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INFLUENCE OF WILDFIRE ON SOIL MICROBIAL COMMUNITY AND CHEMICAL PARAMETERS IN DOLNA BANIA REGION, BULGARIA

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Abstract

Microbial indicators of forest soils affected by forest fire under conifers (*Pinus sylvestris* L., *Larix desidua* Mill.) and mixed (*Pinus sylvestris* L., *Quercus cerris* L.) forests from north slopes of Rila Mountain (Dolna Bania region) have been examined 7 days after a wildfire occurrence. An increased quantity of total microflora in the upper (0–5 cm) layer of fire-affected soil in comparison with that taken from a control (unburned) sampling site was established due to the rise of soil temperature and pH, accompanied with a simultaneous decrease in soil humidity. Results show the highest proportion of the analysed microflora represented by non-spore-forming bacteria, bacilli, bacteria assimilating mineral nitrogen. The quantity of *Actinomycetes* and *Micromycetes* decreased in soil affected by fire. The predominant groups of microorganisms (ammonifying and nitrifying bacteria) play an important role at different stages of mineralization processes of the organic matter in fire-affected soil.

Key words: microbial diversity, Rila Mountain, soils affected by fire.

Introduction

Forest fires are becoming of increasing concern worldwide – both for the ecosystems they occur, and for the population. Some authors associate the increase in the frequency of fires in recent years with the climate change (Halofsky et al. 2020). The anthropogenic interference could also be accepted as a factor for their occurrence through human demography and activities, such as land use and fire management (Syphard et al. 2017, Williams et al. 2019).

Fires affect the soil organic matter, the amount of macro- and micro-nutrients, soil physical properties, soil acidity, and the amount and composition of flora, fauna and soil microbiota (Guerrero et al. 2005, Velizarova and Filcheva 2011). Fire can have many effects on soil organic carbon (SOC) through changing the quantity and quality of carbon inputs to soil, such as forest floor, and affecting conditions that control microbial activity and access to it (Berryman and al. 2020). Through

laboratory experiment was established that high-intensity and high-severity fires decreased SOC stored in macroaggregates in comparison with soils exposed to low temperatures (Araya et al. 2017). Additionally, Albalasmeh et al. (2013) found that in low- and moderate-severity burns, if long enough in duration, soil aggregates can degrade. The extremely high soil temperatures during fire events lead to chemical and microbial community transformations with potential feedbacks to SOC processing (Esquilín et al. 2007). Heat-induced changes also affect soil nitrate ($-NO_3$), ortho-phosphate ($-PO_4$), and sulfate ($-SO_4$) status and facilitate their movement in surface overland flow (Miller et al. 2006). Microbial community responses to fire may correlate with soil physico-chemistry, such as pH or nutrient pool sizes, or availability, exemplifying microbe-environment feedbacks in response to extreme disturbances. Furthermore, selection for disturbance tolerant microbial communities and declines in microbial biomass presumably results in long-term effects on essential ecosystem functions, such as exoenzyme production and decomposition (Dooley and Treseder 2012; Holden et al. 2013).

One of the main factors for the restoration of soil fertility after fires is the state of soil microflora (quantity, quality composition and activity) which plays an important role in restoring ecosystems (Li et al. 2019). It serves as a biomarker in the monitoring of soil (Yakovlev 2000). Soil microorganisms can be affected by fire, either as a direct result of heating or as an indirect effect of changes in soil physico-chemical properties (Docherty et al. 2012).

