Article

Essential Role of Forest Fires in Humic Acids Structure and Composition Alteration

Ekaterina Chebykina *[ID] and **Evgeny Abakumov** [ID]

Department of Applied Ecology, Faculty of Biology, Saint-Petersburg State University, Saint-Petersburg 199178, Russia
* Correspondence: e.chebykina@spbu.ru; Tel.: +7-921-987-70-51

Abstract: Pyrogenic components of soil organic matter are practically not taken into account when assessing the implications of global climate changes on the SOM sequestration rate and its stability. In this regard, both the amount and structural stability of SOM were investigated for postpyrogenic forest–steppe environments. Mature unaffected by fire Psamment Entisols were investigated in comparison with same soil strongly affected by surface and crown fires in 2010 in Tolyatti city. This article discusses postpyrogenic succession when comparing the results of field works in 2010 with 2020. The elemental composition of humic acids as well as ^{13}C NMR spectra were investigated for the studied humic acids. Moreover, for the first time ever, integrated indicators of HAs' molecular composition were presented for postpyrogenic soils. The data obtained showed that there are significant changes in the structural organization of organic matter as a result of forest fires. A simultaneous decrease in the H/C and O/C atomic ratios was identified as a result of fire, which indicates a slight loss of oxygen-containing functional groups, while the aromaticity degree of molecules increases. An increase in the content of oxygen-containing groups in the HA molecules was shown for 10 years after the fires, which was accompanied by the oxidation of the HA molecule with the same increase in the aromaticity degree of the HA molecule. The HA molecules of 2020 turned out to be more resistant to environmental factors compared to analogues in 2010. As a result of fires, the aromaticity degree of HAs molecules significantly increases.

Keywords: wildfire; postpyrogenic succession; soil organic matter; ^{13}C-NMR spectroscopy; crown fire; surface fire

1. Introduction

Fires are a global phenomenon and affect all ecosystems' components, including soils. They have a global nature and can contribute to unexpected changes in atmospheric composition that are difficult to predict [1–4]. Carbon dioxide emissions resulting from an increase in fire frequency could escalate global warming. Considering that, in addition to ecosystem degradation, fires lead to the transfer of forests from carbon stocks to sources of greenhouse gases, then a problem can be expected in the implementation of international agreements and additional economic losses in the future. Nowadays, it is important to decide whether it is necessary to undertake certain measures to restore forests or to allow natural regeneration. Reforestation was shown to accelerate the conversion of the fire-affected areas from carbon source to carbon sink after wildfires [5], although natural regeneration may also increase carbon sequestration [6]. Therefore, the relevance of studies on the fire impact on forest ecosystems has increased significantly in recent years.

Organic matter plays an important role in the global carbon cycle of the planet [7,8]. However, so far pyrogenic components of the soil organic matter (SOM) are practically not taken into account. Moreover, there are global changes in the humosphere under unpredictable effect of fires, and the fingerprints of this key factor on the molecular structure of SOM are underestimated. In order to assess the implications of global climate changes

on the SOM sequestration rate and its stability, both the amount and the structural stability of SOM should be investigated for postpyrogenic environments [9,10]. Therefore, it is essential to predict the resistance degree of organic matter to transformation processes. A qualitative composition of humic acids (HAs) analysis is a powerful instrument for investigation the stability of SOM [7,9]. SOM consists of up to 50% humic acids (HAs), which are formed in soils during the humification of organic residues [8]. The composition of HAs determines the direction of the stabilization processes of organic matter in soil.

Forest fires alter the morphological and physicochemical properties of soils [11–14] and lead to the complete or partial degradation of upper horizons and the formation of so-called pyrogenic horizons [15,16]. During the fire, the dry Oi horizon serves as fuel, burns out, and forms a thin Q_{pyr} horizon in its place, where the main products of the partial pyrolysis of the organic matter was concentrated. However, there are disagreements regarding the study of postpyrogenic soil organic matter.

SOM changes caused by the action of fire or high temperature lead to the formation of the so-called pyrogen-transformed humus (pyromorphic humus) [17–21]. The temperature effect on soil organic matter is a complex and not fully understood issue. In addition to the difficulty of studying the system of humic substances in soil, this is also explained by a number of specific reasons:

1. the dependence on the duration of exposure to different temperature effects, so it is impossible to compare a ten-second exposure to a temperature of 1000 °C and exposure for 30 min at a temperature of 300 °C, but, unfortunately, there are no comprehensive studies on this topic in the scientific literature;
2. the soil temperature during and after the fire in such studies is often an average value, while soil and organic residues can reach temperatures of about 850 °C on the surface;
3. particles of organic matter exposed to different temperatures will be distributed over the entire upper organic horizon even after fires of low intensity;
4. there are no studies about the effect of different types of fires on SOM and its modification during the time after wildfires.

Summarizing the available literature on this issue, it is reasonably safe to suggest that, on the one hand, the humus soil state undergoes certain changes during fires, and on the other hand, it is obvious that there is no consensus on the question of processes occurring with SOM during fires. Previous publications contain rather heterogeneous data on the stability of pyrogen-transformed humus [15]. SOM stabilization includes chemical stabilization, biochemical stabilization, and physical protection [22]. Some papers show a very slow decomposition rate [23], and some admit that black carbon is very stable and practically non-degradable [19], while other authors show that it successfully undergoes chemical [24,25] and microbial [26] oxidation. C.M. Preston and M.W.I. Schmidt showed that pyrogenic SOM is largely resistant to decomposition and therefore is included in a very stable carbon pool in soils and sediments [27]. The alteration of pyrogen-transformed humus stability over time (several years, decades, and so on) has not yet been studied by other researchers. Moreover, the impact of forest fires on SOM in the forest–steppe zone has been studied rather poorly compared to studies of soils in the boreal zone [28–33], but the number of forest fires in the forest–steppe zone increases every year, and they become more catastrophic [34]. Besides, researchers devoted to the processes of organic matter transformation over time (monitoring work) for the same objects have not been previously carried out—this shows the relevance and scientific novelty of this work.

For a deeper study of the fundamental processes of humification and structural fragments in postfire areas, it is necessary to use modern high-precision instrumental methods [35]. NMR spectroscopy has significantly expanded the knowledge on SOM composition and structure and enriched understanding of humification and transformation mechanisms [36]; therefore, this study use these methods for postpyrogenic SOM characterization. There are a few indices for the assessment of humus stability: for example, the degree of aromaticity and the elemental composition of humic substances [37,38].

Therefore, the aim of this work is to study the molecular composition of soil organic matter after wildfires.

To achieve this aim, the following tasks were set:

- to describe the process that occur in SOM after wildfires by way of the determination of the elemental composition of HAs isolated from control and postpyrogenic soils;
- to characterize molecular fragments of HAs isolated from control and postpyrogenic soils according to 13C-NMR spectroscopy;
- to estimate the rate and trend of organic matter transformation during the time (10-year period).

2. Materials and Methods

2.1. The Study Area

The subject of the study is the forest–steppe island pine forests near Tolyatti city, Samara region, Russian Federation, which were affected by catastrophic forest fires in July 2010. This is the territory of the Stavropol pine forest (a former park area between the Komsomolsky, Central and Avtozavodsky districts of Tolyatti city, near the Institute of Ecology of the Volga Basin of the Russian Academy of Sciences (53°29′43.80″ N, 49°20′56.44″ E, 179 m a.s.l.) (Figure 1). The study was carried out in the areas affected by the crown and surface forest fires that occurred in 2010 and in the territory that was not exposed to fire (control). A description of the samples and studied plots is given in Tables 1 and 2. The abovementioned areas were studied in 2010 as quickly as possible after the removal of the emergency regime from the territory, and annual surveys of the territories were also carried out. This article discusses postpyrogenic succession comparing the results of field works in 2010 with the results in 2020 at the same sampling plots.

The forest type at the control plot is grassy pine forest. A grass–gramineous successional stage was noted at the crown fire plot in 2020, and the total projective cover was 60–70% (Table 2). The following plant species were present: *Calamagrostis epigeios* (L.) Roth, *Bromopsis inermis* (Leyss.) Holub, *Artemisia absinthium* L., *Artemisia austriaca* Jacq., *Artemisia campestris* L., *Nonea pulla* (L.) DC., *Sisymbrium loeselii* L. and *Chamaecytisus ruthenicus* (Fisch. ex Wolosz.) Klaska. A shrub steppe with *Chamaecytisus ruthenicus* (Fisch. ex Woł.) Klásk. had begun to form at the surface fire plot (Table 2), and the total projective cover was 90–95%. Plant species: *Chamaecytisus ruthenicus* (Fisch. ex Wolosz.) Klask., *Veronica chamaedrys* L., *Taraxacum officinale* Wigg. s. l., *Saponaria officinalis* L., *Berteroa incana* (L.) DC., *Calamagrostis epigeios* (L.) Roth.

Soils of the studied areas are gray humus sandy and loamy soils with signs of the alpha-humus process development but without the formation of an independent podzolic horizon formed on ancient alluvial Volga sands—Psamment Entisols (according to WRB). Soil diagnostics were carried out according to the "Classification and diagnostics of soils of Russia" [39] and the World Reference Base for Soil Resources, FAO 2015 [40].

