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Selected Biological Properties of the Soil in a Burnt-Out Area under Old Pine Trees Three Years after an Fire

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1. Introduction

Fires, on account of their emotional, economic and cognitive aspects, are of interest to numerous researchers, and their impact is often unpredictable and difficult to investigate. They are among some of the most dynamic factors shaping terrestrial ecosystems (e.g. Bowman et al. 2009). They destroy vegetation and alter the physicochemical and biological properties of soils (e.g. Hauke-Pacewiczowa and Trzcińska 1980, Querner et al. 2010), including those of forest soils (e.g. Certini 2005). They affect the hydrological and thermal properties of the soil, the rate of mineralization, circulation of nutrients, and accelerate soil erosion (e.g. Certini 2005, Köster et al. 2011, Hewelke et al. 2018).

Under the influence of fires, not only vegetation but also litter and the organic soil layer are destroyed (e.g. Certini 2005). Fires affect living organisms directly (causing their death) and indirectly, transforming their living environment (affecting food availability and quality, heterogeneity of the environment, pH increase, etc.) (e.g. Kim and Jung 2008).

Fires significantly influence the abundance of microorganisms and taxonomic biodiversity of bacteria and fungi (e.g. Dooley and Tresender 2012, Knelman et al. 2015), and soil animals, such as springtails and mites (Wikars and Schimmel 2001, Kim and Jung 2008, Malmström et al. 2009).

Soil mesofauna, including springtails and mites, is an important element of the trophic network of every ecosystem, especially forest soils (e.g. Petersen and Luxton 1982, Carrillo et al. 2011). The animals of the mesofauna affect the development and spreading of microorganisms (bacteria and fungi) (e.g. Wallwork 1983, Tiunov and Scheu 2005) and thereby indirectly influence the processes of decomposition of dead organic matter, thus affecting the circulation of nutrients (e.g. Seastedt 1984, Carrillo et al. 2011). The interdependencies between the decomposition processes resulting from the activity of microorganisms, mesofauna and vegetation are the basis for the functioning of ecosystems (e.g. Van der Putten et al. 2001, Eisenhauer and Schadler 2011).

The impact of a fire is manifold, being dependent on, among other things, its intensity, the time of year it occurs, and the incidence rate (Querner et al. 2010, Gongalsky et al. 2012). The fire itself is controlled by many factors, such as the type and moisture content of the fuel, temperature and humidity of the air, wind speed, and the topography of the terrain (Certini 2005). Like the effects of a fire, the restoration of ecosystems after a fire is also influenced by the intensity and frequency of fires, weather conditions, vegetation type, and the physicochemical and biological properties of the soil (e.g. Malmström et al. 2008, Malmström 2010).

It is believed that the restoration of communities of soil organisms takes 2-7 years (e.g Huta et al 1967, Malmström et al. 2008, Kim and Jung 2008, Saifutdinov et al. 2018), and even longer (Malmström 2012, Auclerc et al. 2019). Different groups of soil organisms respond differently to fire-induced disturbances and have different abilities to restore their communities (e.g. Lindberg and Bengtsson 2006, Malmström 2012).

Restoration of communities of soil organisms depends primarily on their chances of surviving a fire and their migration abilities, as well as on the presence of areas unaffected by fire (‘islands’, unburnt areas within the site of the fire, ‘corridors’) (e.g. Gongalsky et al. 2012). Also important are: the intensity of the fire, the time of year when it occurred, and weather conditions (e.g. Malmström et al. 2008).

However, despite many studies, the rate at which communities of soil organisms are restored is still not well known, especially in areas burnt out by anthropogenic fires, which, due to climate change and human activity, occur more and more often (Olejniczak et al. 2017, Górska et al. 2018).

The research on soil microorganisms and mesofauna was conducted in burnt-out areas resulting from anthropogenic fires of different intensity, in the Kampinos National Park in the third year after the fires (which occurred in May and June 2015) in a 200-year-old forest stand.

The aim of the research was to determine the degree of restoration of the abundance of microorganisms and mesofauna in the areas burnt out by fires of different intensity in a two-hundred-year-old pine stand. It had been assumed that: 1) the restoration of mesofauna communities was correlated with the restoration of microbial communities, 2) the restoration of communities of soil organisms can occurs similarly regardless of the intensity of the fire.

2. Study areas and methods

The study was carried out in the Kampinos National Park near Warsaw, in its north-eastern part, in the Kaliszki protection zone. The research sites included a 200-year-old pine stand (fresh coniferous forest habitat, *Peucedano-pinetum*) (Zaniewski and Otręba 2017) located on rusty soils (Brunic Arenosol) with fresh moder-mor humus (Biały et al. 2000, FAO 2015).

