Research Article

Characteristics of humic acids isolated from burned and unburned topsoils in sub-boreal Scotch pine forests by ¹³C-NMR spectroscopy

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Abstract

Postpyrogenic soil dynamics is an informative tool for studying soil elementary processes in extreme temperature conditions and for predicting short time environmental changes in conditions of catastrophic landscape changes. Soil organic matter (SOM) system evolution is the most rapid process of postpyrogenic soil development. In this relation, the focus on humus structure is important for understanding these important dynamics. Soil restoration after spontaneous forest fires near Togljatty City (Samara Region, Russia) was abandoned in 2010 and further monitoring over the next ten years was organised to evaluate the speed of humus accumulation dynamics. The aim of this study was to apply the ¹³C-NMR (nuclear magnetic resonance) spectroscopy to analyse the effect of forest fires on SOM degradation in Scotch pine forests on Psamment Entisols of the fragmented steppe in the Samara Region. Three key soil plots were studied for estimating SOM quality changes under the forest fire effect: surface forest fire, crown forest fire and control, i.e. 18 soil samples of top soil horizons were analysed in three replicates. The data obtained indicate that the humus molecular composition was substantially affected by the wildfires. Investigation of the humic acids' (HAs) molecular structure by ¹³C-NMR showed a relative increase in aromatic compounds and decrease in aliphatic ones. The aromaticity degree of HA molecules increases in 5.7 and 3.8 times in cases of surface and crown forest fires, correspondingly. In general, crown and surface fires plots are not very different in terms of ¹³C-NMR spectra of HAs (p = 0.34); however, HAs of control plot have essential differences from pyrogenic ones (p < 0.05). ¹³C NMR spectra have shown a change in the proportion of oxygen-containing functional groups as well. One of the most important effects observed in wildfire-affected SOM is that the proportion of lignin-like structures increases as a concomitant effect of depletion of *C*,*H*-alkyl groups, especially in the case of crown fires.

Keywords

¹³C-NMR spectroscopy, crown fire, soil cover, soil organic matter (SOM), surface fire, wildfires

Introduction

Forest fire frequency is becoming increasingly relevant in the context of global climate change, with an observed trend of increasing natural forest fire frequency. The number of postpyrogenic territories is increasing annually both in Russia and in the world; and not only peat soils, but also mineral ones are burning. Forest fires play an important role in the study of various aspects of soil degradation due to their specific impact on the environment. Fires are a powerful active ecological factor of modern soil formation. Any fire impact affects both the chemical and biological soil characteristics; moreover, the impact degree is closely related to the intensity of fire events. The pyrogenesis processes are a widespread phenomenon that has a huge impact on soil formation processes, which makes us pay special attention to them when studying natural ecosystems.

The effect of different fire intensity on SOM is a complex and comprehensive issue. However, the effect of different types of fires on SOM is not fully understood. Summarising previous scientific studies (Gonzalez-Perez et al. 2004, Maksimova and Abakumov 2015, Dymov and Gabov 2015, Miesel et al. 2015, Jimenez-Gonzalez et al. 2016, Masyagina et al. 2016, Abakumov et al. 2017 etc.), it can be argued that, on the one hand, the humus soil state undergoes certain changes as a result of fires and, on the other hand, there is obviously no consensus on the processes that occur when fires affect the system of SOM. Both simulation experiments under laboratory conditions and studies of fires in natural environments that are cited in the classic generic literature on the effects of fire (Kang and Sajjapongse 1980, Almendros et al. 1990, Fernandez et al. 2001, Sirotiak et al. 2021 etc.), suggest that the effect of temperature is highly variable depending on the heating time, the moisture of the soil and especially the soil depth. Therefore, modern science requires the data accumulation on postpyrogenic changes in the system of SOM in various combinations of times and fire types.

For a deeper study of the fundamental processes of humus alteration after wildfires, it is necessary to use modern high-precision instrumental methods (Beznosikov and Lodygin 2010, Lodygin et al. 2014). Various methods have been used to investigate the HAs of

SOM in different ecosystems. One of the methods for studying the molecular composition of organic matter is nuclear magnetic resonance (NMR), which enables the study of the qualitative and quantitative characteristics of organic matter (Lodygin and Beznosikov 2010, Ejarque and Abakumov 2016, Chukov et al. 2017, Lodygin et al. 2017). Using ¹³C–NMR spectroscopy, we identify the proportion of aromatic compounds (Celi et al. 1997) in the composition of HAs, to assess the stabilisation of organic matter in postpyrogenic soils.