Data on the main chemical parameters of soils were published in previous works. Based on study results, it was shown that pyrogenic soil formation in the subboreal zone is characterized by the following elementary soil processes [41–43]: litter burnout, accumulation of carbonaceous material, reduction of pore space, an increase of pH of topsoil, deterioration of biological activity and accumulation of humons. The direct fire effect on soil properties occured only on surface horizons up to 20 cm in depth. However, the indirect effect of the pyrogenic factor on soils can manifest with a latent period, gradually spreading to ever deeper layers. Studies have shown that forest fires alter the morphological and physicochemical properties of soils [41,44–46] and lead to the complete or partial degradation of organic horizons (according to C_{total}—Table 1) and the formation of so-called pyrogenic horizons [15,16,47,48].
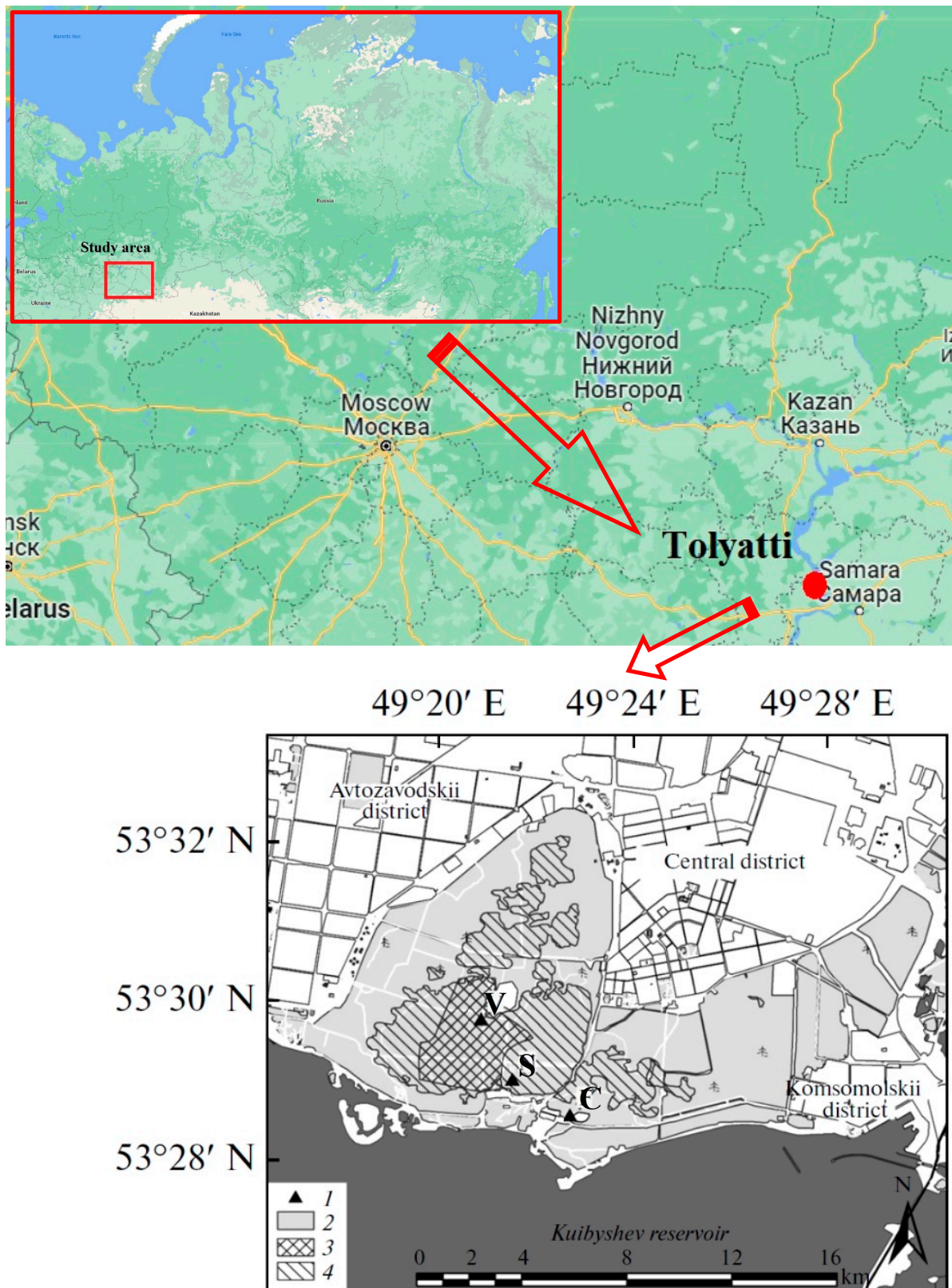
Figure 1. Stavropol pine forest damaged and destroyed by forest fires in 2010. Symbols: 1—▲—location of soil profiles (V—crown fire area, S—surface fire area, C—area on the territory unaffected by the fire); 2—forested areas (before the fire in 2010); 3—territories affected by crown forest fire; 4—territories affected by surface forest fire.

Table 1. The description of soils studied and HAs extracted.

Sample No.	Plot	Year of Sampling	Horizon, Depth, cm	Soil Horizons Description	C_{total}, %	Ash Content of HAs, %
1	Control	2010	O, 0–10	peat litter, consisting of needles, tree bark, leaves and cones, 10 YR 4/2 (Munsell chart method), slightly moistened, gray with whitish dusting, loose, sandy loam, structureless, roots abundance, presence of coal particles	3.94 ± 1.35	10.69
2	Crown fire	2010	Qpyr, 0–5	ash with needles and cones, dry, gray, 10 YR 3/2 (Munsell chart method), loose, sandy loam, loose crumbly structure, roots and coals abundance, undulating boundary	1.42 ± 0.31	2.81
3	Surface fire	2010	Qpyr, 0–4	ash with needles, leaves and burnt pine cones, slightly moistened, loose, gray, 10 YR 3/2 (Munsell chart method) sandy loam, loose crumbly structure, roots and coals abundance, undulating boundary	1.21 ± 0.50	3.93
4	Crown fire	2020	AYpyr, 0–20	color from gray to dark gray, 10 YR 2/1 (Munsell chart method), traces of surface water erosion, slightly moistened, loose, sandy loam, crumbly–nutty structure, roots abundance, presence of coal particles, undulating boundary	2.09 ± 0.20	11.43
5	Surface fire	2020	AYpyr, 0–29	dark gray, 10 YR 3/1 (Munsell chart method), almost black color, no signs of erosion, slightly moistened, loose, sandy loam, crumbly–nutty structure, roots abundance, presence of coal particles, undulating boundary	3.16 ± 0.25	5.69

Table 2. The photo description of studied plots and soils. Sample No. corresponds to Table 1.

Sample No.	Plot Photo	Soil Photo
1 Control 2010		

Table 2. *Cont.*

Sample No.	Plot Photo	Soil Photo
2 Crown fire 2010		
3 Surface fire 2010		
4 Crown fire 2020		

Table 2. *Cont.*

Sample No.	Plot Photo	Soil Photo
5 Surface fire 2020		

2.2. Laboratory Analysis

Studied soil samples were taken from the topsoil at each plot (depth is shown in Table 1). This article compares results of field summer works in 2010 with 2020; therefore, soils were sampled in these periods. Each soil was sampled in threefold replication. Three plots were investigated for each study scenario for statistics, and in this paper, generalized soil data for each situation are described. Soil samples were dried to an air-dry state and sifted through a sieve with a mesh size of 1 mm.

The HA isolation and purification were conducted using the procedure described by M. Schnitzer [49] and Swift [50] and modified by Chukov [51]. At the end of the isolation process, humic powders were treated with hydrofluoric acid to coagulate mineral suspensions and finally remove fine mineral suspensions [52]. The elemental composition (C, N, H) of humic acids (HAs) was determined using a Euro EA1110 (CHNS-O) analyzer in the ecoanalytical laboratory of the Institute of Biology at the Komi Science Centre of the Ural Branch of the Russian Academy of Sciences (IB FRC Komi SC UB RAS). The ash content of humic powders (Table 1) was determined using the gravimetric method, GOST 11306-2013 (Russian Federation).

Calculation of the atomic percentages; atomic ratios C/N, H/N and O/C; and oxidation degree (ω) of humic acids was carried out in accordance with the manual of Orlov and Grishina [53]. C, H, N, and O content was calculated as % of mass, and C/N, H/C, and O/C are mole ratio. The ratio H/C modified (H/C$_{mod}$), which indicates the number of substituted hydrogen atoms in HAs, was used for avoiding errors in the determination of the hypothetical hydrocarbon skeleton of the studied HAs. The H/Cmod was calculated according the following formula [54,55]:

$$H/C_{mod} = H/C + 2\ (O/C) * 0.67 \tag{1}$$

In order to compile the simplest formula of humic acids, the determined mole number of each element is divided by the smallest of them (the smallest number of moles in humic acid is represented by nitrogen). As a result, the simplest atomic multipliers are obtained; the results are expressed in round numbers of tenths in order to simplify calculations, and then the determined values are multiplied by the smallest integer that reduces all values to an integer number of atoms [53,54].

The total relative oxidation or reduction of humic acids is estimated from the differences in the numbers of oxygen and hydrogen atoms per carbon atom in the whole molecule:

$$\omega = (2\,Q_O - Q_H)/Q_C \tag{2}$$

where Q_O is the number of oxygen atoms; Q_H is the number of hydrogen atoms, and Q_C is the number of carbon atoms in a molecule of humic acids.

