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THE INFLUENCE OF FIRES ON THE BIOLOGICAL ACTIVITY OF FOREST SOILS IN VRANCEA, ROMANIA

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Abstract

Soils are influenced by stand composition and hydrological regime as well as by stress abiotic factors, including forest fires. As such, this study analyzed three stands affected by fires of different severities. Soil samples were gathered during the spring of 2017 from three sample areas in Vrancea County, eastern part of Romania. For each area studied, soil profiles were studied in 3 areas affected by fires and in control areas located close to each other. The soil respiration was determined by monitoring CO₂ fluxes with an EGM Environmental Gas Monitor, PP systems. The microbial measures (mesophilic heterotrophic bacteria, fungi and nitrogen fixing bacteria from genus *Azotobacter*) were achieved through the plate count method. The results were statistically interpreted with the ANOVA classical- univariate analysis. The fires affected the humus and total nitrogen quantities at a certain time interval from their apparition, but only for the first 10 centimetres of the soil depth and in the case of low intensity fires. The correlation between high temperatures and CO₂ fluxes was also established. The fires also affected the abundance of fungi. After six years from their apparition, the “unseen” effects of fires on forest soils characteristics are still detected, such as the decrease of soil respiration and bacteria amount, a pH increase and a decrease of humus and total nitrogen.

Key words: dehydrogenases activity; soil respiration; soil bacteria and fungi

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1. Introduction

Over 2/3 of the terrestrial biodiversity is represented by forest ecosystems. This biodiversity is very important for the maintenance of ecosystem services. Baldrian et al. (2015), observed that the species that enter in the composition of stands are the ones that are mostly affecting the biodiversity of soils; the most affected ones being the fungus species, in comparison with other bacteria. Analysing the results of the FunDivEUROPE project (in which 5 European stands were studied, including one from Suceava), Carnol et al. (2015), observed that the soil microbial biomass increases together with the increase of forest

species diversity. The soil type was podzol and dystic cambisol. The Shannon index values calculated by the authors are of 1.06 at the altitude of 600 m, 1.07 at 900 m, with the lowest value of 0.87 at 1200 m (Klimek et al., 2015). Generally speaking, the indexes of the activity of microorganisms from soils are well correlated with the content of organic carbon and nitrogen, partially with the water content and not at all with the soil acidity (Gömöryová et al., 2011). The problem of factors that influence the composition of microbial communities from soils was long debated and was implied that, while at a local level, the microbial soil communities can be explained because of the existent plants (Bezemer et al., 2010; Orwin et

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al., 2010; Thomson et al., 2010; Wei et al., 2018), at a larger, regional and continental level, other factors are more important, namely climate, topography, soil pH and abiotic factors (Ettema and Wardle, 2002; Fierer and Jackson, 2006; Fierer et al., 2009; Lauber et al., 2008).

The answer of microbial processes towards environment stress conditions does not relate only to soil humidity and the availability of nutrients and organic matter, but incorporates also the changes in the structure of microbial communities (Pesaro et al., 2004; Ulea et al., 2017). Soils are very sensitive to the changes that appear in the composition of stands, in hydrologic regime or in the development of basic biological processes (Spâchez et al., 2013; Târziu et al., 2004) and especially in the human impact – including forest fires (Gorbunova et al., 2014).

Forest fires are one of the main factors that reduce the taxonomy and functional diversity of soil microbial communities (Bárcenas-Moreno et al., 2011; Smith et al., 2008; Xiang et al., 2014). From this perspective, Fernández-Gonzalez et al. (2015), have analysed microbial communities associated with oak and hornbeam stands at three years from a forest fire. They have established the most affected bacteria populations, as well the most resilient ones to fires by realizing genetical studies on the resilient bacteria and by determining the enzymes that can play a crucial role in this process.

Toxic products from the soil, such as polyaromatic hydrocarbons are redistributed, which leads to the mortality of soil organisms. Grasso et al., 1996, have found an increase of some microbial populations with 2.7 times after 10 days, in comparison with the control situations, and have explained this fact by a change in the participation ratio of the soil heterotroph respiration in the carbon circuit. The immediate effect of fires on soil organisms consists in a reduction of their biomass (Certini, 2005). Actually, the maximum temperatures are often so high that they can lead to the killing of most living beings (DeBano et al., 1998). Generally speaking, the direct fire effects on invertebrate that live in the soil are less important than the ones on microorganisms, due to their higher mobility which allows invertebrates to escape from heating by penetrating deep in the soil. However, the fire's indirect effects, by reducing the litter, can lead to a dramatic decrease of the number of invertebrate species that live in the soil (Certini, 2005). Another study (Klimek et al., 2015), realized in Poland, (Carpathian Mountains), focused on the diversity of bacterial communities in an altitudinal transect and the altitudes selected were: 600 m, 900 m and 1200 m. Mixture stands of common beech, hornbeam, oak and birch can be found at the altitude of 600 m, followed by mixture stands of common beech and fir at 900 m and Norway spruce stands with few mountain ash species at 1200 m. The recursion to normality for the number of species is not predictable as it is linked to soil humidity after the fire (Neumann and Tolhurst, 1991). A subject that was not considered before is represented by soil behavior after a fire in

areas subjected to drought. As such, this interplay between fire and drought and its effects are not yet known. This applies especially to soil nutrients or microbial action (Beier et al., 2012; Luo et al., 2008; Wu et al., 2011). Fire occurrences are highly influenced by raised temperatures or decreased precipitations, leading to considerable and dangerous fires (Bedia et al., 2014; Bradstock et al., 2010; Westerling et al., 2006; Xistrakis et al., 2014). In addition, this means that post-fire recovery of these ecosystems could also be affected by drought.