In August 2018, three years after fire of different intensity, nine test plots (10×10 m) were selected. The degree of burn-out of the organic layer was adopted as the criterion of fire intensity (Zaniewski and Otręba 2017). In the areas affected by the strong fire (**S**), the fire had tough impact and almost all the organic layer burnt and in the areas of the weak fire (**W**), the fire had only partially damaged the organic layer. The test plot in the unburnt areas was located 20 m away from the fire boundary.

The plots were designated on each burnt-out site: after a weak fire (**W**) and after a strong fire (**S**), and also in adjacent unburnt, control (**C**) areas.

The soil samples were collected in the organic layer (down to a depth of 5 cm) and mineral layer (from a depth between 5 cm and 25 cm). Soil samples were collected from six randomly selected points for each of the plot and a collective sample prepared for each of the layer.

Total organic carbon was measured using a Shimadzu TOC-V analyser with a solid-sample module (Shimadzu TOC 5000 A) by a non-dispersive infrared method. Nitrogen level was determined using the Kjeldahl method (analyser Kjeltec-Tecator). The soil pH in H_2O and in 1 m KCl was measured potentiometrically, while the soil moisture content was gravimetrically determined.

The physicochemical properties of the soils are given in Table 1.

Microbiological analysis

Microbiological samples were collected from the same each test plot into sterile containers, making sure aseptic conditions were maintained. The samples were taken from 6 randomly selected points of each plot, separately from the organic layer (0-5 cm) and the mineral horizon (5-25 cm) of the soil. The soil and litter were subjected to microbiological analyses to determine the total number of culturable heterotrophic soil bacteria on the Bunt and Rovira medium (Bunt and Rovira 1955) and of microscopic fungi on Martin's medium (Martin 1950) by bottom inoculation in agar. The abundance (number) of microorganisms was expressed in colony forming units (cfu) per kg dry litter or soil.

Table 1. Physical and chemical properties of the soils in the organic and mineral layers in the test areas: unburnt (control, **C**), burnt out by the weak fire (**W**), and burnt out by the strong fire (**S**)

Study area	Soil pH		$C\ g \cdot kg^{-1}$	$N\ g \cdot kg^{-1}$	C:N	Soil moisture content $g \cdot g^{-1}$
	pH_{KCL}	pH_{H2O}				
Soil organic layer						
Control (C)	3.11	3.68	419.2	16.38	26	38.35
Weak Fire (W)	3.09	3.76	326.2	14.39	23	30.14
Strong Fire (S)	3.18	3.91	296.4	13.21	22	34.53
Soil mineral layer						
Control (C)	3.31	3.61	24.11	1.13	21	7.19
Weak Fire (W)	3.26	3.57	38.15	1.85	21	11.70
Strong Fire (S)	3.63	3.83	22.02	0.94	24	5.14

Analysis of soil mesofauna

As in the case of microorganisms, 6 samples were taken from each test plot separately from the two layers, organic (0-5 cm) and mineral (5-10 cm), using a 10 cm^2 steel corer. A total of 108 samples were collected. The collected soil samples were used to extract mesofauna – mites (*Acari*) and springtails (*Collembola*), in a MacFadyen's apparatus, which were then preserved in 70% ethanol.

Mathematical analysis

The results of microbiological tests were verified by one-way analysis of variance; homogeneous groups were distinguished by the Tukey test for $\alpha = 0.05$ using the Statgraphics ver. plus 4.1 program.

The principal component analysis (PCA) was used to investigate the interdependencies between the examined traits and multi-trait variations among the objects studied.

3. Results

The abundance of bacteria and microscopic fungi in the soil and litter, three years after the fires, in the soil genetic horizons depended on the intensity of the fire (Tab. 2).

Table 2. Numbers of microorganisms and mesofauna in the test areas on the following sites: unburnt (control – C), burnt out by the weak fire (W), burnt out by the strong fire (F), in the soil organic layer (0-5 cm) and mineral layer (0-25 cm, in the case of mesofauna 5-10 cm), and the effect of fire intensity on the abundance of the studied edaphone groups in the tested soil layers (homogeneous groups were distinguished by the Tukey test for α = 0.05)

Study area	Heterotrophic bacteria cfu · 10^6 · kg^{-1} DW_s	Microscopic fungi cfu · 10^6 · kg^{-1} DW_s	Mites *Acari* N · 10^3 · m^{-2}	Springtails *Collembola* N · 10^3 · m^{-2}
Soil organic layer				
Unburnt (**C**)	2 000.0 b	1 514.0 a	13.1 a	3.2 a
Weak Fire (**W**)	1 120.0 a	1 593.0 a	8.2 a	4.2 a
Strong Fire (**S**)	1 350.0 a	1 480.0 a	10.0a	2.8 a
Soil mineral layer				
Unburnt (**C**)	127.0 a	117.0 a	3.0 a	0.2a
Weak Fire (**W**)	239.0 b	257.0 b	0.7a	0.1a
Strong Fire (**S**)	335.0 c	143.0 a	1.4a	0.3a