The aim of this study was to discuss the factors that influence fire damage levels. In order to achieve this goal, the following tasks were set: a) to demonstrate the application of the ¹³C-NMR spectroscopy for comparison of the structural composition of the SOM from both burned and unburned topsoils; b) to evaluate the stabilisation rate of postpyrogenic soils and to analyse a soil degradation in dry Scotch pine forests resulting from forest fires; c) moreover, one of the study objectives was to study in detail the changes in the properties of organic matter and to detect the depth at which these changes manifest.

The working hypothesis of the study is that wildfires in sub-boreal environments significantly change molecular composition of the SOM and the depth of its changes is 4–5 cm.

Material and methods

¹³C-NMR spectra of HAs isolated from the upper soil horizons affected by forest fires (in 2010) in Togljatty City, Samara Region, were analysed to study postpyrogenic changes of SOM. Authors carried out monitoring studies of postpyrogenic soils in the forest-steppe zone in the period from 2010 to 2020 using the example of Scotch pine forest in Togljatty City (Maksimova et al. 2014, Maksimova et al. 2019).

Scotch pine forests (*Pinus sylvestris* L.) around Togljatty City are formed on sandy and sandy loam deposits of eolian or alluvial origin in a subboreal climate. This is the territory of the Stavropol pine forest (a former park zone between the Komsomolsky, Central and Avtozavodsky Districts of the City, near the Institute of Ecology of Volga Basin of the Russian Academy of Sciences (53°29'43.80" N, 49°20'56.44" E, 179 m a.s.l.). Investigated soil type at each study plot was Psamment Entisols. Soil diagnostics were carried out according to the "Classification and diagnostics of soils of Russia" (Shishov et al. 2004) and the World Reference Base for Soil Resources, FAO 2015 (IUSS Working Group WRB 2015).

Postfire dynamics studies of soil cover were carried out in areas affected by surface and crown forest fires in 2010: plot No. 1 - a middle-aged pine forest in Togljatty City (surface fire was at the end of July 2010 – there was a burnout of the lower plant stratum with partial damage to the stand); plot No. 2 - middle-aged pine forest in Togljatty City (crown fire was at the end of July 2010 – there was a complete burnout of all vegetation); plot No. 3 – similar forest plots with the same soil type, but not subjected to forest fire (about 1 km away from the pyrogenic effect) were used as control – the soils of natural scotch pine forests. These three sampling scenarios were similar in terms of geology, topography, soil

and vegetation before fire. Three soil pits were put in each study plot. In case of current study, two top soil horizons were investigated: 18 soil samples of topsoils (Apir (0–10 cm) and AY (10–15 cm)) were analysed in three replicates. The descriptions of the vegetation and soil profiles, as well as the sampling procedures, were performed in accordance with the common methodological recommendations. Soil samples were air-dried and sieved through a 1-mm sieve. The data presented in this paper are the analysis results of HAs' spectra isolated from upper horizons of soil samples taken in 2019.

Humic substances isolation was carried out in accordance with the standard methods of the International Humic Substances Society IHSS (http://www.humicsubstances.org/). HAs were extracted from soil samples according to R.S. Swift (Swift 1996) modified by S.N. Chukov et al. (Chukov et al. 2017). Briefly, humic acids were extracted from soil sample (weight is 100 g) with 0.1 M sodium hydroxide (NaOH) solution (soil/solution ratio 1:10) under nitrogen gas. After that, the solution was acidified to pH 1 and the HAs were separated by centrifugation at high speed (> 15000 rpm) for 20 min. Then HAs were demineralised by shaking overnight in strong hydrogen fluoride (HF) solution (45%) and washed with deionised water; after that they were freeze-dried.