The aim of the study was to determine the influence of fires from the last 5–6 years on the chemical and microbiological activity of the forest soils. Three plots were selected in the eastern part of Romania, (Vrancea County). Chemical parameters (pH, humidity index, humus, total nitrogen and carbonates) together with microbial analyses (soil respiration, dehydrogenase activity, total number of mesophilic bacteria, total number of fungi, and total number of nitrogen fixing bacteria from genus *Azotobacter*) were determined to study the fire influence on biological activity and microbial communities of the selected soil. The hypothesis from which the study starts is to identify the response of certain soil parameters to the stress caused by fires of different severities.

2. Material and methods

2.1. Research field

The investigations were realized in Vidra and Focșani Forest Districts from Vrancea County. The forests from these districts are mainly composed of Pine stands (*Pinussylvestris L.* and *Pinusnigra J.F. Arnold*) (Silvestru-Grigore et al., 2018), sea buckthorn (*Hippophaesalicifolia D. Don*) (Constandache et al., 2016a) and other species and are situated on degraded lands (Constandache et al., 2016b).

Three stands affected by fires (from the last 5–6 years) of different severities were analysed as follows:

Plot 1. Vidra Forest District, Țifești plot, U.P. I Bolotești, u.a. 166 – strong litter fire on a surface of 6000–7000 m² – from 2011. The stand composition is 100% black pine (*Pinusnigra*).

Plot 2. Vidra Forest District, ValeaSării plot, U.P. III ValeaSării, u.a. 105 A – weak-medium litter fire on a surface of 3000–4000 m² – from 2012. The stand composition is 90% black pine and 10% other species.

Plot 3. Focșani Forest District, Suraia plot, U.P. IX Bilești, u.a. 83B – medium litter and plantation canopy fire on a surface of approximately 5 ha – from 2011. The stand composition is 70% ash (*Fraxinus excelsior L.*) and 30% pedunculate oak (*Quercusrobur L.*).

The parcel data from the areas in which sample surfaces were situated for determining the influence of fire on the characteristics of forest soils are rendered in Table 1. The soil types from the three surfaces are:

phaeozem for Suraia and ValeaSării and cambic chernozem for Țifești, soils characteristic for the plain area (Spârchez et al., 2011; Crișan et al., 2017). Phaeozem occupies 3% of the total forest soil surface from Romania, while chernozem occupies 1% (Dincă et al., 2014). Both soils are characterized by a high content of humus (Dincă et al., 2015).

2.2. Soil sampling and analysing

During field works from the spring of 2017 (at the beginning of March), soil samples were gathered from the three plots described in previous section (plot 1, plot 2 and plot 3) represented by different fire intensities. In each plot, three soil profiles (with two standard horizons: 0–10 and 10–20 cm) were made in the areas affected by fires (In) and three profiles were made in the witness areas (unaffected by fires) – M (witness). Finally, we obtain 36 soil samples having for each plot making 6 profiles (3 In and 3 M) on 2 horizons (Eq. 1):

$$\begin{aligned} \text{Total soil samples} &= 3 \text{ plots} * \\ & * \{ [3 \text{ profiles(In)} + 3 \text{ profiles(M)}] * \\ & * 2 \text{ horizons} \end{aligned} \quad (1)$$

Furthermore, a main soil profile (PP) was realized for plot 1, plot 2 and plot 3, from which samples on pedo-genetic horizons were then gathered in order to determine the main type of soil.

In the laboratory the samples were left maximum 72 hours for drying. When used for microbial counts, the samples were sifted with 2-mm mesh sieves and pieces of root, leaves, small stems and insects were eliminated. The soil samples were then moistened and incubated three up to five days at 37°C, followed by microbiological analysis. This was a standard procedure whenever a new group of organisms was studied. The chemical property analysis of soil samples (pH, humus, total nitrogen and carbonates) was realized based on renowned national and international methodologies (Dincă et al., 2012).

2.3. Environmental parameters and respiration measurements of soils

Monitoring CO₂ fluxes from the forest areas and from the ones affected by fires was realized with the help of a portable device that directly analyses the

carbon dioxide (CO₂) concentrations in a small immovable soil chamber respiration (SRC-1). This soil chamber respiration has a volume of 1171 cm³ and a coverage surface of 78 cm², for which a rubber sleeve was attached in order to connect the PVC rings (colars) permanently installed in the soil. The device’s two components are interconnected, while the measuring procedure is controlled by the operating system from the main module. This device (EGM – Environmental Gas Monitor; PP-systems, USA) is based on the existence of an infrared sensor that measures the CO₂ concentration in the camera (IRGA). Furthermore, the method used for determining concentrations and, implicitly, the soil respiration (equivalent to the CO₂ flux, CO₂ assimilation) employs the technique of dynamic closed cameras (Pumpanen et al., 2004).

The soil respiration (SR) measurements were taken periodically, but atmospheric conditions were also taken into account. As such, during the last 24 hours before the measurements, precipitations must not be recorded in order to discourage a short intensification of the rhizosphere activity. For each sample surface (‘p’ meaning perturbed/affected and ‘np’ meaning non-perturbed), 20 samples are taken, situated at 2 meters one from the other and by taking into account the field configuration and the position of the affected/unaffected tree. With a week before the start of measurements, the position of the future concentration measurements was materialized in the field by situating PVC rings of a standard dimension (110 mm in diameter) for each sample surface. This was realized in order to avoid soil perturbation and for keeping an initial measurement position, as well as for avoiding possible contamination or gas releases in the surrounding environment. Each measurement campaign was realized on the duration of two days. The device measures the soil CO₂ concentrations for each repetition for 2 minutes.

In addition, the device has attached a temperature sensor (STP-1) with continuous recording, that was used for determining soil temperature at a depth of 3 cm, this being the most affected level in regard with fine roots and microorganisms. More than this, each repetition is accompanied by soil and atmosphere temperature and humidity determinations. Soil water content (SWC) used time domain technique (TDR 300, Sensotch, USA) for establishing the soil volumetric humidity at the depth of 20 cm.