In the organic soil layer in the burnt-out areas, irrespective of fire intensity, lower bacterial abundance were still recorded three years after the fire, in comparison with the control (unburnt) areas. In contrast to the bacteria, the numbers of microscopic fungi did not differ significantly in the burnt-out and unburnt areas (Tab. 2). The soil mineral layer from the areas affected by the weak fire and strong fire in the old pine-tree stand was characterized by a significantly higher number of heterotrophic soil bacteria, compared with the control soil (Tab. 2). The abundance of fungi in the soil mineral horizon, three years after the fire, was the highest in the soil in the areas affected by the weak fire, compared with the soils in the other test areas (Tab. 2).

In the case of soil mesofauna, there was no significant effect of fire intensity on the abundance of invertebrates three years after the fire (Tab. 2). The numbers of springtails and mites were many times lower in the mineral layer than in the organic layer, regardless of the strength of the fire (Tab. 2).

The PCA analysis revealed that in the organic soil layer the number of fungi showed a negative relationship with soil acidity (Fig. 1). By comparison, the numbers of bacteria were strongly correlated with high soil moisture, C and N contents, and the C/N ratio (Fig. 1). Based on the PCA analysis, contrary to bacteria, the occurrence of fungi in the soil mineral layer was strongly positively correlated with the C and N contents, and with the highest moisture content of the humus layer (Fig. 2).

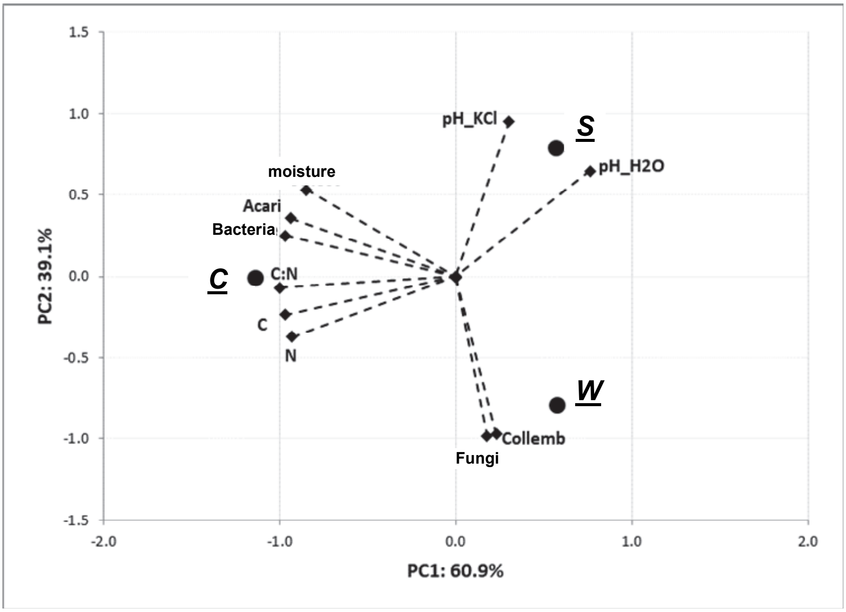

Fig. 1. Differences among the test areas in terms of the traits examined: soil properties (C and N contents, soil pH, moisture), abundance of microorganisms (bacteria and fungi) and of mesofauna (*Acari* and *Collembola*) in the soil organic layer, three years after the fire. **C** – control, **W** – weak fire, **S** – strong fire

In the organic layer, the numbers of springtails (*Collembola*) were strongly positively correlated with those of fungi and negatively correlated with soil pH, which was also the case with fungi (Fig. 1). However, the occurrence of mites (*Acari*) and bacteria was strongly positively interrelated, as well as being positively correlated with the level of moisture, C and N contents, and C : N ratio (Fig. 1). In the case of soil mineral layer, the occurrence of mites and springtails was strongly positively interrelated, as well as being positively correlated with soil acidity (pH) (Fig. 2).