The combustion value of humic acids was determined by the Aliev formula [55]:

$$Q = 90\,C + 34.4\,H - 50\,(0.87\,O - 4\,N) \tag{3}$$

where Q is the combustion value (cal/g); and C, H, O, and N are the content of carbon, hydrogen, oxygen, and nitrogen in the humic acids (%).

Van Crevelen diagrams of humic acids were compiled in accordance with the method of Orlov [54].

^{13}C-NMR spectra of humic acids were determined using a Bruker Avance 500 NMR spectrometer (Karlsruhe, Germany, 2003). The data from NMR spectroscopy was obtained from the "Center of Chemical Analyses and Materials and Center of Magnetic Resonance Research", Scientific Park of Saint-Petersburg State University. Solid-phase samples were placed in a 3.2 mm zirconium oxide rotor and spun at a frequency of 12 kHz at the magic angle. The contact time was 2 m; the delay time was 3 s, and the number of scans was 8000. The relative contributions of the various carbon groups were determined by the integration of the signal intensity in their respective chemical shift regions. Data were processed using MestReNova. Groups of different compounds were distinguished using chemical shift values. Spectra were divided into the following main chemical shift regions [56–63]: alkyl C (0–47 ppm), methoxyl C (O-CH3) (47–60 ppm), O,N-alkyl C (60–108 ppm), aromatic C (108–164 ppm: 108–144 ppm—C,H-arom and 144–164 ppm—O,Narom), atoms of carboxyl fragments and carbonyl-amides (164–183 ppm), atoms of quinone fragments (183–190 ppm), and carbonyl groups of ketones and aldehydes with signals in the ranges of 190 to 204 ppm. The relative intensity for each chemical-shift region was obtained with the integration routine of the spectrometer. The following parameters were used to standardize the quantitative characteristics of HA molecules: the ratio of the aromatic structures' carbon to the aliphatic chains' carbon, (Ar/AL) [57,64], the aromaticity degree (Ar/(Ar + AL)) [61], and the SOM decomposition degree (*C,H*-alkyl/*O,N*-alkyl) [65,66]; in addition, Lodygin et al. [57] proposed an integral index of HAs' hydrophobicity ($AL_{H,R} + AR_{H,R}$), which is the total content of unoxidized carbon atoms (substituted by hydrogen atoms or other aliphatic fragments), which makes it possible to indirectly estimate the amphiphilic properties of HAs, calculated as the sum of the signals at 0–47 and 108–144 ppm. The signals of aromatic structures (Ar) were summarized over the areas of 108–164, 183–190 ppm, aliphatic (AL)—0–105, 164–183, and 190–204 ppm.

The normal distribution of the data was verified, and a variance analysis (ANOVA) and post hoc test (Fisher's least significant difference) were performed. Differences between several scenarios (control, immediately after wildfire (surface and crown forest fires) and 10 years after fires (surface and crown)) were considered significant at $p < 0.05$. Statistical data processing and analysis were carried out using standard methods in software packages MS Excel 2016, Past (version 3.20), and Statistica 64 (version 10).

3. Results and Discussion

3.1. Elemental Composition of Studied HAs

The elemental composition of HAs is the most important indicator that determines the progress of humification, oxidation, and the degree of condensation of HAs [57,67]. The elemental composition of HAs varies within a fairly wide range, which is associated with the high heterogeneity of the local conditions for the formation and maturation of HAs in the soil [68]. The main factors influencing the formation of HAs are the composition of the precursors of humification, the rate of humification, and the climatic parameters of the

territory [68]. There are results of high temperatures' effect on the elemental composition of humic acids as a result of forest fires.

Apart from the mass % of elements (C, H, N, O) and atomic percent (Table 3), the molar ratios of the studied elements were used in order to provide more detailed information. The C/N ratio is an indicator of the degree of carbon enrichment with nitrogen. The H/C ratio is an indicator of the carbon degree enrichment with hydrogen and indicates the type of structure of the HAs. The O/C ratio is an indicator of the degree of oxidation of HAs [58].

Table 3. The elemental composition of studied HAs.

Sample No. *	C, %	N, %	H, %	O, %	C:N	O:C	H:C	H/C_{mod}	Formula of Humic Acids	Combustion Value, cal/g	Oxidation Degree
1	54.7	4.5	5.5	35.3	14.15	0.48	1.20	1.85	$C_{99}H_{119}O_{48}N_7$	4481	−0.23
2	57.5	5.6	4.0	32.9	11.98	0.43	0.84	1.41	$C_{84}H_{70}O_{36}N_7$	5005	0.02
3	55.3	5.6	4.4	34.7	11.50	0.47	0.94	1.57	$C_{58}H_{54}O_{27}N_5$	4740	0.01
4	54.3	4.7	3.5	37.5	13.48	0.52	0.77	1.46	$C_{40}H_{31}O_{21}N_3$	4316	0.27
5	53.7	4.1	3.5	38.7	15.28	0.54	0.78	1.50	$C_{107}H_{83}O_{58}N_7$	4090	0.31
Post hoc test	0.24	$p \ll 0.05$	$p \ll 0.05$	$p \ll 0.05$	0.16	0.20	0.15	0.34		$p \ll 0.05$	$p \ll 0.05$
Significance of differences	Insign.	Sign.	Sign.	Sign.	Insign.	Insign.	Insign.	Insign.		Sign.	Sign.

* Sample No. corresponds to Table 1: 1—Control; 2—Crown fire, 2010; 3—Surface fire, 2010; 4—Crown fire, 2020; 5—Surface fire, 2020.

The mass percent of carbon is 54.7% in humic acids of the control plot and 53.7–57.5% in postpyrogenic soils. The oxygen content in the HAs of control soils is 35.3%, and for postfire soils it varies from 32.9% to 38.7%. The nitrogen content in HA molecules after fires change in the range of 4.1 to 5.6%, while for the control, it is 4.5%.

The type of molecule carbon skeleton structure can be approximately identified using the H/C ratio [54]. According to D. van Krevelen [69], H:C > 2 ratios are characteristic of paraffins and H:C ratios from 1.5 to 1.8–2.0 are characteristic of cycloparaffins; the H:C ≪ 1.0 ratio is characteristic of aromatic hydrocarbons. This ratio shows that the HA molecules of the control plot have a significant proportion of aliphatic fragments, while, as a result of fire, their number noticeably decreases; moreover, the proportion of oxygen-containing and aromatic components increases. Therefore, the aromaticity degree of organic substances formed during fires increases, and after 10 years, this trend continues. There is an increase in the HAs molecules carbonization (carbon content) in the range: control > surface fire > crown fire, but after 10 years, this indicator returns to control values. However, the H/C ratio indicates that the hydrogenation degree decreases in the range: control > surface fire 2010 > crown fire 2010 > fire 2020.

Figure 2 shows how three groups of humic acids are clearly distinguished: the control site and two separate groups after a forest fires—during sampling in 2010 and in 2020. It should be remembered that the atomic ratio diagram allows concluding only the total result of the HA transformation process and does not reveal its stages. Samples after fires are characterized by the dehydrogenation of HA molecules, and the same is true for the crown fires (in both situations—2010 and 2020) to a greater extent. With a decrease in the proportion of hydrogen, there is a loss of C-H, CH_2, and CH_3 groups and the formation of more stable fragments of HAs [35], which is especially expressed in case of samples from 2020. Dehydrogenation can occur either as a result of hydrogen loss (absolute dehydrogenation) or as a result of oxidation (relative dehydrogenation). The high oxygen content is associated with the better solubility of oxygen-enriched hydrophilic HA molecules [57]. An increase in the carbon content and a decrease in the hydrogen content in the structure of humic acids have been shown by many authors, in particular, for example, N.V. Chukhareva [70]. Reduced oxygen content and a decrease in the O/C ratio after fires in 2010 indicates the loss of peripheral fragments by HA molecules, which changes the redox balance in the molecules, which will be discussed below. The oxidation

degree of HA is an important characteristic. On the contrary, the samples of 2020 show an increase in the oxygen content, as well as the O/C ratio, which indicates the oxidation of HA molecules 10 years after the fires (Figure 2). It is also confirmed by a change of the oxidation degree in a strongly positive direction (Table 3), whereas HA molecules of the control plot are restored. Active processes of organic matter transformation take place in soils under oxidative conditions according to the oxidation degree. The higher activity of biota in postfire soils 10 years later is likely to promote the oxidation of HA molecules and the accumulation of the most stable oxygen-containing products.
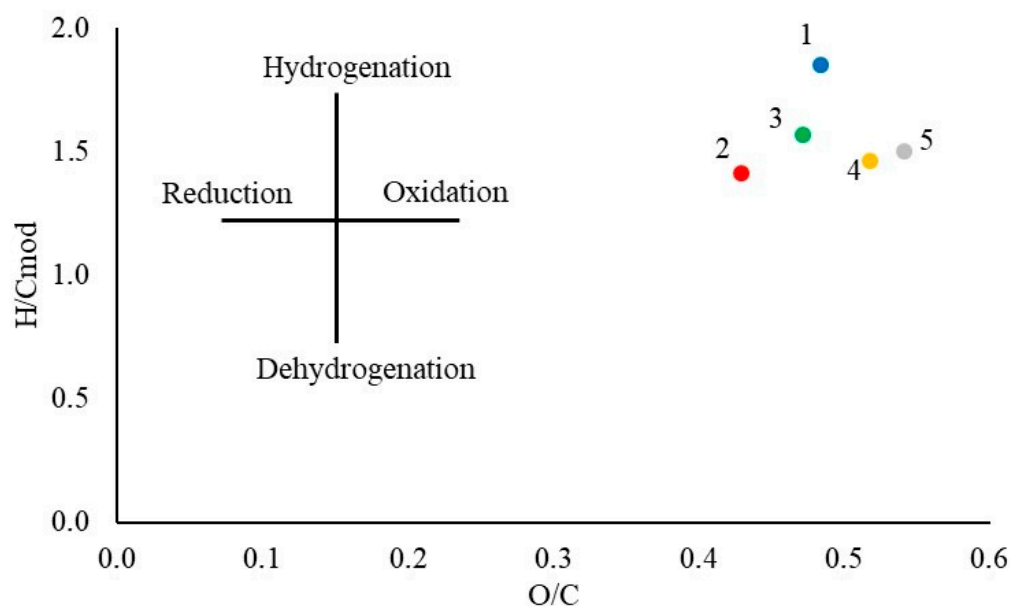