Table 1. Characteristics of the areas affected by fire

Name	OS	UP	ua	Area (ha)	Forest site type	Fores t type	Relief	Altitude (m)	Slope	Comp.	Age (years)	Prod . class	Consistency
Plot 1 Țifești	Vidra	I	166	3.3	9110	8117	Clough	170	S	10Pin	30	4	0.8
Plot 2 ValeaSării	Vidra	III	105 A	18.6	5132	5131	Slope	590	S	9Pin1D T	45	3	0.8
Plot 3 Suraia	Focșani	IX	83B	7.1	9612	9312	Low meadow	200	-	7Fr3St	6	3	0.4

The measurements were realized in three periods, one during autumn (2016) and the other two during spring (2017).

2.4. Analyses of soil microorganisms

Total number of fungi, total number of mesophilic heterotrophic bacteria and total number of nitrogen-fixing bacteria from genus *Azotobacter* were analysed in the selected soils. The soil samples were submitted to decimal dilutions. The evaluation of the total number of mesophilic heterotroph bacteria, the total number of fungi and the total number of nitrogen fixing bacteria from genus *Azotobacter* was realized through the plate count method. The Plate Count Agar environment was used for counting the mesophilic aerobic bacteria (pH 7.5, incubation at 3 days at 37°C) (Atlas, 2004). The total number of fungi was determined on the Agar Sabouraud culture environment with an insertion of 0.5% chloramphenicol (pH 5.4, incubation at 4–5 days at 25°C) (Dehghan, 2014). The nitrogen fixing non-symbiotic bacteria from genus *Azotobacter* were cultivated in an Ashbys Mannitol Agar culture environment (pH 7.4, incubation at 5 days at 35–37 °C) (Jiménez et al., 2011). The Petri plates with the inoculate cultures were incubated in the Memmert UNB 100 incubator. After the incubation period, the colonies of microorganism were counted with the help of a POL EKO LKB2002 colony counter. The obtained values were multiplied with the dilution factor (10^9 for the mesophilic heterotroph bacteria, 10^4 for fungi and 10^6 for the *Azotobacter* bacteria). As such, the number of units that form colonies (CFU) was obtained at one soil gram (Bölter, 2002).

2.5. Dehydrogenase activity of soil microorganisms

The dehydrogenase activity reflects the intensity of the microbial metabolism in the soil, as well as the microbial activity.

Dehydrogenases are involved in the oxidation of the soil's organic substrate through the transfer of hydrogen from here to a series of inorganic NAD (nicotinamide adenine dinucleotide) or NADP (nicotinamide adenine dinucleotide phosphate) acceptors (Subhani et al., 2001). Being part of the enzymatic system of the microorganism involved in cellular metabolism, they can be considered indexes of the microbial oxidative system (Brezińska et al., 1998).

The dehydrogenase activity is caused by organic substances present in the soil. As such, 6 grams of soil were taken from each soil variant (6 variants of 3 repetitions), from each repetition. These samples were mixed with 1 ml TTC 3% solution (chloride of 2,3,5-trifenylnitrazol) and 2 ml of distilled water. The samples were incubated 48 h at 32°C. During the incubation, TTC, which is a colourless compound, is transformed in a red compound (trifenilformazan) under the action of the hydrogen

transferred by the dehydrogenase. The formazan is extracted with methanol so that, after the incubation period, 10 ml of methanol was added in all samples. After this, the samples were stirred with the magnetic stirrer and centrifuged 10 minutes at 4000 rpm. The supernatant was spectrophotometrically determined at the wave length of 485 nm. As such, the highest the formazan concentration is, the highest the dehydrogenase activity is, which is expressed in mg formazan/g soil (Alef and Nannipieri, 1995; Thalmann, 1968).

2.6. Statistical analyses

The statistical significance of the differences between the average values of the parameters between the investigated experimental areas was studied by using the ANOVA classical-univariate analysis (one-way ANOVA, $N = 3$, $P \leq 0.05$). This analysis offers results for a single variable (quantitative parameter) in order to multiply compare the samples (post-hoc Tukey test).

The research results were generated with the GraphPad Prism software, version 5.00 (GraphPad Software, San Diego). The variation analysis describes the influence of the stress abiotic factor in the variation of the biological properties of the studied soils. In order to study the relations between the soil's dehydrogenase activity and the abundance of the studied eco-physiological microorganism's groups, correlations between the studied parameters were realized by calculating the Pearson coefficient (GraphPad Prism software, version 5.5).

3. Results and discussion

3.1. The chemical properties of forest soils from areas affected by fires

It can be observed (Table 2) that the soil's pH is higher in the areas affected by fire, while the humus quantity is smaller in the fire areas and the total nitrogen quantity is generally smaller in the fire area, with the exception of the weak severity fire; in this case the total nitrogen quantity from the soil is higher in the affected area due to its small severity, as well as due to the apparition of abundant herbaceous vegetation in the area in which recent plantations were realized.

These trends are constant, regardless of the fire's severity. Differences between the type of fires are not observable, with the exception of the soil's nitrogen quantity from the weak severity fire, due to its reduced severity as well as it relatively long-time period registered from its apparitions. If the data obtained on the two depth categories are analyzed, it can be observed that the same variations of the soil's chemical properties are recorded on the first 10 centimeters of the soil (an increase of pH and the decrease of humus and total nitrogen quantities—for the areas affected by fires).

Table 2. Analytical soil data from Valea Sării, Suraia and Țifești areas

Nr. crt.	Suprafață	Surface type	pH	Humus (%)	Total Nitrogen (%)	Carbonates (g/kg)
1	Valea	Weak fire	7.987	5.357	0.300	16.122
2	Sării	Witness	7.870	5.508	0.282	10.034
3	Suraia	Medium fire	8.032	2.905	0.149	6.975
4		Witness	7.941	3.885	0.199	7.099
5	Țifești	Strong fire	7.828	4.184	0.215	7.591
6		Witness	7.814	4.497	0.231	6.078

However, at deeper depths, the difference between the areas affected by fires and those not affected by fires in regard with the humus and total nitrogen quantities disappears in certain cases (namely in fires with a weak intensity). As such, it seems that fires are affecting some soil properties (the quantity of humus and total nitrogen) at a certain time interval from its apparition, but only on the first 10 centimeters of the soil profile in the case of low severity of the fires.