¹³C-NMR spectra of HAs were obtained using a Bruker Avance 500 NMR spectrometer, Billerica, Massachusetts, United States in a 3.2 mm zirconium oxide (ZrO₂) rotor. The magic angle rotation speed was 12 kHz. The repetition delay was 3 sec. The data of NMR spectroscopy has been obtained from the "Center of Chemical Analyses and Materials and Center of Magnetic Resonance Research", Scientific Park of Saint-Petersburg State University.

¹³C-NMR spectra analysis of HA powders from studied soils made it possible to identify the ranges of chemical shifts belonging to carbon atoms of various functional groups and molecular fragments of HAs (Emsley et al. 1965, Lodygin and Beznosikov 2010, Kholodov et al. 2011, Lodygin et al. 2014, Chukov et al. 2017). Various molecular fragments were identified including:

0-47 ppm - C, H-substituted aliphatic fragments;

47-60 ppm - methoxy and O, N-substituted aliphatic fragments;

60–105 ppm – aliphatic fragments, doubly substituted by heteroatoms (including carbohydrate) and methine carbon of ethers;

105–144 ppm – C, H-substituted aromatic fragments;

144–164 ppm – O, N-substituted aromatic fragments;

164–183 ppm – carboxyl group, esters, amides and their derivatives;

183–204 ppm – quinone, aldehydes and ketones groups.

The aromaticity degree was calculated as the sum of signals in the 105–164 and 164–183 ppm regions, while aliphatic compounds are characterised by the 0–105 and 183–204 ppm regions (Kovalev and Kovaleva 2013, Lodygin 2016).

The following parameters were used in order to standardise the quantitative characteristics of HAs' macromolecules: the ratio of the aromatic structures carbon to the aliphatic chains carbon – Ar/AL (Lorenz et al. 2006) and a decomposition degree of SOM (*C*,*H*-alkyl/*O*,*N*-alkyl) (Baldock and Preston 1995, Pedersen et al. 2011). Moreover, E.D. Lodygin (Lodygin 2016) has proposed an integral index of HAs' hydrophobicity ($AL_{H,R}+AR_{H,R}$), which is the total proportion of unoxidised carbon atoms (substituted by hydrogen or other atoms of aliphatic fragments), which makes it possible to indirectly evaluate the amphiphilic properties of SOM (Lodygin et al. 2014, Beznosikov et al. 2015).

Statistics. Data normal distribution was verified and a variance analysis (ANOVA) and post hoc test (Fisher's least significant difference) were performed. Differences were considered significant at p < 0.05. Hierarchical clustering. Ward's method. This analysis uses a method of variance to estimate the distances between clusters. Statistical data processing and analysis were carried out using standard methods in software packages MS Excel 2016, Past (version 3.20), Statistica 64 (version 10).

Results

¹³C-NMR spectra obtained from the isolated HA powders are shown at Figs 1, 2. The results are consistent with previously obtained information in this area (Almendros et al. 1992, Certini et al. 2011, Jimenez-Gonzalez et al. 2016) that the aliphatic part is the main proportion in HAs' composition of natural soils, which is a typical zonal feature of SOM in the forest-steppe zone, while as a result of fires, the aromaticity degree of HA molecules increases significantly in 5.7 and 3.8 times in cases of surface and crown forest fires correspondingly (Abakumov et al. 2009).

In general, crown and surface forest fires plots do not differ in the ¹³C-NMR spectra of HAs from the upper burnt Apir horizons; however, HAs of the control plot differ significantly from pyrogenic ones (Table 1, Figs 1, 2). 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 tetra-substituted 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 HAs' 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 (Maksimova and Abakumov 2017).

The lowest concentration amongst all structural groups was characteristic for the carbonyl group, which corresponds to the results by Rovira et al. (Rovira et al. 2012). The most