Figure 2. The van Krevelen diagram of the elemental composition of HAs. Sample No. corresponds to Table 1: 1—Control; 2—Crown fire, 2010; 3—Surface fire, 2010; 4—Crown fire, 2020; 5—Surface fire, 2020.

The percentage of hydrogen decreases as a result of fires and continues to decrease for 10 years, which is the reason for the dehydrogenation of HA molecules. The effect of the crown fire leads to stronger dehydrogenation (Figure 2) due to a more complete loss of hydrogen. At the same time, the amount of carbon in the HA molecules in soils after forest fires increases, which indicates that there is a partial degradation of the peripheral part of HA molecules, and that is another reason for dehydrogenation. Therefore, a hardly hydrolyzable black carbon component, the so-called "black" organic carbon, formation in postpyrogenic soils is obvious—highly aromatic compounds formed as a result of oxidative processes.

The simultaneous decrease in the H/C and O/C atomic ratios during fires indicates a significant loss of oxygen-containing functional groups with an increase in the aromaticity degree of HA molecules. The number of oxygen-containing groups increases after 10 years, molecules become more oxidized, but the aromaticity degree does not change. As shown by previous researchers, high-temperature-induced dehydration and polymerization reactions lead to the accumulation of a large number of aromatic structures, including nitrogen-containing heterocyclic compounds [19,20,71]. There is an increase in the O/C ratio and the oxidation degree of HA molecules when comparing humic acids in 2010 and 2020. HA samples changed the oxidation degree from reduced compounds in case of the control plot to compounds with zero oxidation degree immediately after the fires (2010), while the 2020 HA samples clearly show oxidized compounds (Table 3).

The enrichment degree of humic acids with nitrogen, determined by the C/N ratio, narrows as a result of fires, which indicates that the structure and composition of HA molecules are being transformed, which is accompanied by a slight reduction (Figure 2)

and an increase in the nitrogen content in the HA molecule. This can be due to the formation of heterocycles with nitrogen. However, after 10 years, this indicator approaches the control plot values. Conjugated reactions of the breaking C-C bonds, dehydration, and saponification, as well as the hydrolysis and substitution of NH_2 groups, associated with organic nitrogen compounds, occur during the process of the destruction of organic matter under the influence of high temperatures, excess water, and its vapors as chemical agents [72]. As a result, NH_3, acid amides, hydroxy compounds of pyridine bases with a high tendency for condensation, volatile and non-volatile phenols, benzol, and other aromatic compounds are formed, that is, the necessary primary sources of structural units of the humic acid molecule [72].

Moreover, as a result of the calculations carried out, the simplest HA formulas for postfire soils, which are presented in Table 3, were composed. When compiling the simplest formulas of humic substances, it should be always remembered that their conventionality follows primarily from the complex fractional composition of the analyzed powders. One of the advantages of the simplest formulas is the clarity of the composition and the evaluation of atomic ratios. These formulas are no less convenient when studying the distribution of individual atoms over atomic (functional) groups.

The humic acids of postfire soils in 2010 are characterized by increased caloric content and after 10 years, humic acids are reduced in relation to the control, according to combustion value data. This fact is in good agreement with the decrease in the content of oxygen-containing groups and the decrease in the O/C ratio. This is probably due to the fact that during fires, as shown by researchers [20], there is a significant, up to sixfold, increase in the fraction of soil lipids, which are high in calories. Some lipids are plant growth promoters or inhibitors, and waxes and similar substances are the essential cause of soil water repellency [19]. Another explanation is also possible—the calorific capacity of HA molecules increases due to a relative decrease in the oxygen content, because in the case of oxidation, the oxygen atoms play a significant role in this reaction, which reduces the total calorific capacity of organic matter.

An array of data on the elemental composition of soil HAs after fires was obtained according to the studies carried out, which made it possible to reveal the patterns of the transformation of organic matter. The obtained data clearly show that humic acids in the soils of the postfire and control plots form rather heterogeneous groups. The main difference in the degree of the heterogeneity of HA groups is the ratio of hydrogen, oxygen, and carbon. There is a simultaneous decrease in the H/C and O/C atomic ratios at high temperatures, which indicates a slight loss of oxygen-containing functional groups (moreover, the loss is greater during a crown fire), while the aromaticity degree of molecules increases. Changes also occur in the HA molecules 10 years after the fires; these manifest mainly in an increase in the content of oxygen-containing groups, which is accompanied by the oxidation of the HA molecule with the same increase in the aromaticity degree of the HA molecule.

3.2. Molecular Structure of Studied HAs

Analysis of the ^{13}C-NMR spectra of humic acid from the studied soils made it possible to identify the ranges of chemical shifts belonging to carbon atoms of various functional groups and molecular fragments of HA [60,61,73–76] (Figure 3). The results of the ^{13}C-NMR-spectroscopy analysis of functional groups and molecular fragments are given in Figure 4.
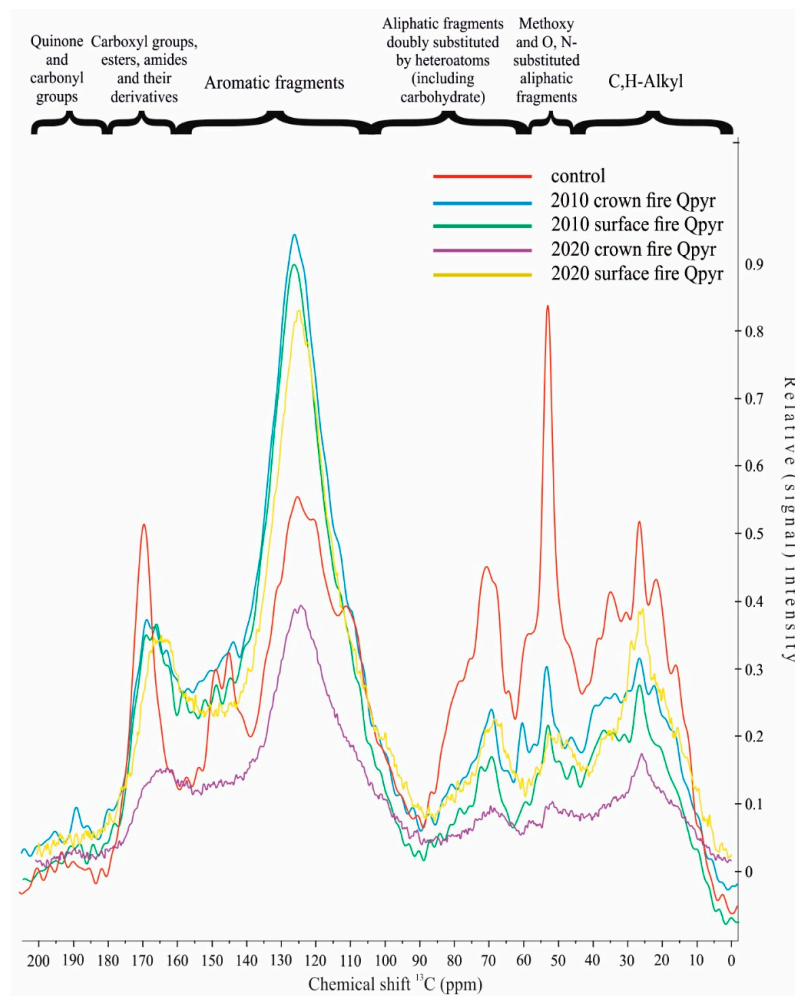
Figure 3. The ^{13}C NMR spectra of HAs isolated from studied soils.
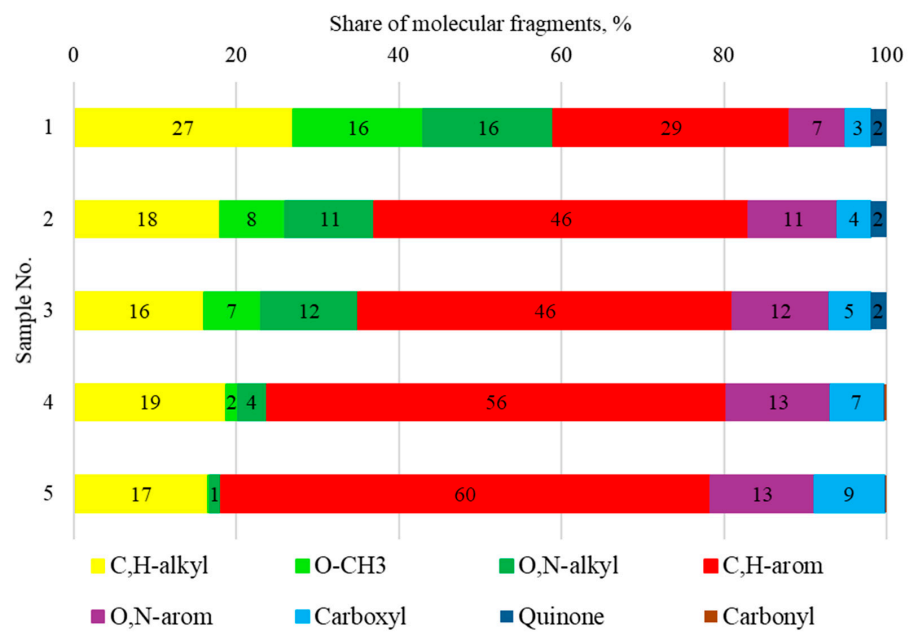