3.2. Pedoclimatic parameters and soil respiration

For the measurements realized during fall, the average soil temperature varied between 13.11°C in Valea Sării (SP affected) and 16.99°C in Suraia (SP affected). For the two measurements realized during spring (namely in March and April 2017), the soil temperatures have recorded similar values, with small differences. As such, the minimal temperature of 4.67°C was recorded on April in Suraia (SP affected), while the maximum temperature of 5.70°C was recorded on March in Țifești (Table 3). For the measurements realizing during fall, the SWC has recorded a minimum value of 27.92% in Țifești (SP affected), and a maximum value of 53.96% in Suraia (SP unaffected). For the spring interval, due to climatic conditions (low temperatures and low precipitation quantities), the SWC levels have known low variations; as such, the minimum recorded value

was of 27.36% in Suraia (control plot) on March, while the maximum recorded value was 41.24 % in Valea Sării (control) on April (Table 4). Average SWC for Valea Sării location, significant differences between the surface affected by fire and the control surface were recorded both in September and in March. For Țifești location, significant differences between the two surfaces were encountered only during the spring months, while for Suraia location the significant difference level between the surfaces was determined only in April.

The soil respiration has recorded during autumn a minimum value of 3.59 $\mu\text{mol}/\text{m}^2/\text{s}$ in Suraia (control plot) and a maximum value of 5.16 $\mu\text{mol}/\text{m}^2/\text{s}$ in Țifești (control plot). For the measurements realized during spring, the minimum recorded value was 1.06 $\mu\text{mol}/\text{m}^2/\text{s}$ on March in Valea Sării (control plot), while the maximum value was of 2.13 $\mu\text{mol}/\text{m}^2/\text{s}$ in Țifești (control plot), for the same recorded period (Table 5).

Average values and the standard deviations were calculated for each sample surface for the measured parameters, namely soil temperature at 3 cm (ST), soil humidity at 20 cm (SWC) and soil respiration (SR). Furthermore, possible significant differences between the areas affected by fires and the witness ones were determined for each experimental location by analyzing the variation (One Way Anova variant). The ANOVA application was possible thanks to the Levene assumption test.

Table 3. Average soil temperature and standard deviations value for each measured period and location (bold number indicate statistically significant differences ($p < 0.05$))

Location	State	Average temperature (* C) and standard deviations		
		sept.2016	mar.2017	apr.2017
Valea Sării	affected	13.11 (± 1.21)	5.70 (± 0.21)	5.14 (± 0.22)
	control	13.51 (± 0.52)	5.69 (± 0.32)	5.08 (± 0.29)
Țifești	affected	14.58 (± 0.43)	5.37 (± 0.30)	4.89 (± 0.13)
	control	15.46 (± 0.43)	5.72 (± 0.25)	4.95 (± 0.17)
Suraia	affected	16.99 (± 1.12)	4.87 (± 0.27)	4.67 (± 0.23)
	control	16.17 (± 0.63)	4.77 (± 0.18)	4.70 (± 0.11)

Table 4. Average soil water content and standard deviation value for each measured period and location (bold number indicate statistically significant differences ($p < 0.05$))

Location	Forest state	Average soil water content (%) and standard deviation		
		sept.2016	mar.2017	apr.2017
Valea Sării	affected	39.06 (± 7.35)	38.68 (± 1.98)	37.58 (± 3.24)
	control	33.25 (± 7.08)	39.83 (± 2.26)	41.24 (± 1.89)
Țifești	affected	31.88 (± 5.18)	33.15 (± 2.04)	31.59 (± 1.65)
	control	27.92 (± 4.09)	27.76 (± 3.33)	30.13 (± 1.63)
Suraia	affected	52.78 (± 4.16)	31.58 (± 1.64)	30.89 (± 2.03)
	control	53.96 (± 4.41)	27.36 (± 3.45)	29.75 (± 2.70)

Table 5. Average soil respiration and standard deviation values for each measured period and location (bold number indicate statistically significant differences ($p < 0.05$))

Location	Forest state	Average soil respiration ($\mu\text{mol m}^{-2} \text{s}^{-1}$) and standard deviation		
		sept.2016	mar.2017	apr.2017
ValeaSării	affected	3.84 (± 1.51)	1.13 (± 0.27)	1.49 (± 0.39)
	control	4.72 (± 1.78)	1.06 (± 0.37)	1.71 (± 0.44)
Țifești	affected	4.93 (± 0.86)	1.80 (± 0.42)	1.78 (± 0.41)
	control	5.16 (± 1.83)	2.13 (± 0.86)	1.98 (± 0.47)
Suraia	affected	4.27 (± 1.11)	1.33 (± 0.39)	1.51 (± 0.29)
	control	3.59 (± 0.88)	1.79 (± 0.91)	1.85 (± 0.68)

The Tukey test was applied for favourable cases, clarifying the existence of significant differences ($p < 0.05$) for each analyzed parameter. Average soil temperature do not register significant variations ($p > 0.05$) for most of the periods in which measurements were realized, with the exception of Suraia during September 2016 and Țifești in March where significant differences ($p < 0.05$) have existed between the surface affected by fires and the control surface.

The same analysis type as for the parameters discussed above was also applied for the description of significant differences for the average values determined for the soil respiration. As such, the ANOVA analysis and the Tukey test have shown that significant differences between the surface affected by fire and the control surface were recorded only in a few cases. The carbon dioxide production from the soil's level have shown significant differences in ValeaSării for the measurements realized in March and April, while for Țifești the same situation was encountered only in September. Significant differences were not recorded for Suraia location (Table 5).