Brown et al. (2019) and Yeager et al. (2005) found that in soils affected by fire, the amount of nitrogen-fixing and ammonia-oxidizing bacteria is lower, while the number of species of *Clostridium* and *Bacillus* are higher than that in the soil from control sampling site. They established that these trends are kept during fourteen months after the fire. The growth of microorganisms is activated in the topsoil affected by fire. According to some authors their quantity at a 0–5 cm depth is 3 times higher than in the control soil (Bogdanov et al. 2003). Neary et al. (1999) report that the effect of heating on microbes is most significant in the surface soil layer, where microorganisms are most abundant. Other authors (Mabuhay et al. 2006) found that bacteria are more resistant to heat and recover faster after fire than *Micromycetes*. And in both groups of microorganisms they have proven fire resistant species – bacteria (*Massilia* sp., *Arthrobacter* sp.) and *Micromycetes* (*Penicillium* sp., *Fusicladium* sp.) (Whitman et al. 2019). The ratios of alpha-, beta- and gamma-proteobacteria, and *Cytophaga-Flavobacterium* group to total Eubacteria increased immediately after the wildfire, and the other eubacterial proportions decreased in the surface and middle layer soils (Kim et al. 2004). Richness of bacterial community composition tended to be lower in the high burn soils in mixed conifer forests relative to unburned soils, while it was similar across all soils in pine forests (Weber et al. 2014). Microbes differ in their sensitivity to heat caused by fire (Gema and Bååth 2009). Forest fires are one of the main factors that change the taxonomy and functional diversity of soil microbial communities. According to some authors, the microbial diversity decreases in soils after a fire (Smith et al. 2008, Bárcenas-Moreno et al. 2011, Xiang et al. 2014), while according to others – it increases. The heat-resistant species – mainly spore-forming appear after fire (Yeager et al. 2005, Brown et al. 2019)

which is an important indicator of soil state (Yakovlev 2000). Therefore, the study of microbial diversity in the dynamics after a fire is of great importance.

The aim of study was to assess the initial response – a week after wildfire occurrence of microbial and chemical indicators of forest soils (Chromic Luvisols) affected by crown and surface fire under conifers and mixed forests from north slopes of Rila Mountain (region of Dolna Bania).

Materials and Methods

Soil sampling

Soil sampling have been performed at five experimental sites as follows – two affected by crown fire and forest vegetation: Scots pine (*Pinus sylvestris* L.) – sampling site No1 (SS No1), European larch (*Larix decidua* Mill.) – SS No2; two experimental sites affected by surface fire and vegetation: Scots pine (*Pinus sylvestris* L.) – SS No3 and mixed forest – Scots pine (*Pinus sylvestris* L.) and Turkey oak (*Quercus cerris* L.) – SS No4. The control experimental site was established in an unaffected Scots pine (*P. sylvestris*) forest – SS No5. The studied sampling sites are situated at similar altitude, slope exposure and soil characteristics (Molla and Velizarova 2016). The soils are Chromic Luvisols (LV-cr) (WRBSR 2015).

The sampling was performed a week after forest fire. Soil samples were taken from 0–5 cm and 5–20 cm depth of soil profile mineral part. Those from surface mineral soil have been collected randomly at each sampling site. Sampling of the accumulated forest litter was carried out simultaneously. Within each plot for soil sampling, the litter was collected from the area with size 0.25 m × 0.25 m outlined by wooden frame. Forest litter collected from fire-affected sampling sites was not possible to separate by fractions because of partly or fully carbonization. Litter samples from unaffected sampling site were separated into three fractions – litter layer (L), fermentation layer (F), and humus layer (H). They were dried and milled. All samples were transported and analysed within 48 h. Sampling and sample preparation processing have been performed following the standard methodological approach (Cools and De Vos 2020). The soil from observed sites belongs to Haplic Luvisols (WRBSR 2015). Identification and classification were performed according to the morphological peculiarities of the established soil profiles and based on the data received from the following laboratory analyses. The samples for microbial analysis were taken with a sterile instrument from 0–5 cm, 5–20 cm of soil mineral part, and from the forest litter affected by fire, in sterile paper bags.

Microbiological analyses

Microbiological studies include the determination of non-spore-forming bacteria, bacilli, *Actinomycetes*, *Micromycetes* and bacteria assimilating mineral nitrogen. The analysed groups of microorganisms were determined on the respective nutrient media according to their morphological characteristics. The identification of species is subject to additional analyses. Appropriate dilutions of soil samples were inoculated on specific culture media plates: meat–peptone agar for non-spore-forming bacteria and bacilli, starch–ammonium agar for *Actinomycetes* and bacteria assimilating mineral nitrogen and Czapek's agar for *Micromycetes*. Agar plates were then incubated for 2 (non-spore-forming bacteria, bacilli, bacteria assimilating min-

eral nitrogen) and 10 (*Actinomycetes*, *Micromycetes*) days in thermostat and typical morphological colonies were counted with colony counter. The number of tested microorganisms was expressed as colony-forming units (cfu) per 1 g of dry soil (Zvyagintsev et al. 1980). The samples for microbiological analysis were taken with a sterile instrument, in sterile paper bags. They were transported and investigated the latest up to 48 h, as up to the moment of the culture they were stored in a refrigerator at 4–10 ºC. The mineralization coefficient was calculated by dividing bacteria assimilating mineral nitrogen to non-spore-forming bacteria + bacilli, according Mishustin and Runov (1957), Nustorova and Malcheva (2020).