Figure 4. The molecular composition of HAs isolated from studied soils. Sample No. corresponds to Table 1: 1—Control; 2—Crown fire, 2010; 3—Surface fire, 2010; 4—Crown fire, 2020; 5—Surface fire, 2020.

The data obtained confirm many studies in this field [17,21,77] that the aliphatic part is the main share in the humic acids' composition of soils that are not affected by forest fire, while, as a result of fires, the aromaticity degree of HA molecules significantly increases, which is a typical zonal feature of soil organic matter in the forest–steppe zone [78].

All ^{13}C NMR spectra have one relatively sharp peak in the 0–47 ppm region, which can be related to methylene atoms of carbon in positions α, β, δ, and ε from methyl end-groups in alkyl chains [79]. The data obtained show a significant decrease in the content of these functional groups as a result of fires from 27% to 16–18% compared to control, and there were no changes after 10 years. The share of O,N-alkyl C, predominantly represented by cellulose C, decreased to 12% after forest fires (2010) compared to control, and after 10 years it continued to decline to 2%. The proportion of methoxyl C (47–60 ppm), probably associated with lignins, decreased from 16% in control plot up to 8% after wildfires in 2010 and to 0% in 2020. Moreover, the difference between a crown and a surface forest fire in this case was not so noticeable, which does not confirm previous studies that, with heating, the thermal destruction of hemicelluloses takes place first, followed by the destruction of cellulose and lignin with further increasing temperature [14,80].

Based on data obtained, we can note that, in the studied control HAs, aliphatic fragments predominate up to 62% in sample No. 1. Sharp peaks are noted in the region of 108–164 ppm after forest fires—there is a significant increase in aromatic fragments in the HA molecules of postpyrogenic soils—up to 60% for samples in 2010 (crown and surface fires do not differ from each other), and there is a further increase in the aromaticity degree of HA molecules after 10 years—up to 73%. Moreover, it was previously shown that this change concerns only the upper horizons, and the underlying humus horizons do not differ in HA composition compared to the control soils [81]. Therefore, in the process of humification in the studied samples, there is a decrease in the proportion of aliphatic and the accumulation of aromatic (C-C/C-H-AR, COO/N-C=O).

As a result of forest fires, the content of groups present in lignins and methyl groups of aromatic and aliphatic carbon decreases, and the content of carbon atoms associated with oxygen, secondary alcohols, hydrocarbons, and *C,H*-alkyl and tetrasubstituted carbon groups slightly decreases. However, the content of fragments of C–C bonds, C,H-substituted aromatic carbon groups, and aromatic carbon of phenolic esters and phenols increases in postpyrogenic areas. These results are consistent with the data of HA elemental analysis: fires cause a significant loss of oxygen-containing functional groups (due to their effect on hydrophobicity, retention of bases, and, mainly, colloidal properties and solubility in humic substances) and an accumulation of a large number of aromatic structures.

Aromatic structures (AR) in the humic substances' composition determine their low water solubility and high stability. Stability to the biodegradation of new formed pyromorphic humus substances has been discussed by Gonzalez-Perez et al. [19,71]. An increase in the proportion of aromatic fragments of HAs leads to the stabilization of organic matter in soils and its low availability for soil microorganisms and, thus, to further transformation [82].

An analysis of the integral indicators of the humic substances' molecular composition showed that HAs isolated from samples taken in 2020 are generally more mature and resistant to oxidation (including microbial) compared to 2010 (Table 4). Some researchers proclaim the opposite opinion, that it successfully undergoes chemical [24,25] and microbial [26] oxidation. The total share of unoxidized carbon atoms in HAs is about 77%, while for 2010 this value is noticeably lower—62%. This indicates that periodically repeating low-intensity fires in forest–steppe ecosystems can contribute to the accumulation of stable forms of pyrogenic carbon in soils.

Table 4. Integral indicators of the molecular composition of HAs in postpyrogenic soils.

Sample No. *	AR, %	AL, %	Aromaticity Degree	AR/AL	$AL_{H,R} + AR_{H,R}$	C,H-Al/O,N-Al
1	38	62	0.38	0.61	56	0.84
2	59	41	0.59	1.44	64	0.95
3	60	40	0.60	1.50	62	0.84
4	69	31	0.69	2.27	75	3.74
5	73	27	0.73	2.71	77	10.89
Post hoc test	$p \ll 0.05$	$p \ll 0.05$	$p \ll 0.05$	$p \ll 0.05$	$p \ll 0.05$	0.33
Singnificance of differences	Significant	Sign.	Sign.	Sign.	Sign.	Insign.

* Sample No. corresponds to Table 1: 1—Control; 2—Crown fire, 2010; 3—Surface fire, 2010; 4—Crown fire, 2020; 5—Surface fire, 2020.

The H/C index correlates well with the data on humic substance aromaticity obtained through ^{13}C-NMR spectroscopy [59,62,73].

Some differences are noted in the C,H-alkyl/O,N-alkyl ratio reflecting the degree of organic matter decomposition: 2020 samples have relatively high values up to 10.9, which indicates their greater degree of humification compared with samples immediately after the fires (2010). The humification of humic substances 10 years after fires leads to an increase in the thermodynamic stability of HA molecules, which is shown below.

It should be noted that almost all integral indicators differ significantly for all scenarios.

3.3. Stabilization of Organic Matter in the Studied Soils

The method of graphical data representation in the coordinates C,H-alkyl/O,N-alkyl − $AL_{H,R} + AR_{H,R}$ (Figure 5) was used for the numerical description of HA structure, which serves as a convenient example for demonstrating the contribution of oxidation processes (humification) and condensation (stability).
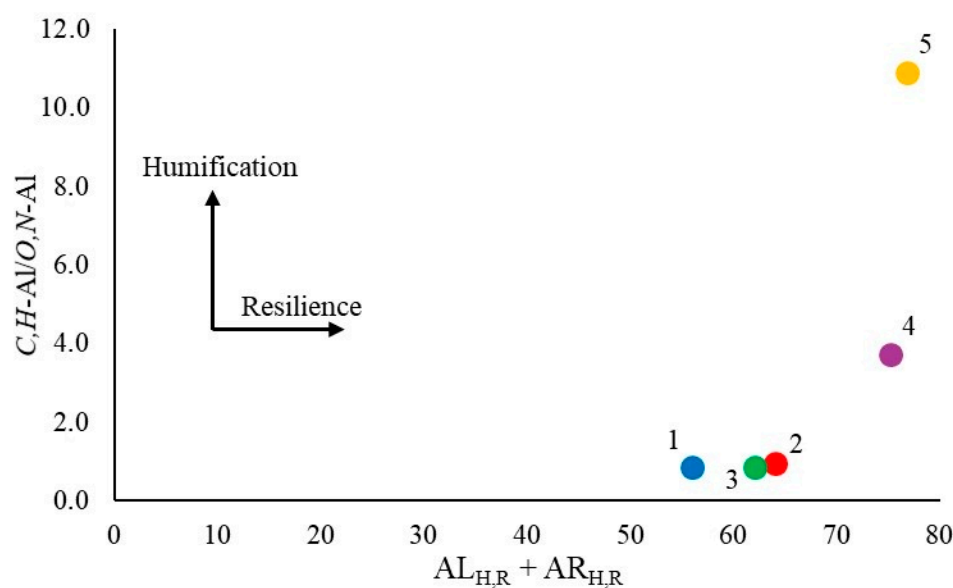

Figure 5. A diagram of the integrated indicators of HAs' molecular composition. Sample No. corresponds to Table 1: 1—Control; 2—Crown fire, 2010; 3—Surface fire, 2010; 4—Crown fire, 2020; 5—Surface fire, 2020.

Graphical and statistical analysis demonstrates a greater humification degree of samples in 2020 compared to analogues in 2010, and they are also more resistant to environmental factors.