The lowest percentage values for the variation coefficient (CV) were determined for soil temperature, being gathered between 2.4% (witness area) and 9.2% (affected area) (Fig. 1). The coefficient of variation calculating for SWC has recorded an intermediate value position compare with ST and SR, with minimum value registered in March at Suraia site, affected plot and a maximum value registered in September at ValeaSării site, control plot (Fig. 2). As it was expected, the highest percentage values had

recorded for soil respiration, with the maximum variation coefficient values reaching 50.8% in ValeaSării, in March, in the control area (Fig. 3). The minimum value for the same soil respiration statistical indicator was 17.6%, value determined in Țifești, in September, for the area affected by fire. Furthermore, it can be observed that the minimum CV values for two locations (Țifești and Suraia) were determined during September, for the areas affected by fire, while the maximum CV values were recorded for all locations during March.

-As such, a larger spatial variability of the soil respiration can be observed at the level of the surfaces affected by fire, suggesting altogether a more intense biological activity in the soil in these surfaces, especially during March, as significant microclimatic soil differences were recorded only for ValeaSării, for the same measurement period mentioned above. This fact considers the bidirectional effect of both climatic and fauna factors from the soil as being decisive on the soil respiration in the case of the effect caused by the past litter fire. As such, if for ValeaSării the fire effect has decreased the carbon dioxide production from the soil surface during the spring periods, for Suraia location the same effect has happened only during the autumn period.

For Țifești, where the fire effect was superficial (forest management data), its effects were insignificant. Further, Ryu et al. (2009), confirm the fact that forest fires lead to a decrease of soil respiration in comparison with the effect caused by exploitation works that lead to its increase. This fact is caused by the change of the roots biomass as well as the diversity of the soil microbial community.

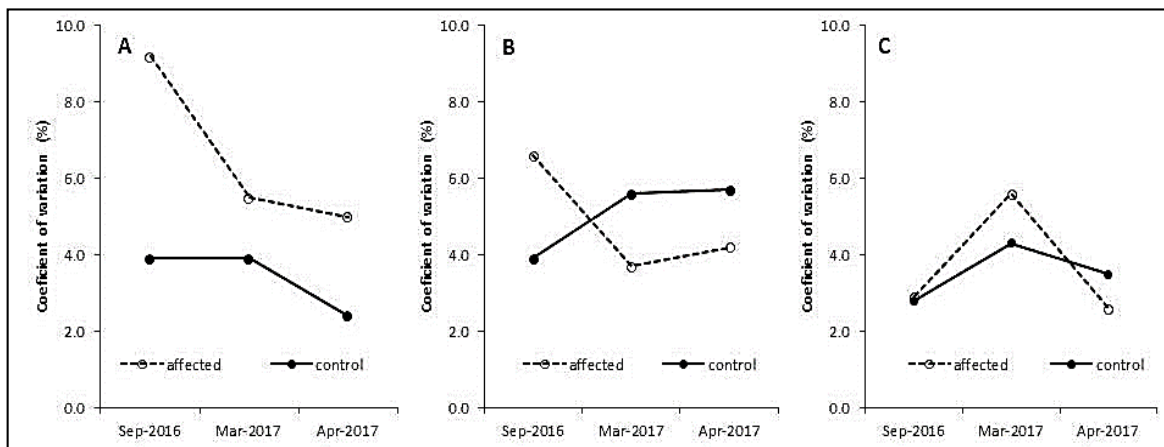


Fig. 1. The temporal variability of CV for soil temperature at ValeaSării site (A), Suraia site (B) and Țifești site (C)

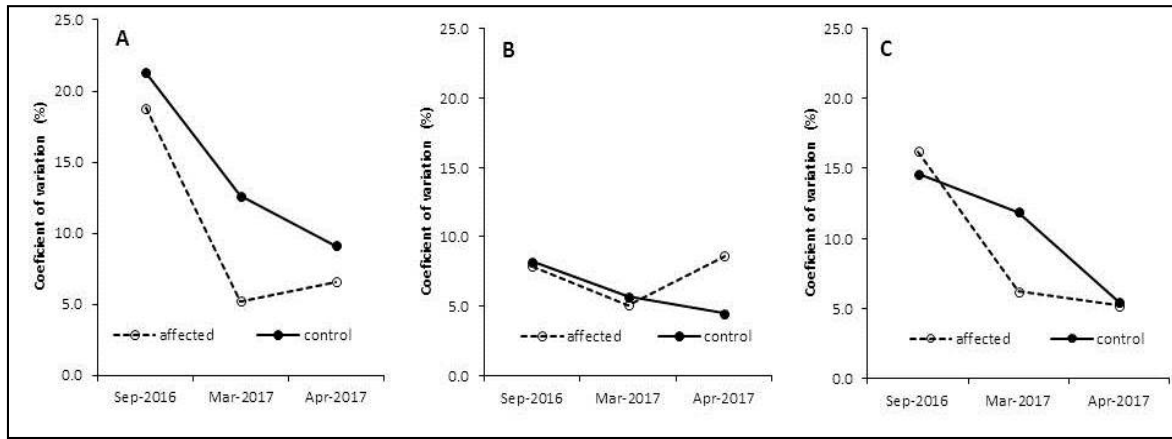


Fig. 2. The temporal variability of CV for soil water content at Valea Sării site (A), Suraia site (B) and Tifești site (C)