Chemical analyses of soil

Soil samples were taken according to Bulgarian State Standard ISO 18400-104:2019 and ISO 18400-102:2019. The active reaction of soils (pH) was measured in water extract in compliance with Bulgarian State Standard ISO 10390:2011. The quantity of humus is determined as per method of Turin according to the methodology, represented in Donov et al. (1974). The content of nitrogen is reported photometrically with Nitrospectral as per international standard ISO 14255:2002, in compliance with methods of 'Merck' company. The moisture of the soil is specified as per weight method through usage of thermostat and drying at temperature 105 ºC up to a constant weight. Soil density was measured using metal ring pressed into the soil (intact core), and determining the weight after drying, detailed described in Donov et al. (1974). The same methods were used for analyses of forest litter chemical parameters.

Statistical data processing includes calculation of the average values and coefficient of variation (±CV) by three replications for each group of microorganisms. Correlation analysis was applied to determine the relationships between soil chemical and microbiological indicators. Excel 2010 program for Windows was used for the statistical analysis.

Results and Discussion

The results obtained show that the studied soils and forest litter differ in the total amount of microorganisms, their variability by groups and the degrees of soil organic matter mineralization (tables 1 and 2).

The active reaction of soil (pH) after forest fire is higher in all experimental variants compared to the control pH values (SS No5). More pronounced statistically significant differences were observed for the upper 5 cm of soil and for all sampling sites, affected by crown fire.

The soil bulk density values increased in the fire influenced soil in comparison with that of the control sampling site. This increase is higher for the surface 5 cm layer. The fire burns biomass and soil organic matter resulting in carbon decrease in crown fire affected sampling sites (SS No1 and SS No2), while surface fire SS No3 and SS No4 provoked an increase in soil organic carbon amount in comparison to that in control SS No5.

The data from Table 1 show that soils affected by fire contain a higher amount of total microflora compared with soil from the control SS – No5, except for the surface soil layer of SS No4. This trend is not typical for the amount of *Actinomycetes* in the upper soil layer and for the bacteria assimilating mineral nitrogen for both layers. The amount of these groups of micro-

Table 1. Soil indices, qualitative and quantitative composition of soil microflora.

SS No	Depth, cm	C, %	N, %	pH (H_2O)	Moisture, %	Density, g/cm^3	Total microflora	Non-spore-forming bacteria	Bacilli	*Actinomycetes*	*Micromycetes*	Bacteria, assimilating mineral nitrogen	Coefficient of mineralization
							Colony-forming units per g of dry soil (x10^3 cfu/g soil), ± Coefficient of variation (CV), in brackets – as percentage of total microflora, %						
1	0÷5	1.66	0.042	6.06	0.87	1.66	5200	4120±0.49 (79)	680±2.94 (13)	100±9.172 (2)	300±3.33 (6)	1800±3.38	0.38
	5÷20	1.38	0.031	5.53	1.07	2.04	450	380±9.49 (84)	44±7.87 (10)	8±12.50 (2)	18±5.56 (4)	784±0.51	1.85
2	0÷5	1.93	0.085	6.42	0.84	1.92	20260	19400±0.45 (96)	560±7.14 (3)	140±7.14 (1)	160±6.25 (1)	3120±0.80	0.16
	5÷20	1.24	0.07	6.12	1.85	1.97	316	202 ±4.32 (64)	80±7.50 (25)	14±7.14 (4)	20±10.00 (6)	168±1.19	0.60
3	0÷5	2.35	0.095	5.81	1.36	2.15	3300	2260±2.34 (68)	760±5.26 (23)	120±8.33 (4)	160±2.50 (5)	3360±1.19	1.11
	5÷20	1.79	0.082	5.40	1.77	1.43	484	368±1.09 (76)	90±8.01 (19)	0	26±7.69 (5)	1200±1.76	2.62
4	0÷5	3.45	0.13	6.12	1.93	1.40	1760	800±2.5 (46)	860±4.65 (49)	60±8.82 (3)	40±5.00 (2)	3640±1.10	2.19
	5÷20	2.21	0.098	5.85	5.93	1.40	238	90±8.01 (38)	84±4.76 (35)	26±7.69 (11)	38±5.26 (16)	144±1.39	0.83
5	0÷5	2.07	0.093	5.47	1.61	1.34	1860	780±1.28 (42)	540±1.85 (29)	380±3.16 (20)	160±1.25 (9)	3660±1.09	2.77
	5÷20	1.10	0.052	5.26	13.1	1.78	160	32±6.25 (20)	94±4.26 (59)	10±10.00 (6)	24±4.17 (15)	88±2.27	0.70