A ternary graph with the use of a tri-plot from MS Excel was used for a numerical description of the structural-group composition of studied humic acids (Figure 6). The vertex of the triangle x(C,H-AL) is C,H-substituted aliphatic fragments (0–47 ppm); the corner x(Ar) is carbon atoms of aromatic fragments (108–144, 144–164 and 183–190 ppm), and the corner x(O,N-AL) is carbon atoms of O,N-substituted aliphatic fragments (47–60, 60–108, 164–183, and 190–204 ppm). Any point on the surface of the triangle defines the composition of the functional groups and molecular fragments of studied HAs [59,83].
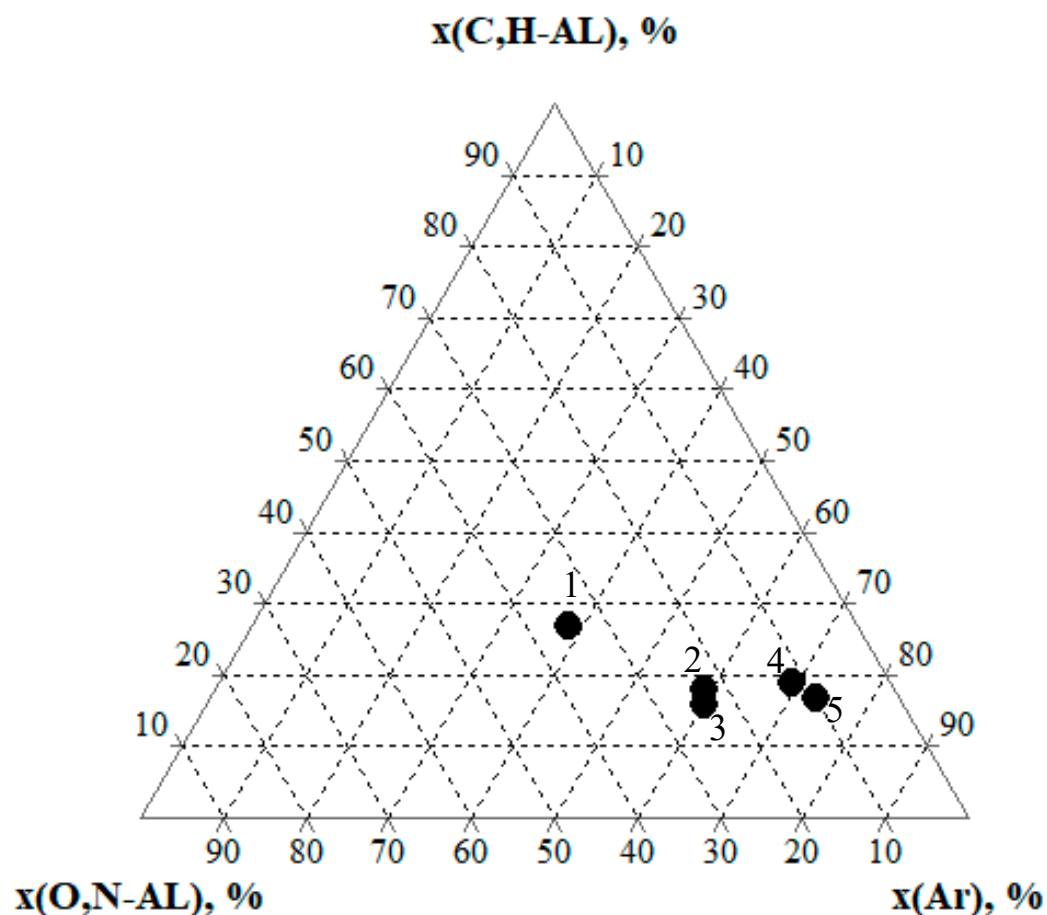

Figure 6. Diagram of structural and functional composition of HSs from studied soils. Sample No. corresponds to Table 1: 1—Control; 2—Crown fire, 2010; 3—Surface fire, 2010; 4—Crown fire, 2020; 5—Surface fire, 2020.

Therefore, Figure 6 clearly shows that there are significant changes in the structural organization of organic matter as a result of combustion (forest fires). Three heterogeneous groups of HAs are clearly distinguished—isolated from the control soil, from postfire soils: in 2010 and 2020. The accumulation of aromatic compounds in humic substances is obvious, due to a relative decrease in the aliphatic part or, in some cases, due to a small accumulation of carboxyl and carbonyl groups.

4. Conclusions

The assessment of the composition of HAs' main functional groups and molecular fragments proves that there is significant variation in their molecular composition when comparing control soil with postfire. There is a simultaneous decrease in the H/C and O/C atomic ratios at high temperatures (from 1.85 in case of control to 1.41 in case of fire),

which indicates a slight loss of oxygen-containing functional groups (the loss is greater during a crown fire), while the aromaticity degree of HA molecules increases. There were no significant differences between surface and crown fires. However, it was noted that there was an increase in the oxygen content in HA composition 10 years after the fires, as well as the O/C ratio, which indicates the oxidation of HA molecules, which is also confirmed by a change in the oxidation degree to a strongly positive direction (0.27–0.31), while the control HAs are restored (−0.23).

According to ^{13}C-NMR spectroscopy data, it was found that, along with quantitative changes, a significant transformation of the carbon skeleton of HA molecules occurs in postpyrogenic soils as a result of fires, which is expressed as a relative increase in the total content of aromatic structures (from 38% in control soils to 60–70% in postpyrogenic soils), especially their highly condensed varieties, due to a relative decrease in the aliphatic part or in in some cases due to the slight accumulation of carboxyl and carbonyl groups. The humification processes in soils 10 years after the fires contribute to the formation of "aggressive" humic acids enriched in oxygen-containing functional groups with a high content of aromatic fragments.

A comprehensive study of macromolecular organic compounds in postpyrogenic soils and the use of highly sensitive spectroscopic methods will contribute to the understanding of the fundamental processes of humus formation and the creation of new ideas about the complex composition and structure of natural molecular compounds in soils after fires. It becomes more relevant in the context of the essential role of SOM that plays an important in the global carbon cycle and global changes in the humosphere under the effect of unpredictable fires.

References

1. Doerr, S.H.; Santın, C. Global trends in wildfire and its impacts: Perceptions versus realities in a changing world. *Philos. Trans. R. Soc. B. Biol. Sci.* **2016**, *371*, 20150345. [CrossRef] [PubMed]
2. Flannigan, M.D.; Krawchuk, M.A.; de Groot, W.J.; Wotton, B.M.; Gowman, L.M. Implications of changing climate for global wildland fire. *Int. J. Wildland Fire* **2009**, *18*, 483–507. [CrossRef]
3. Tarasov, P.A.; Ivanov, V.A.; Ivanova, G.A.; Krasnoshchekova, E.N. Postpyrogenic changes in the hydrothermal parameters of soils in middle-taiga pine forests. *Euras. Soil Sci.* **2011**, *44*, 731–738. [CrossRef]
4. Bento-Goncalves, A.; Vieira, A.; Ubeda, X.; Martin, D. Fire and soils: Key concepts and recent advances. *Geoderma* **2012**, *191*, 3–13. [CrossRef]
5. Magnani, F.; Mencuccini, M.; Borghetti, M.; Berbigier, P.; Berninger, F.; Delzon, S.; Grelle, A.; Hari, P.; Jarvis, P.G.; Kolari, P.; et al. The human footprint in the carbon cycle of temperate and boreal forests. *Nature* **2007**, *447*, 849–851. [CrossRef]
6. Amiro, B.D.; Orchansky, A.L.; Barr, A.G.; Black, T.A.; Chambers, S.D.; Lii, F.C.; Goulden, M.L.; Litvak, M.; Liu, H.P.; McCaughey, J.H.; et al. The effect of post-fire stand age on the boreal forest energy balance. *Agric. For. Meteorol.* **2006**, *140*, 41–50. [CrossRef]

7. Dymov, A.A.; Startsev, V.V.; Milanovsky, E.Y.; Valdes-Korovkin, I.A.; Farkhodov, Y.R.; Yudina, A.V.; Donnerhack, O.; Guggenberger, G. Soils and soil organic matter transformations during the two years after a low-intensity surface fire (Subpolar Ural, Russia). *Geoderma* **2021**, *404*, 115278. [CrossRef]

8. Lal, R. Forest soils and carbon sequestration. *For. Ecol. Manag.* **2005**, *220*, 242–258. [CrossRef]

9. Routh, J.; Hugelius, G.; Kuhry, P.; Filley, T.; Tillman, P.K.; Becher, M.; Crill, P. Multi-proxy study of soil organic matter dynamics in permafrost peat deposits reveal vulnerability to climate change in the European Russian Arctic. *Chem. Geol.* **2014**, *368*, 104–117. [CrossRef]

10. Fritz, M.; Deshpande, B.N.; Bouchard, F.; Högström, E.; Malenfant-Lepage, J.; Morgenstern, A.; Nieuwendam, A.; Oliva, M.; Paquette, M.; Rudy, A.C.A.; et al. Brief Communication: Future avenues for permafrost science from the perspective of early career researchers. *Cryosphere* **2015**, *9*, 1715–1720. [CrossRef]

11. Imeson, A.C.; Verstraten, J.M.; van Mulligen, E.J.; Sevink, J. The effects of fire and water repellence on infiltration and runoff under mediterranean type forest. *Catena* **1992**, *19*, 345–361. [CrossRef]