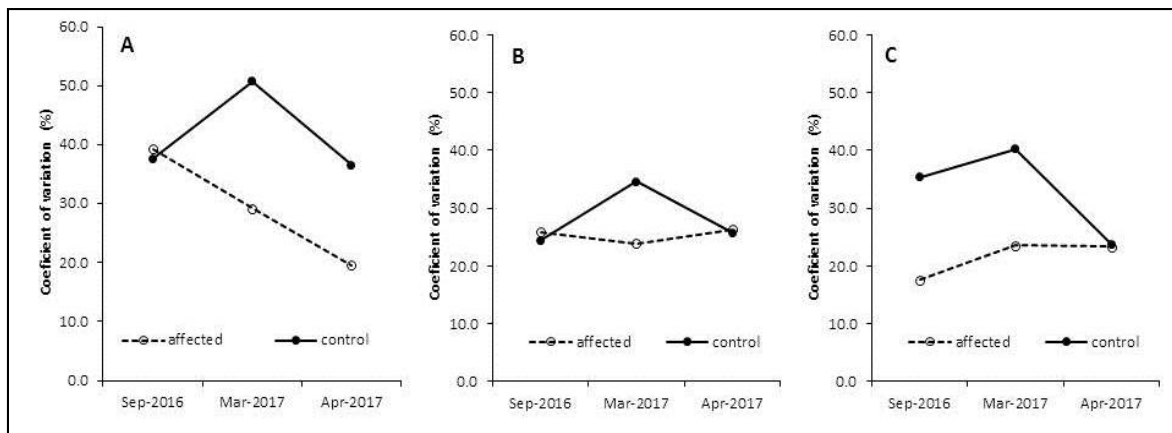


Fig. 3. The temporal variability of CV for soil respiration at Valea Sării site (A), Suraia site (B) and Tifești site (C)

3.3. Biological properties of forest soils

Table 6 presents the results of biological parameters from the soil variants studied under the average \pm standard deviation form, in order to emphasize the significantly statistical differences between the average values, based on one-way ANOVA variation analysis.

The significance of differences between each parameter's averages (for the *Fire* factor) are marked with letters, after each average value. Different letters designate significant statistical differences between the corresponding average values. A multiple average comparison was performed with the post-hoc Tukey test ($P \leq 0.05$) within the variation analysis (ANOVA).

Soil enzyme activities are an integrative indicator of a change in the biology and biogeochemistry of the soil. In all three studied areas, the values of the actual dehydrogenase activity values record a decrease tendency in the variants affected by fire. However, these differences are insignificant from a statistical point of view (Table 6).

In areas affected by fire (Tifești and Valea Sării), the values' variation amplitude is higher comparative with the control variants (Fig. 4). Significantly statistical differences were emphasized in Tifești area between the witness variant and the variant affected by fire in the case of the fungi and

Azotobacter abundance, who have recorded lower values in the variant affected by fire (Table 6). Furthermore, in Suraia area, the *Azotobacter* bacteria have presented lower values in the soil variant affected by fire, significantly statistical comparative with the control area unaffected by the fire (Table 6). The mesophilic bacteria did not vary significantly between the studied areas (Table 6). The values' variation amplitude was higher in the control variants, namely in Tifești and Valea Sării (Fig. 5).

Azotobacter is widespread only in soils that have a pH ranges in the 6.4–7.9 interval. At a more acid soil solution reaction, they are not found at all or can be found only in small quantities. As the pH increases, the *Azotobacter* starts to develop. The smallest number of *Azotobacter* cells can be encountered during winter. The nitrogen fixing bacteria's activity decreases after the depth of 20 cm. The optimum respiration temperature for these bacteria is between 20–30°C, its limits being gathered between 10–50°C. In areas affected by fires such as Tifești and Suraia, *Azotobacter* has recorded significantly lower values in comparison with the values determined in areas not affected by fire (Table 6, Fig. 6).

In Tifești area, fungi were affected by fire, recording significantly lower values in the fire area than in the control one (Table 6, Fig. 7). In this area,

the values variation amplitude was much higher in the control area, unlike Suraia area, where, in the variant affected by fire the values have a higher variation amplitude.

The correlative interpretation of the soil's biological parameters in the variants not affected by fire has emphasized average and statistically significant correlations between the actual dehydrogenase activity and the heterotrophic mesophilic bacteria ($R = 0.6907$; $p = 0.03$) as well as between *Azotobacter* bacteria and the total number of fungi ($R = 0.6777$; $p = 0.04$) (Table 7).

3.4. Discussions

Fire is a major ecosystem driver across many regions worldwide (Bowman et al., 2009). Severe fires have an important impact on physical, chemical and biological properties of soils (Caon et al., 2014; Certini, 2005; Neary et al., 1999). Soil properties and dynamics after fire can be affected by post-fire changes in both total and patterns of precipitation (Certini, 2005). More frequent correlations between the studied microorganism groups have been determined from the areas affected by fire, as well as between them and the soil's dehydrogenases activity.

Table 6. The values of studied parameters expressed under the average \pm standard deviation form based on the post-hoc Tukey multiple comparison tests from within the one-way ANOVA analysis for the **Fire** factor

Soil Variant	DHA	Mesophilic bacteria (CFU/g soil) ($\times 10^9$)	Fungi (CFU/g soil) ($\times 10^3$)	Azotobacter (CFU/g soil) ($\times 10^6$)
ValeaSării Ctrl A	1.755a ± 0.359	432.0a ± 97.000	70.0bc ± 10.000	439.0b ± 86.000
ValeaSării	1.368a ± 0.629	429.0a ± 27.000	13.3c ± 5.774	433.0b ± 72.000
Țifești Ctrl A	1.559a ± 0.229	423.0a ± 89.000	223.3a ± 68.069	735.0a ± 67.000
Țifești	1.246a ± 0.349	408.0a ± 77.000	37.3c ± 28.361	420.0b ± 70.000
SuraiaCtrl A	0.283b ± 0.076	256.0a ± 43.000	165.3ab ± 37.220	659.7a ± 119.918
Suraia	0.215b ± 0.009	247.0a ± 60.000	115.7abc ± 53.295	149.0c ± 29.000

Note: For each column, different letters denote statistical significant differences between the means