Table 2. Forest litter chemical and microbiological indices.

SS No	Sub-layer	C, %	N, %	pH (H_2O)	Moisture, %	Stock, g/cm^2	Total microflora	Non-spore-forming bacteria	Bacilli	Actinomycetes	Micromycetes	Bacteria, assimilating mineral nitrogen	Coefficient of mineralization
							Colony-forming units per g of dry litter (x10^3 cfu/g litter), ± Coefficient of variation (CV), in brackets – as percentage of total microflora, %						
1		41.55	0.449	7.44	0.48	3252	6220	5960±0.67 (96)	260±7.69 (4)	0	0	5160±1.16	0.83
2	BFL*	30.60	0.369	8.01	1.41	6154	8260	6400±3.13 (77)	160±6.25 (2)	1620±1.23 (20)	80±2.50 (1)	3200±3.13	0.49
3		54.45	0.658	7.28	0.38	6020	6740	6400±1.56 (95)	100±6.00 (1.5)	20±10.00 (0.3)	220±5.45 (3.3)	3840±1.56	0.59
4		40.65	0.292	6.80	1.71	4910	4940	3640±1.65 (74)	1080±1.85 (22)	0	220±4.55 (4)	4000±3.50	0.85
5	L	59.70	1.565	5.06	3.14	2505	1680	1160±3.63 (71)	160±5.00 (17)	20±5.00 (2)	340±1.56 (9)	2880±1.20	2.18
	F	45.90	0.688	5.08	7.67	2442	15880	13760±0.58 (87)	1540±2.25 (10)	180±5.56 (1)	400±2.50 (3)	5720±1.40	0.37
	H	22.20	0.150	5.37	0.94	6041	5060	3600±2.78 (69)	860±4.65 (10)	120±3.33 (1)	480±1.67 (20)	9800±0.61	2.20

Note: *BFL – burned forest litter.

organisms is lower in the soils affected by fire in comparison to the control sample. It was found that both the total microflora and the quantity by groups is higher at a depth of 0–5 cm, as compared to the lower layer of soil, and the drop is statistically significant in SS No2 – 64 times. It should be noted that the amount of total microflora is higher in the top-soil layer from both sampling sites influenced with crown fire (SS No1 and 2) in comparison with that affected by surface fire (SS No3 and 4). At the deeper soil layer (5–20 cm), however, this quantity sharply decreased. According to some authors (Keeley 2009, Pérez-Izquierdo et al. 2021) the crown fire leads to accumulation of a bigger amount of biomass, respectively, organic compounds in soil surface layer, which in turns impacts fungal biomass and community.

The results for the number of microorganisms in the samples from forest floor show maxima in the fermentation layer (F layer), wherein the amount of non-spore-forming bacteria, bacilli and *Actinomycetes* is highest compared to L and H layers (Table 2). This is important for understanding the destruction of organic and inorganic compounds. According to research of Grishakina et al. (2006) the processes of organic nitrogen mineralization were the most intensive in the upper (L and F) sub-horizons of the litter. However, according to the results of our study the higher quantity of microorganisms does not always imply a higher activity, which is confirmed by values of mineralization coefficient – it is obstructed in the fermentation bed, as the ratio between the amonifying bacteria and bacteria assimilating mineral nitrogen is 3:1. This fact determines low rate of degradation of organic substance and, respectively – a low coefficient of mineralization in F layer, which is 6 times lower than that of L and H layers from control sampling site.