12. Robichaud, P.R. Fire effects on infiltration rates after prescribed fire in northern Rocky Mountain forests, USA. *J. Hydrol.* **2000**, *231*, 220–229. [CrossRef]

13. Fultz, L.M.; Moore-Kucera, J.; Dathe, J.; Davinic, M.; Perry, G.; Wester, D.; Schwilk, D.W.; Rideout-Hanzak, S. Forest wildfire and grassland prescribed fire effects on soil biogeochemical processes and microbial communities: Two case studies in the semi-arid Southwest. *Appl. Soil Ecol.* **2016**, *99*, 118–128. [CrossRef]

14. Santin, C.; Doerr, S.H.; Merino, A.; Bryant, R.; Loader, N.J. Forest floor chemical transformations in a boreal forest fire and their correlations with temperature and heating duration. *Geoderma* **2016**, *264*, 71–80. [CrossRef]

15. Krasnoshchekov, Y.N.; Cherednikova, Y.S. Postpyrogenic transformation of soils under *Pinus sibirica* forests in the southern Lake Baikal basin. *Eurasian Soil Sci.* **2012**, *45*, 929–938. [CrossRef]

16. Dymov, A.A.; Abakumov, E.V.; Bezkorovaynaya, I.N.; Prokushkin, A.S.; Kuzakov, Y.V.; Milanovsky, E.Y. Impact of forest fire on soil properties (review). *Theor. Appl. Ecol.* **2018**, *4*, 13–23. [CrossRef]

17. Jimenez-Gonzalez, M.A.; De la Rosa, J.M.; Jimenez-Morillo, N.T. Post-fire recovery of soil organic matter in a Cambisol from typical Mediterranean forest in Southwestern Spain. *Sci. Total Environ.* **2016**, *572*, 1414–1421. [CrossRef]

18. Krasilnikov, P.V. Persistent carbon compounds in soils: Origin and functions. *Eurasian Soil Sci.* **2015**, *9*, 1131–1144.

19. Gonzalez-Perez, J.A.; Gonza'lez-Vila, F.J.; Almendros, G.; Knicker, H. The effect of fire on soil organic matter—A review. *Environ. Int.* **2004**, *30*, 855–870. [CrossRef]

20. Almendros, G.; Gonzalez-Vila, F.J.; Martın, F. Fire-induced transformation of soil organic matter from an oak forest: An experimental approach to the effects of fire on humic substances. *Soil Sci.* **1990**, *149*, 158–168. [CrossRef]

21. Almendros, G.; González-Vila, F.J.; Martín, F.; Fründ, R.; Lüdemann, H.-D. Solid state NMR studies of fire-induced changes in the structure of humic substances. *Sci. Total Environ.* **1992**, *117*, 63–74. [CrossRef]

22. Chen, H.Y.H.; Shrestha, B.M. Stand age, fire and clearcutting affect soil organic carbon and aggregation of mineral soils in boreal forests. *Soil Biol. Biochem.* **2012**, *50*, 149–157. [CrossRef]

23. Liang, B.; Lehmann, J.; Solomon, D.; Sohi, S.; Thies, J.E.; Skjemstad, J.O.; Luizão, F.J.; Engelhard, M.H.; Neves, E.G.; Wirick, S. Stability of biomass-derived black carbon in soils. *Geochim. Cosmochim. Acta* **2008**, *72*, 6069–6078. [CrossRef]

24. Cheng, C.-H.; Lehmann, J.; Engelhard, M.H. Natural oxidation of black carbon in soils: Changes in molecular form and surface charge along a climosequence. *Geochim. Cosmochim. Acta* **2008**, *72*, 1598–1610. [CrossRef]

25. Cheng, C.-H.; Lehmann, J.; Thies, J.E.; Burton, S.D.; Engelhard, M.H. Oxidation of black carbon by biotic and abiotic processes. *Org. Geochem.* **2006**, *37*, 1477–1488. [CrossRef]

26. Knicker, H.; Hilscher, A.; de la Rosa, J.M.; González-Pérez, J.A.; González-Vila, F.J. Modification of biomarkers in pyrogenic organic matter during the initial phase of charcoal biodegradation in soils. *Geoderma* **2013**, *197*, 43–50. [CrossRef]

27. Preston, C.M.; Schmidt, M.W.I. Black (pyrogenic) carbon: A synthesis of current knowledge and uncertainties with special consideration of boreal regions. *Biogeosciences* **2006**, *3*, 397–420. [CrossRef]

28. Han, C.-L.; Sun, Z.-X.; Shao, S.; Wang, Q.-B.; Libohova, Z.; Owens, P.R. Changes of Soil Organic Carbon after Wildfire in a Boreal Forest, Northeast CHINA. *Agronomy* **2021**, *11*, 1925. [CrossRef]

29. Scholten, R.C.; Jandt, R.; Miller, E.A.; Rogers, B.M.; Veraverbeke, S. Overwintering fires in boreal forests. *Nature* **2021**, *593*, 399–404. [CrossRef]

30. Ziegler, S.E.; Sharon, A.; Billings, S.A.; Chad, S.; Lane, C.S.; Li, J.W.; Fogel, M.L. Warming alters routing of labile and slower-turnover carbon through distinct microbial groups in boreal forest organic soils. *Soil Biol. Biochem.* **2013**, *60*, 23–32. [CrossRef]

31. Bouchard, M.; Pothier, D. Long-term influence of fire and harvesting on boreal forest age structure and forest composition in eastern Québec. *For. Ecol. Manag.* **2011**, *261*, 811–820. [CrossRef]

32. Simard, D.G.; Fyles, J.W.; Paré, D.; Nguyen, T. Impacts of clearcut harvesting and wildfire on soil nutrient status in the Quebec boreal forest. *Can. J. Soil Sci.* **2001**, *81*, 229–237. [CrossRef]

33. Kelly, J.; Ibáñez, T.S.; Santín, C.; Doerr, S.H.; Nilsson, M.-C.; Holst, T.; Lindroth, A.; Kljun, N. Boreal forest soil carbon fluxes one year after a wildfire: Effects of burn severity and management. *Glob. Chang. Biol.* **2021**, *27*, 4181–4195. [CrossRef] [PubMed]

34. Geraskina, A.P.; Tebenkova, D.N.; Ershov, D.V.; Ruchinskaya, E.V.; Sibirtseva, N.V.; Lukina, N.V. Wildfires as a factor of the loss of biodiversity and functions of forest ecosystems. *For. Sci. Issues* **2021**, *4*, 1–76. [CrossRef]

35. 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.

36. Uyguner, C.S.; Hellriegel, C.; Otto, W.; Larive, C.K. Characterization of humic substances: Implications for trihalomethane formation. *Anal. Bioanal. Chem.* **2004**, *378*, 1579–1586. [CrossRef]

37. Abakumov, E.; Alekseev, I. Stability of soil organic matter in Cryosols of the maritime Antarctic: Insights from ¹³C NMR and electron spin resonance spectroscopy. *Solid Earth* **2018**, *9*, 1329–1339. [CrossRef]

38. Lodygin, E.; Beznosikov, V.; Abakumov, E. Humic substances elemental composition of selected taiga and tundra soils from Russian European North-East. *Pol. Polar Res.* **2017**, *38*, 125–147. [CrossRef]

39. Shishov, L.L.; Tonkonogov, V.D.; Lebedeva, I.I.; Gerasimova, M.I. *Classification and Diagnostics of Soils in Russia*; Ojkumena: Smolensk, Russia, 2004; p. 342.

40. IUSS Working Group WRB. *World Reference Base for Soil Resources International Soil Classification System for Naming Soils and Creating Legends for Soil Maps*; World Soil Resources Reports No. 106; FAO: Rome, Italy, 2015.

41. Maksimova, E.Y.; Tsibart, A.S.; Abakumov, E.V. Soil properties in the Tol'yatti pine forest after the 2010 catastrophic wildfires. *Eurasian Soil Sci.* **2014**, *47*, 940–951. [CrossRef]

42. Chebykina, E.; Abakumov, E. Changes in key physical soil properties of post-pyrogenic forest ecosystems: A case study of catastrophic fires in Russian sub-boreal forest. In *Advances in Understanding Soil Degradation. Innovations in Landscape Research*; Saljnikov, E., Mueller, L., Lavrishchev, A., Eulenstein, F., Eds.; Springer: Cham, Switzerland, 2022; pp. 687–700. [CrossRef]

43. Chebykina, E.; Abakumov, E.; Shamilishvilly, G. Polycyclic aromatic hydrocarbons accumulation and toxic equivalency factors (TEFs) in postpyrogenic soils. In Proceedings of the VIII Congress of the Dokuchaev Soil Science Society, Syktyvkar, Russia, 19–24 July 2021; Volume 862, p. 012094. [CrossRef]

44. Abakumov, E.; Maksimova, E.; Tsibart, A. Assessment of postfire soils degradation dynamics: Stability and molecular composition of humic acids with use of spectroscopy methods. *Land Degrad. Dev.* **2017**, *29*, 2092–2101. [CrossRef]

45. Maksimova, E.; Abakumov, E. Wildfire effects on ash composition and biological properties of soils in forest-steppe ecosystems of Russia. *Environ. Earth Sci.* **2015**, *74*, 4395–4405. [CrossRef]

46. Maksimova, E.; Abakumov, E.; Shamilishvili, G. Sustainable development of forest ecosystems in urbanized territories as a way of wildfire control in Russia. In *Urbanization: Challenge and Opportunity for Soil Functions and Ecosystem Services*; SUITMA; Vasenev, V., Dovletyarova, E., Cheng, Z., Prokof'eva, T., Morel, J., Ananyeva, N., Eds.; Springer: Cham, Switzerland, 2019; pp. 279–288. [CrossRef]

47. Certini, G. Fire as a soil-forming factor. *Ambio* **2014**, *43*, 191–195. [CrossRef] [PubMed]

48. Dymov, A.A.; Gabov, D.N.; Milanovskii, E.Y. ¹³C-NMR, PAHs, WSOC and repellence of fire affected soils (Albic Podzols, Russia). *Environ. Earth Sci.* **2017**, *76*, 1–10. [CrossRef]

49. Schnitzer, M. Organic matter characterization. In *Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties. Agronomy Monograph No. 9.*; Page, B.L., Miller, R.H., Keeney, D.R., Eds.; Soil Science Society of America: Madison, WI, USA, 1982; pp. 581–594.