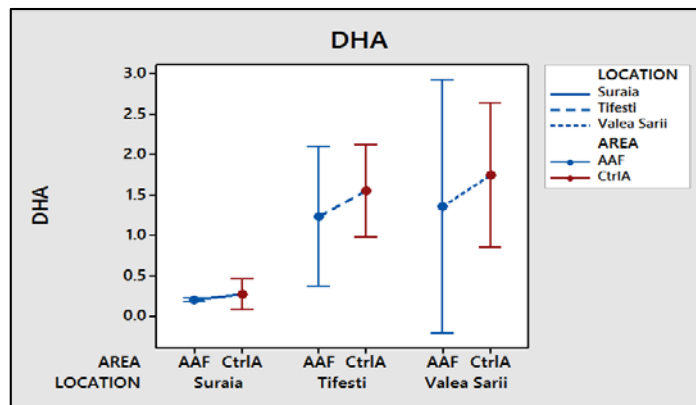


Fig. 4. Graphical representation of the dehydrogenase activity variation (mg formazan/10 g soil) in the control soil variants (Ctrl A) and in areas affected by fire (AAf)

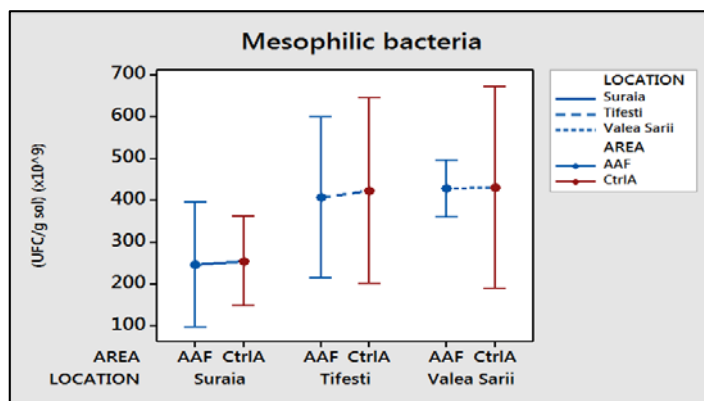


Fig. 5. The dynamic of heterotroph mesophilic bacteria abundance (CFU/g soil) in soil variants affected by fire and in the control soil variants

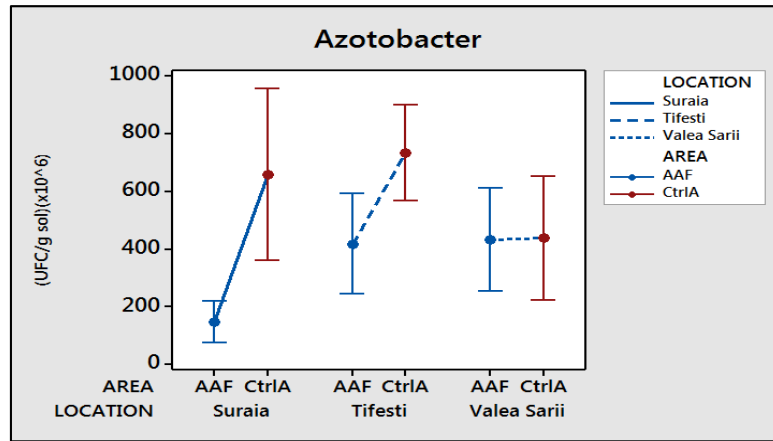


Fig. 6. Variation of the total number of non-symbiotic nitrogen fixing bacteria from genus *Azotobacter* (CFU/g soil) in the soil variants affected by fire and in the control soil variants

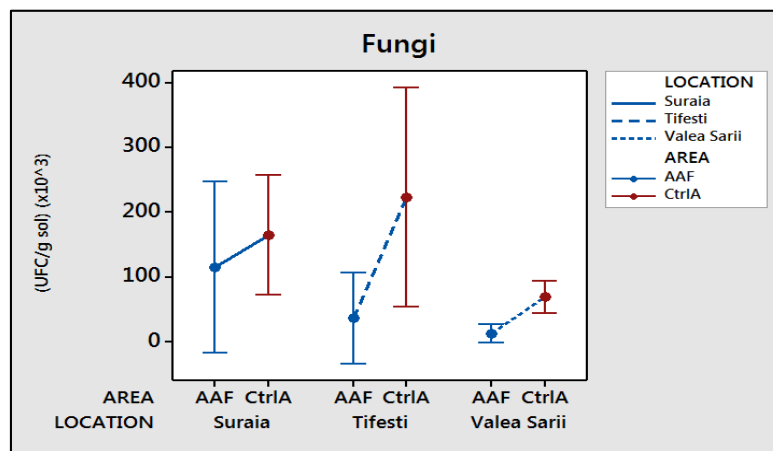


Fig. 7. Variation in the total number of fungi (CFU/g soil) in soil variants affected by fire and in the control soil variants

Table 7. Correlative interpretation of the values of the studied biological parameters in the areas affected and not affected by fire

<i>Ctrl A</i>	<i>R\values p</i>	<i>DHA</i>	<i>Mesophilic bacteria</i>	<i>Fungi</i>	<i>Azotobacter</i>
	DHA		0.0394	0.7138	0.3337
	Mesophilic bacteria	0.6907		0.8298	0.9061
	Fungi	-0.1429	-0.0840		0.0449
	Azotobacter	-0.3653	0.0462	0.6777	
<i>AAF</i>	<i>R\values p</i>	<i>DHA</i>	<i>Mesophilic bacteria</i>	<i>Fungi</i>	<i>Azotobacter</i>
	DHA		0.0020	0.0169	0.0018
	Mesophilic bacteria	0.8753		0.0060	0.0001
	Fungi	-0.7623	-0.8265		0.0070
	Azotobacter	0.8787	0.9418	-0.8181	

Note: *R values* (the ones that are under the main diagonal) marked with bold are statistically significant ($p < 0.05$); CTRL= Witness Area; ZIA=Area affected by fires; Correlation interval: weak correlation: < 0.6 ; average correlation: $0.6-0.85$; strong correlation: > 0.85

