRef]
20. Hatcher, P.G.; VanderHart, D.L.; Earl, W.L. Use of solid-state 13C NMR in structural studies of humic acids and humin from Holocene sediments. *Org. Geochem.* **1980**, *2*, 87–92. [CrossRef]
21. Lodygin, E.D.; Beznosikov, V.A.; Vasilevich, R.S. Molecular Composition of Humic Substances in Tundra Soils (C-13-NMR Spectroscopic Study). *Eurasian Soil Sci.* **2014**, *47*, 400–406. [CrossRef]
22. Kholodov, V.A.; Konstantinov, A.I.; Kudryavtsev, A.V.; Perminova, I.V. Structure of humic acids in zonal soils from 13C NMR data. *Eurasian Soil Sci.* **2011**, *44*, 976–983. [CrossRef]
23. Kovaleva, N.O.; Kovalev, I.V. Lignin phenols in soils as biomarkers of paleovegetation. *Eurasian Soil Sci.* **2015**, *48*, 946–958. [CrossRef]
24. Lobanov, V.G.; Aleksandrova, A.V.; Shurai, K.N. Structural and functional characteristics of soils in Krasnodar Krai. *Sci. J. Kuban State Agrar. Univ.* **2015**, *109*, 1–10.
25. Polyakov, V.; Abakumov, E.V. Humic Acids Isolated from Selected Soils from the Russian Arctic and Antarctic: Characterization by Two-Dimensional 1H-13C HETCOR and 13C CP/Mas NMR Spectroscopy. *Geosciences* **2020**, *10*, 15. [CrossRef]
26. Kechaikina, I.O.; Ryumin, A.G.; Chukov, S.N. Postagrogenic transformation of organic matter in soddy-podzolic soils. *Eurasian Soil Sci.* **2011**, *44*, 1077–1089. [CrossRef]
27. Kurganova, I.; Merino, A.; Lopes de Gerenyu, V.; Barros, N.; Kalinina, O.; Giani, L.; Kuzyakov, Y. Mechanisms of carbon sequestration and stabilization by restoration of arable soils after abandonment: A chronosequence study on Phaeozems and Chernozems. *Geoderma* **2019**, *354*, 113882. [CrossRef]

28. Vishnyakova, O.; Ubugunov, L. Changes in Molecular Structure of Humic Substances in Cambisols under Agricultural Use. *Agronomy* **2023**, *13*, 2299. [CrossRef]
29. Artemyeva, Z.S.; Danchenko, N.N.; Kolyagin, Y.G.; Varlamov, E.B.; Zasukhina, E.S.; Tsomaeva, E.V.; Kogut, B.M. Chemical Structure of Organic Matter of Agrochernozems in Different Slope Positions. *Eurasian Soil Sci.* **2023**, *56*, 705–714. [CrossRef]
30. Sarker, T.C.; Incerti, G.; Spaccini, R.; Piccolo, A.; Mazzoleni, S.; Bonanomi, G. Linking organic matter chemistry with soil aggregate stability: Insight from 13C NMR spectroscopy. *Soil Biol. Biochem.* **2018**, *117*, 175–184. [CrossRef]
31. Jahn, R.; Blume, H.P.; Spaargaren, O.; Schad, P. *Guidelines for Soil Description*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2006; p. 98.
32. WRB. *IUSS Working Group WRB World Reference Base for Soil Resources 2014, Update 2015*; WRB: Rome, Italy, 2015; p. 195.
33. Orlov, D.S. *Soil Chemistry: A Textbook*; Moscow State University: Moscow, Russia, 1985; p. 376.
34. FAO. *Standard Operating Procedure for Soil Total Carbon—Dumas Dry Combustion Method*; GLOSOLAN-SOP-03; FAO: Rome, Italy, 2019; p. 12.
35. Bowman, G.; Hutka, J. Particle Size Analysis. In *Soil Physical Measurment and Interpritation for Land Evaluation*; McKezie, N., Coughlan, K., Cresswell, H., Eds.; CSIRO Publishing: Victoria, BC, Canada, 2002; pp. 224–239.
36. Vasilevich, R.S.; Beznosikov, V.A.; Lodygin, E.D. Molecular Structure of Humus Substances in Permafrost Peat Mounds in Forest-Tundra. *Eurasian Soil Sci.* **2019**, *52*, 283–295. [CrossRef]
37. van Krevelen, D.W. Studies of gas absorption. VI. A graphical representation for the efficiency of physical absorption. *Recl. Des Trav. Chim. Des Pays Bas* **1950**, *69*, 503–508. [CrossRef]
38. Dymov, A.A.; Dubrovskiy, Y.A.; Startsev, V.V. Postagrogenic development of Retisols in the middle taiga subzone of European Russia (Komi Republic). *Land Degrad. Dev.* **2018**, *29*, 495–505. [CrossRef]
39. Polyakov, V.; Abakumov, E. Estimation of Carbon Stocks and Carbon Sequestration Rates in Abandoned Agricultural Soils of Northwest Russia. *Atmosphere* **2023**, *14*, 1370. [CrossRef]
40. Shpinova, N.V.; Sartakov, M.P.; Komissarov, I.D.; Efanov, M.V. Elemental composition of humic acids of initial and thermo-processed sipropels of lakes of the Surgut region KhMAO-Ugra. *Innov. Investig.* **2018**, *9*, 161–164.
41. Stekolnikov, K.E.; Kotov, V.V.; Donskikh, I.N.; Gridyaeva, E.S. Elemental composition of humic acids of chernozem leached under different types of anthropogenic impact. *Bull. Voronezh State Univ. Ser. Chem. Biology. Pharm.* **2006**, *2*, 103–107.
42. Zavyalova, N.E.; Vasbieva, M.T. Elemental Composition and Structure of Humic Acids in Virgin and Arable Soddy-Podzolic Soils of the Cis-Urals. *Eurasian Soil Sci.* **2021**, *54*, 1575–1580. [CrossRef]
43. Gorbov, S.N.; Bezuglova, O.S.; Tischenko, S.A.; Gorovtsov, A.V. Organic Matter and Elemental Composition of Humic Acids in Soils of Urban Areas: The Case of Rostov Agglomeration. In *Megacities 2050: Environmental Consequences of Urbanization. ICLASCSD 2016*; Vasenev, V.I., Dovletyarova, E., Chen, Z., Valentini, R., Eds.; Springer: Cham, Switzerland, 2018; pp. 80–98. [CrossRef]
44. Beznosikov, V.A.; Lodygin, E.D. High-molecular organic substances in soils. *Trans. Komi Sci. C Cent. Ural. Branch Russ. Acad. Sci.* **2010**, *1*, 24–30.
45. Mohammed, I.; Kodaolu, B.; Zhang, T.; Wang, Y.; Audette, Y.; Longstaffe, J. Analysis of Molecular Structure Changes in Humic Acids from Manure-Amended Soils over 17 Years Using Elemental Analysis and Solid-State 13C Nuclear Magnetic Resonance Spectroscopy. *Soil Syst.* **2023**, *7*, 76. [CrossRef]
46. Kukuļs, I.; Kļaviņš, M.; Nikodemus, O.; Kasparinskis, R.; Brūmelis, G. Changes in soil organic matter and soil humic substances following the afforestation of former agricultural lands in the boreal-nemoral ecotone (Latvia). *Geoderma Reg.* **2019**, *16*, e00213. [CrossRef]