50. Swift, R.S. Organic matter characterization. In *Methods of soil Analysis. Part 3. Chemical Methods*, 1st ed.; Sparks, D.L., Ed.; SSSA: Madison, WI, USA, 1996; Volume 1, pp. 1001–1069.

51. Chukov, S.N.; Ejarque, E.; Abakumov, E.V. Characterization of humic acids from tundra soils of northern Western Siberia by electron paramagnetic resonance spectroscopy. *Eurasian Soil Sci.* **2017**, *50*, 30–33. [CrossRef]

52. Goncalves, C.N.; Dalmolin, S.D.; Dick, D.P.; Knicker, H.; Klamt, E.; Kögel-Knabner, I. The effect of 10% HF treatment on resolution of CPMAS ¹³C NMR spectra and on the quality of organic matter in Ferralsols. *Geoderma* **2003**, *116*, 373–392. [CrossRef]

53. Orlov, D.S.; Grishina, L.A. *Workshop on the Humus Chemistry*; Publishing House of Moscow State University: Moscow, Russia, 1981; 272p.

54. Orlov, D.S.; Sadovnikova, L.K.; Sukhanova, N.I. *Soil Chemistry*; Higher School: Moscow, Russia, 2005; p. 558.

55. Orlov, D.S. *Soil Chemistry*; Publishing House of Moscow State University: Moscow, Russia, 1985; p. 376.

56. Mathers, N.J.; Xu, Z.H. Solid-state ¹³C NMR spectroscopy characterization of soil organic matter under two contrasting residue management regimes in a 2-year-old pine plantation of subtropical Australia. *Geoderma* **2003**, *114*, 19–31. [CrossRef]

57. 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]

58. Orlov, D.S. *Soil Humic Acids and General Theory Humification*; Moscow State University: Moscow, Russia, 1990; p. 325.

59. Lodygin, E.; Vasilevich, R. Environmental aspects of molecular composition of humic substances from soils of northeastern European Russia. *Pol. Polar Res.* **2020**, *41*, 115–135. [CrossRef]

60. Lodygin, E.D.; Beznosikov, V.A. The ¹³C NMR study of the molecular structure of humus acids from podzolic and bog-podzolic soils. *Eurasian Soil Sci.* **2003**, *36*, 967–975.

61. Liang, B.C.; Gregorich, E.G.; Schnitzer, M.; Schulten, H.R. Characterization of water extracts of two manures and their absorption on soils. *Soil Sci. Soc. Am. J.* **1996**, *60*, 1758–1763. [CrossRef]

62. Vasilevich, R.; Lodygin, E.; Abakumov, E. The molecular composition of humic acids in permafrost peats in the European Arctic as paleorecord of the environmental conditions of the holocene. *Agronomy* **2022**, *12*, 2053. [CrossRef]

63. Li, L.; Huang, W.L.; Peng, P.A.; Sheng, G.Y.; Fu, J.M. Chemical and molecular heterogeneity of humic acids repetitively extracted from a peat. *Soil Sci. Soc. Am. J.* **2003**, *67*, 740–746. [CrossRef]

64. Lorenz, K.; Preston, C.M.; Kandeler, E. Soil organic matter in urban soils: Estimation of elemental carbon by thermal oxidation and characterization of organic matter by solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy. *Geoderma* **2006**, *130*, 312–323. [CrossRef]

65. Baldock, J.A.; Preston, C.M. Chemistry of carbon decomposition processes in forests as revealed by solid-state carbon-^{13} nuclear magnetic resonance. In *Carbon Forms and Functions in Forest Soils*; McFee, W.W., Kelly, J.M., Eds.; Soil Science Society of America: Madison, WI, USA, 1995; pp. 89–117.

66. Pedersen, J.A.; Simpson, M.A.; Bockheim, J.G.; Kumar, K. Characterization of soil organic carbon in drained thawlake basins of Arctic Alaska using NMR and FTIR photoacoustic spectroscopy. *Org. Geochem.* **2011**, *42*, 947–954. [CrossRef]

67. Mao, J.; Cao, X.; Olk, D.C.; Chu, W.; Schmidt-Rohr, K. Advanced solid-state NMR spectroscopy of natural organic matter. *Prog. Nucl. Magn. Reson. Spectrosc.* **2017**, *100*, 17–51. [CrossRef] [PubMed]

68. Piccolo, A.; Spaccini, R.; Savy, D.; Drosos, M.; Cozzolino, V. The soil humeome: Chemical structure, functions and technological perspectives. In *Sustainable Agrochemistry*; Vaz, J.S., Ed.; Springer: Chambrige, UK, 2019; pp. 183–222.

69. Krevelen, v.D. Graphical and statistical method for studying the structure and processes of coal formation. In *Chemistry of Solid Fuel*; Publishing House of Foreign Literature: Moscow, Russia, 1951; Volume 2.

70. Chukhareva, N.V. Pyrolysis of humic acids in air-dry and heat-treated high-moor cottongrass-sphagnum peat. In Proceedings of the VI All-Russian Scientific Conference with International Participation "Humic Substances in the Biosphere", Syktyvkar, Russia, 6–10 October 2014; pp. 83–85.

71. Schulze, E.D.; Wirth, C.; Heimann, M. Managing forests after Kyoto. *Science* **2000**, *289*, 2058–2059. [CrossRef] [PubMed]

72. Efremova, T.T.; Efremov, S.P. Pyrogenic transformation of organic matter in soils of forest bogs. *Eurasian Soil Sci.* **2006**, *39*, 1297–1305. [CrossRef]

73. Kholodov, V.A.; Konstantinov, A.I.; Kudryavtsev, A.V.; Perminova, I.V. Humic acids structure in zonal soils according to ^{13}C NMR spectroscopy data. *Eurasian Soil Sci.* **2011**, *9*, 1064–1073.

74. Chukov, S.N. *Structural and Functional Parameters of Soil Organic Matter under Anthropogenic Impact*; Publishing House of St. Petersburg State University: St. Petersburg, Russia, 2001; 216p.

75. Emsley, J.; Feeney, J.; Sutcliffe, L. *High Resolution Nuclear Magnetic Resonance Spectroscopy*; Elsevier: Amsterdam, The Netherlands, 1968; Volume 2, 468p.

76. Lodygin, E.D.; Beznosikov, V.A.; Chukov, S.N. *Structural and Functional Parameters of Humic Substances in Podzolic and Bog-podzolic Soils*; Nauka: St. Petersburg, Russia, 2007; p. 145.

77. Certini, G.; Nocentini, C.; Knicker, H. Wildfire effects on soil organic matter quantity and quality in two fire-prone Mediterranean pine forests. *Geoderma* **2011**, *167*, 148–155. [CrossRef]

78. Abakumov, E.V.; Fujitake, N.; Kosaki, T. Humus and humic acids of Luvisol and Cambisol of Jiguli ridges, Samara Region, Russia. *Appl. Environ. Soil Sci.* **2009**, *2009*, 671359. [CrossRef]

79. Simpson, A.J.; Simpson, M.J. Nuclear magnetic resonance analysis of natural organic matter. In *Biophysico-Chemical Processes Involving Natural Nonliving Organic Matter in Environmental Systems*; Senesi, N., Xing, B., Huang, P.M., Eds.; John Wiley & Sons Inc.: Chichester, UK, 2009; pp. 589–650.

80. Kosheleva, Y.P.; Trofimov, S.Y. Characteristics of the biochemical composition of plant litter at different stages of decomposition (according to thermal analysis data). *Biol. Bull.* **2008**, *1*, 64–69. [CrossRef]

81. Chebykina, E.; Abakumov, E. Characteristics of humic acids isolated from burned and unburned topsoils in sub-boreal Scotch pine forests by ^{13}C-NMR spectroscopy. *One Ecosyst.* **2022**, *7*, e82720. [CrossRef]

82. Beyer, L.; Sorge, C.; Blume, H.P.; Schulten, H.R. Soil organic matter composition and transformation in gelic histosols of coastal continental antarctica. *Soil Biol. Biochem.* **1995**, *27*, 1279–1288. [CrossRef]

83. Graham, D.J.; Midgley, N.G. Graphical representation of particle shape using triangular diagrams: An Excel spreadsheet method. *Earth Surf. Process. Landf.* **2000**, *25*, 1473–1477. [CrossRef]