As such, the fire process changes the microbial dynamic from the soil and the relations between groups of microorganisms even though the relation between the soil's dehydrogenases activity and the microbial biomass is not always evident, especially in complex systems such as soils (Salazar et al., 2001). The dehydrogenases activity was strongly and significantly correlated with the heterotrophic mesophilic bacteria ($R=0.8753$; $p=0.0020$) and with *Azotobacter* ($R=0.8787$; $p=0.0018$). An average, negative, significantly statistical correlation was

emphasized between fungi and the dehydrogenases activity ($R=-0.7623$; $p=0.0169$). The total number of fungi was negatively and statistically significant correlated (average correlation) with heterotrophic mesophilic bacteria ($R=-0.8265$; $p=0.0060$). A strong, significantly statistical correlation was also recorded between *Azotobacter* bacteria and heterotroph mesophilic bacteria ($R=0.9418$; $p=0.0001$). Furthermore, an average, negative and statistically significant correlation was also established between fungi and the total number of

nitrogen fixing bacteria ($R = -0.8181$; $p = 0.0070$). Fire affects the soil's properties as the organic matter is rapidly submitted to the "combustion" process. The changes that appear in the organic matter influence the physical, chemical and microbiological properties of soils. While some nutrients are volatilized and lost, others become more accessible. As such, fire acts as a fast mineralization agent (DeBano, 1989). The very high soil temperature affect microorganism, destroying or even altering their reproductive capacity. The relation between the soil fire process and its microbiological properties is a complex one that depends on the fire's duration and severity, as well as on the maximum temperature and the soil's humidity. The microbial groups differ in regard with their sensibility towards high temperatures.

The most sensitive ones in this case are the nitrification bacteria and the nitrogen fixing ones (Dunn et al., 1985). The microorganism populations from the humid soils, active from a physiological point of view, are much more sensitive than the dormant (latent) populations from the dry soil. Another important group of sensitive microorganisms towards fire is represented by ectomycorrhizal (Harvey et al., 1989). In the burned soils, the relative amount of actinomycetes is higher and can be related to the ability of actinomycetes (as well as many bacteria) to form spores, making them more heat resistant and consequently less affected by fire (Bárceñas-Moreno et al., 2011). The results obtained in regard with the influence of fires on the soil's biological activity have revealed that in Țițești, an area strongly affected by a 2011 strong litter fire on a surface of 6000–7000 m², aerobic bacteria and *Azotobacter* have recorded the lowest values, significantly statistical from the control area, unaffected by fire. Furthermore, in Suraia area, affected in 2011 by an average litter and canopy plantation fire on approximately 5 ha, *Azotobacter* bacteria and fungus have recorded significantly lower values in comparison with the control area. The microorganisms' answer towards fire varies from undetected effects, in the case of fires with low severities, towards the total sterilization of the soil's superficial stratum in strong fires.

After the fire, a decrease of the microorganisms' abundance is recorded, even though the remaining ones can present higher activity levels than the ones belonging to the microorganisms before the fire (Poth et al., 1995). Forest fires also change the specific composition of microbial communities from the soil. By analysing the phospholipids of fat acids after a fire from a coniferous forest, it was demonstrated that mushrooms were more reduced than bacteria (Bååth et al., 1995). In general, the symbiosis between vascular plants and mycorrhiza suffers from the heat (Vilariño and Arines, 1991). The significant differences encountered in Valea Sării regarding the R_{sol} average values can be correlated with the soil temperature and humidity. These coordinates have registered important differences between the affected surface and the control one,

determinations that were achieved during the first part of spring (March 2017). Solar radiation differences have possibly encouraged an increase in the ST and SWC spatial variability. This fact confirms changes in regard with soil respiration, with possible effects on the microbial activity, responsible for mineralizing the soil carbon in CO₂ fluxes (Moyano et al., 2012). A different situation was encountered in the other two surfaces during the same measurement period. In this case, the presence of a more consistent herbaceous cover resulted from the presence of a young forest (10 years old) has favoured the apparition of a competitiveness among herbaceous plants. Their light and water requirements were almost similar so that the effect of the past fire did not have significant effects on the CO₂ soil production.

4. Conclusions

Amongst the soil properties, temperature and humidity do not present significant differences between the surfaces affected by fires and those not affected by them. However, pH has higher values in the areas affected by fires, even though the humus and total nitrogen contents are much lower in these surfaces. These two last soil properties present significant differences only in the first 10 centimetres of the soil's profile.

In areas not affected by fires, the CO₂ flux has higher values due to the participation of roots and the participation of mycorrhiza. The number of bacteria was much lower in the areas with fires, a fact that has led to the significant decrease of respiration rate. The effect of fires on the CO₂ flux and on the soil's physical-chemical properties also depends on other factors such as pedo-climatic factors, history of the fire's severity and frequency.

In regard with the soil's biological properties, significant differences between the two categories were obtained for the nitrogen fixing bacteria (*Azotobacter* genus), as well as for fungi. The nitrogen fixing bacteria have been identified in large quantities in surfaces with strong and average fires, while have recorded significant differences only in comparison with the surface that was affected by the strongest fire. On the other hand, aerobic bacteria did not vary significantly between the two types of field (affected by fire and not affected by fire). In regard with the actual dehydrogenase activity, this has recorded a decreasing tendency in the fire surfaces, but insignificant from a statistical point of view.

A larger number of significant correlations amongst the biological factors were obtained for the areas affected by fires, in comparison with the ones not affected by fires. As such, positive significant correlations were recorded between DHA and aerobic bacteria, between DHA and *Azotobacter* bacteria and between aerobic bacteria and *Azotobacter*. Significantly negative correlations were recorded between DHA and fungi, between fungi and aerobic bacteria and between fungi and the total number of

nitrogen fixing bacteria. As a total, 6 significant correlations were obtained in the affected areas and only two significant correlations in the control areas.

As a general conclusion, it can be stated that even though six years have passed between the moment of the fire and the present study and the fire's intensity was reduced (only the litter has burned, but not the forest), the 'unseen' effects of this damage can still be seen: a reduced soil respiration, a lower number of bacteria, an increase of pH and a decrease of humus and total nitrogen quantities.

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