pronounced changes in the structural composition of HAs are associated with an increased proportion of fragments of C-C bonds, alkylaromatic compounds and C,H-substituted aromatic carbon. A similar trend was established by Certini et al. (Certini et al. 2011) and Knicker (Knicker 2007). Moreover, there is an increase of carboxyl groups after fires and a slight decrease of aldehyde and ketone groups. A higher content of C3 and C5 syringyl compounds and C3 and C4 guaiacyl compounds at the chemical shift of 153-148 ppm was characteristic for surface fire more than in the case of crown forest fire and was significantly higher than the control. The remaining aromatic carbon signals, including lignin, were found between 140 and 110 ppm. This area shows the fragments content of C-C bonds associated with aromatic compounds. A lignin transformation was revealed in the case of forest fires, especially crown fires, that can be recognised in the O.Nsubstituted aromatic region, although the signals of the methoxyl groups overlap with those of the proteins. Changes in the ratio between C,H-substituted-to-O,N-substituted aromatic C types are an important effect of fire. Moreover, quite visible in the spectra presented, there is a decrease in the content of the following functional groups due to fire effect: 46-64% less of carboxyl, methoxyl/amide carbons (56 ppm), 25-35% and 49-64% less of carbons in glucopyranoside rings in carbohydrate-derived structures (63 ppm (C6), 73 ppm (C2,3,5), correspondingly). These are characteristic for all studied postpyrogenic samples, except for the sub-superficial layer in the case of crown forest fire.

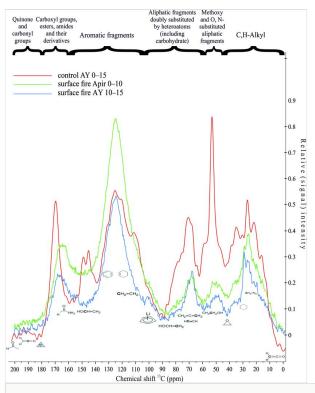


Figure 1.

¹³C-NMR spectra of the isolated HA powders in case of: surface fire.

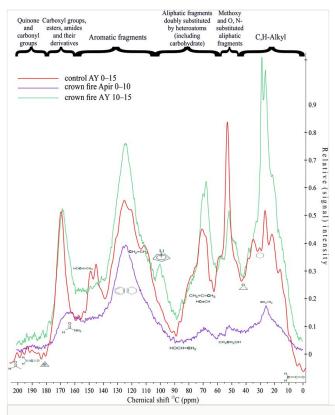


Figure 2.

¹³C-NMR spectra of the isolated HA powders in case of: crown fire.

Table 1.

The aromatic and aliphatic proportion in HAs' molecules of studied soils.

Sample	Aromatic %	Aliphatic %	Ar/ AL	$AL_{H,R}$ + $AR_{H,R}$	<i>C,H-</i> alkyl/ <i>O,N-</i> alkyl)
Control AY	38	62	0.60	65	0.84
Surface fire Apir	81	19	4.25	84	6,53
Surface fire AY	69	31	2.20	79	2.90
Crown fire Apir	74	26	2.83	83	2.70
Crown fire AY	43	57	0.75	74	3.65
Post hoc test Control – Surface fire	p < 0.05				
Post hoc test Control – Crown fire	p < 0.05				
Post hoc test Surface fire – Crown fire	0.34				
Results of One way ANOVA, differences between plots	F = 0.03401	p = 0.476			

Therefore, the accumulation of aromatic compounds in humic substances is obvious after wildfires, due to the relative decrease in the aliphatic part or, in some cases, due to a small accumulation of carboxyl and carbonyl groups. The 126–129 ppm signal has often been assigned to polyaromatic hydrocarbons (PAHs) (Knicker 2007), while the tendency in subsequent studies is to consider that, under standard acquisition conditions, ¹³C-NMR does not distinguish between isolated aromatic rings or polycyclic compounds (Almendros et al. 2018). However, the signal intensity at 126–129 ppm has also been reported to parallel the quantitative increase in PAHs content, which occurs as a characteristic effect of wildfires (Maksimova et al. 2014).

It should also be noted that the radical re-arrangement of HAs' composition as a result of forest fires concerns precisely the upper Apir layer, while the underlying layers, as a whole, are more or less similar in spectra to the control. This mostly concerns the case of a crown forest fire, where the two upper horizons differ significantly in HAs' composition in terms of the aromaticity degree (Table 1). Therefore, below 10 cm, it is difficult to detect changes in the properties of organic matter, no matter what type of fire and, therefore, how high the temperature was.