Mineralization ratio expresses the degree and direction of the transformation processes in soil. It is a consequence of different roles of microorganisms in transformation of organic and inorganic substances. For all studied variants, non-spore-forming bacteria and bacilli dominate, which is a conformation or confirmation for slow mineralization processes of organic matter. The lowest mineralization coefficient of 0.16 and the highest amount of non-spore-forming bacteria was found for the surface soil horizon of SS No2. A similar trend of that coefficient was observed for other sampling sites under coniferous trees. Obviously, the leaves of broadleaf species in forest litter of SS No4 decompose faster than those of conifers, due to the lower content of compounds which are difficult to be decomposed. Similar results were presented by Prescott et al. (2000), studying decomposition rate of broadleaf and needle litter in forests of British Columbia.

At all sites tested the highest share of total microorganisms is composed of non-spore bacteria and bacilli and the trend is again a decrease in depth. The lowest quantity is the one of *Micromycetes* and *Actinomycetes* which were developed mainly in high humidity and low pH. These groups of microorganisms are present in higher amounts in the soil of SS No 2, 3 and 4 in burnt organic matter – presumably as an initial response where several soil parameters simultaneously changed – elevated temperatures, low humidity, and higher pH values, accumulation of burnt plant residues (forest litter) and soil organic carbon. For all studied cases, the bacilli quantitate in the surface layer of 0–5 cm of soil and in F layer of forest litter of the control sampling site is higher in comparison with the deeper layer of 5–20 cm.

The quantity of bacilli in the soil after fire is higher in comparison with the soil of the control trial plot, which is determined by the fact that they are spore-forming microorganisms. The number of bacteria, assimilating mineral nitrogen is highest in the topsoil and have the biggest decrease in depth (42 times) in the control soil, which correlates with the most pronounced reduction in depth of the nitrogen in it (2 times) in comparison to the soils affected by fire. Forest fires change the specific composition of microbial communities from soil. After them mushrooms are more reduced than bacteria (Bååth et al. 1995, Onet et al. 2019). In the burned soils, the relative number of spore-forming bacteria is higher as they are more heat resistant and consequently less affected by fire (Bárcenas-Moreno et al. 2011).

A lower quantity of microorganisms in the soil was found in cases of surface fire compared to the results, obtained in variants with crown fire (Table 1). Obviously, the difference in soil heating during different fire type of propagation patterns influences the metabolism and productivity of microorganisms. The higher soil moisture values, measured in 5–20 cm soil layer of SS No4, affected by surface fire could be in result of an increased hydrophobicity, when the soil organic carbon transforms. This phenomenon occurs during the combustion process when distilled aliphatic hydrocarbons migrate along the soil profile and condense on soil particles forming a water-repellent layer. MacDonald and Huffman (2004) found that the soil surface in sites affected by fire at high and moderate severity was significantly more water repellent than soil surface in unburned sites.

The highest amount of burnt forest litter was found for sampling site No 2, for which a large amount of total microflora was found. For it, the lowest values of mineralization coefficient were established (Table 2). The mineralization of litter depends on temperature and humidity, as well as on chemical composition of plant material (Kuiters and Sarink 1986). Changes of water-holding capacity and physical properties of soil affected by fire are resalted in reduction in water resistance of the aggregate's stability, which in turn contributes to their degradation and soil compaction. Under these conditions, the multiplication of anaerobic microorganisms' groups increases in the areas affected by fire.

Correlation analysis is presented in tables 3 and 4, figures 1 and 2.

Table 3. Correlation coefficient (R) for forest litter.

Forest litter	C	N	pH	Moisture	Stock	Total microflora	Coefficient of mineralization
C	1						
N	0.8289	1					
pH	-0.1757	-0.4443	1				
Moisture	0.2539	0.3390	-0.6322	1			
Stock	-0.5577	-0.6345	0.5283	-0.6565	1		
Total microflora	-0.1034	-0.2441	-0.0737	0.7026	-0.1960	1	
Coefficient of mineralization	-0.0727	0.3236	-0.5888	-0.1958	-0.0640	-0.6877	1

Table 4. Correlation coefficient (R) for soil.