The following parameters were used in order to standardise the quantitative characteristics of HAs' molecules: the ratio of aromatic to aliphatic carbon (degree of decomposition of organic matter) *C*-alkyl/*O*-alkyl and the integral index of HAs hydrophobicity ($AL_{H,R}+AR_{H,R}$) (Fig. 3Table 1). The analysis of molecular composition integral indicators of SOM showed that HAs of postfire plots are quite resistant to oxidation (including microbial) compared to HAs of the control plot (Table 1). The total proportion of unoxidised carbon atoms in the control case is about 65%, while for the postpyrogenic case, this indicator is noticeably higher - 74–84%. The most hydrophobic are HAs of topsoils in postfire plots. Moreover, HAs are characterised by a decrease in the degree of hydrophobicity in the case of transition from organogenic topsoil to mineral horizons.

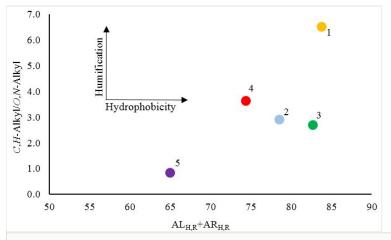
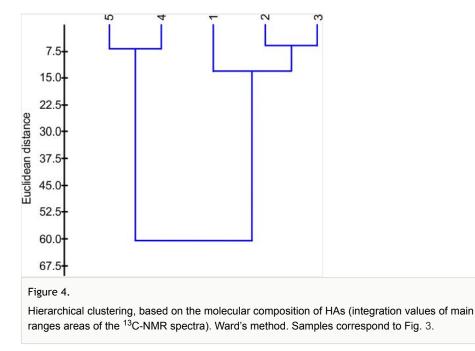


Figure 3.

Integrated indicators of HAs' molecular composition: 1 - surface fire Apir; 2 – surface fire AY; 3 – crown fire Apir; 4 – crown fire AY; 5 – control.

The *C*,*H*-alkyl/*O*,*N*-alkyl ratio, which characterises the decomposition degree of SOM, is maximum for postpyrogenic topsoils, especially for surface fire and ranges from 3.65 to 6.53, while ratio values in the case of the control plot are significantly lower at about 0.84. This indicator sharply decreases when moving from organogenic to mineral horizons, which indicates a greater degree of destabilisation of the SOM in topsoil horizons.

Moreover, we provided a cluster analysis (Ward's method) (Fig. 4) in order to identify relationships between the studied samples. The following groups can be distinguished according to this figure: from samples 1, 2 and 3; samples 4 and 5. The first group characterises HAs formed in the upper and lower soil horizons in the case of surface and crown forest fires. The second group is represented by control soils and the most similar to them – lower soil horizon in the case of crown wildfire.



For this reason, the ¹³C-NMR spectrum analysis data show significant changes in the structural organisation of organic matter as a result of pyrogenic transformation.

Conclusions

The analysis of postpyrogenic SOM showed that the humus state undergoes certain changes during forest fires. The SOM of wildfires and control plots forms rather heterogeneous groups. The main difference in the heterogeneity degree of HA groups is an increase of aromaticity degree (in 5.7 and 3.8 times in the cases of surface and crown forest fires, correspondingly) and a decrease of the aliphatic part proportion in HAs' molecules. Moreover, there is a decrease of methoxy and O,N-substituted aliphatic

fragments after fires and a slight decrease of aldehyde and ketone groups. A significant decrease in the content of carboxyl, methoxyl/amide carbons (46–64% less) and carbons in glucopyranoside rings in carbohydrate-derived structures (25–64% less) was shown as a result of fire effect. A higher content of syringyl compounds and guaiacyl compounds was characteristic for the postfire area. A lignin transformation was revealed in the case of forest fires. Changes in the ratio between C,H-substituted-to-O,N-substituted aromatic C types are an important effect of fire. These regularities are characteristic for all studied postpyrogenic samples, except for the sub-superficial layer in the case of crown forest fire. Below 10 cm, it is generally difficult to detect changes in the properties of organic matter according to ¹³C-NMR spectra, no matter what type of fire and, therefore, how high the temperature was.

Therefore, investigations on SOM after wildfires can further become a part of postfire monitoring system as an element of ecological management of degraded lands.

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Author contributions

E.Ch. conceptualisation and expedition; E.A. expedition with fieldwork and soil sampling. E.Ch. and E.A. wrote the paper. All authors have read and agreed to the published version of the manuscript. The authors declare that they have no conflict of interest.

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