Soil	Soil depth	C	N	pH	Mois-ture	Soil density	Total mi-croflora	Coefficient of mineralization
Soil depth	1							
C	-0.5810	1						
N	-0.3978	0.8606	1					
pH	-0.4784	0.3546	0.3005	1				
Moisture	0.4707	-0.3191	-0.1483	-0.4940	1			
Soil density	0.0521	-0.4270	-0.4644	0.1906	-0.0970	1		
Total microflora	-0.5272	0.0727	0.0706	0.6428	-0.3085	0.2550	1	
Coefficient of mineralization	-0.0010	0.3579	0.3188	-0.5360	-0.2298	-0.5210	-0.4622	1

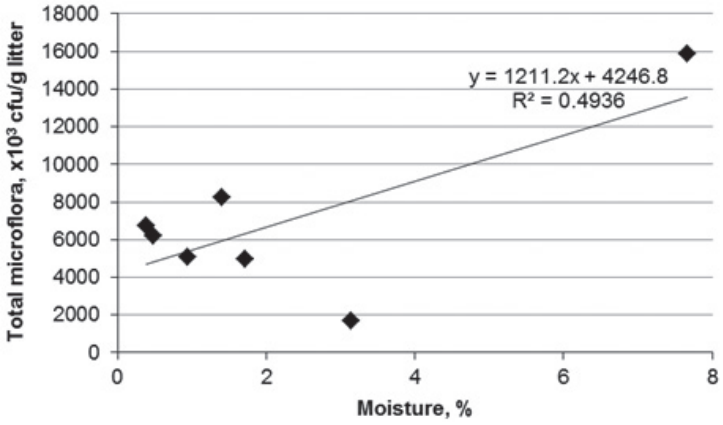

Fig. 1. Dependence between total microflora and humidity (forest litter samples).

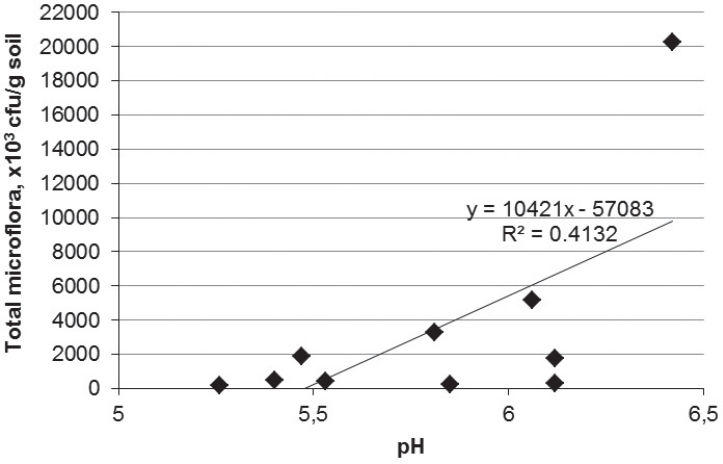

Fig. 2. Dependence between total microflora and pH (soil samples).

The applied correlation analysis showed the strongest correlation between total microflora and moisture content for forest litter samples and between pH and total microflora for soil samples.

Conclusions

Changes in soil bulk density induce changes in soil carbon movement processes of transformed soil organic compounds and fine soil particles. Forest fires influence soil organic carbon content depending on type of fire – crown or surface.

The total amount of microorganisms is higher in fire-affected soil in comparison to that of control unaffected one. This specificity is influenced both by the type of forest and by the type of fire. A higher quantity of microbes was isolated from the soil under European larch and from sites affected by crown fire.

The composition of microbial coenosis is not affected by fires in relation to non-spore forming bacteria and bacilli – these groups of microorganisms dominate both the affected and unaffected by fire soils. There is change in the degree of dominance of *Actinomycetes* and *Micromycetes* – their percentage is higher in the composition of microbial coenosis in the control soil compared to burned soils.

A close correlation of certain indicators (total microflora, non-spore-forming bacteria, mineralization activity) has been established between soils affected by surface fires and the control soil, which is premised on their close values of organic carbon, nitrogen, pH and moisture. These indicators vary considerably in soils affected by crown fire and the control soil. Correlation analysis revealed the strongest dependence between total microflora and forest litter moisture and pH for soil samples.

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