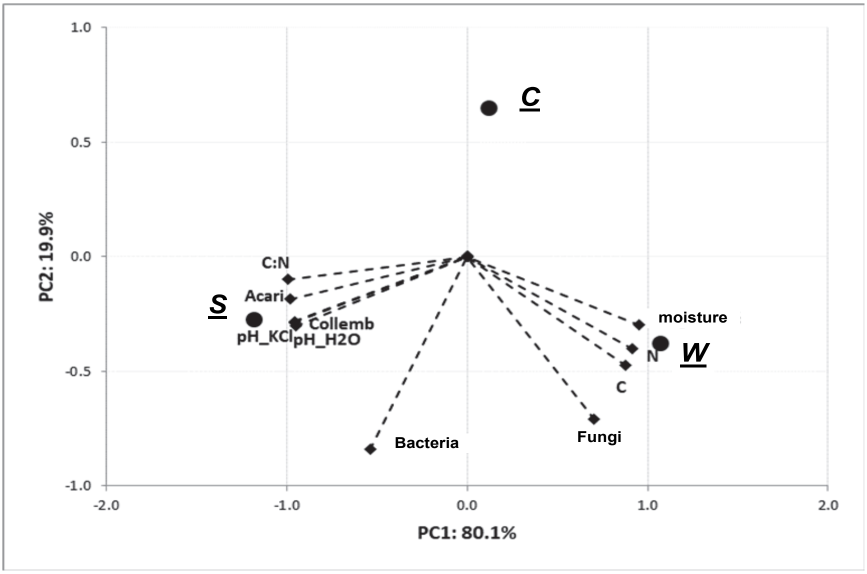

Fig. 2. Differences among the test areas in terms of the traits examined: soil properties (C and N contents, soil pH, moisture), abundance of microorganisms (bacteria and fungi) and of mesofauna (*Acari* and *Collembola*) in the soil mineral layer, three years after the fire. **C** – control, **W** – weak fire, **S** – strong fire

4. Discussion

The dependence of the abundance of bacteria and microscopic fungi in the soil and litter, three years after fire may be attributed to various factors, such as changes in soil chemical properties, as well as in the composition of the flora recolonizing the areas tested and of the various exudates discharged by their root system.

In the areas burnt out by the strong fire, three years after the fire, the mineral layer of the soil was found to contain a significantly greater number of bacteria relative to the areas affected by the weak fire, and which was almost twice as large as that for the unburnt (control) areas. Many factors may have contributed to this, including the amount and chemical composition of root secretions of the plants recolonizing the soil after the fire, as well as competition for an ecological niche of heterotrophic bacteria, which, in contrast to fungi, multiply much faster in a substrate with a lower moisture content. One of the abiotic factors that can limit the development of microorganisms in burnt forest stands is the availability of water. It was observed that the organic layer in the unburnt area had 25% more moisture than on the site where there had been a weak fire and 10% more in relation to the site of the strong fire. On the surface of the site of the strong fire, the fire had almost completely burnt the litter, and on the site of the weak fire, the fire had considerably damaged the organic layer. After the removal of (damage to) the insulating layer of moss, the soil in the burnt forest stand dries faster after a rainfall. Soil microorganisms in burnt-out areas experienced greater stress associated with lack of moisture. This claim is confirmed by studies in which significant decreases in soil moisture were recorded after forest fires (Harden et al. 2006, Holden et al. 2015).

The amount of organic carbon in the soil affected by fire can be higher or lower, than immediately after the fire, depending on the nature of the fire, soil type and moisture, as well the nature of the burned materials (González-Pérez et al. 2004). In the area of the strong fire, in the third year after fire, the contents of organic matter in the organic soil layer, were approximately 70% lower than in control plots and in areas with weak fire 77% than in the control organic soil layer.

The highest abundance of fungi in the soil mineral layer after the weak fire may have been influenced by the strongly acidic soil pH, as well as the high organic carbon content of the substrate, which create optimal conditions for the growth and development of microscopic fungi. The contents of organic carbon in that plot were over 50% higher than in control one. In addition, the amount of moisture in that soil layer was more than twice as high as that in the area of the strong fire and over 1/3 higher in relation to the control area. Our data suggest that one of the abiotic factors that can have an influence on increasing the abundance of fungi in burnt forest stands is the availability of water.

It is known that fires, especially very intense ones, have a significant impact on communities of soil microorganisms (Fioretto et al. 2005, Knelman et al. 2015). It has been shown that even a weak fire significantly affects microorganisms (Dooley and Treseder 2012). The rate of regeneration of microorganisms, apart from the environmental conditions of the soil, may also be determined by their ability to survive fires.

Wang et al. (2015) had shown that microorganisms were characterized by considerable resistance to high temperatures. Therefore, it can be assumed that the microorganisms on the burnt-out sites examined in this study may have survived the fires, especially the weak one. A no less important factor contributing to the restoration of microbial communities is their ability to move around. But even poor colonizers, such as microorganisms, are able to restore their communities relatively quickly on burnt-out sites (Jalaludin 1969). In summary, in the case of the presented study, it can be concluded that the communities of bacteria in the soil after the fires had been regenerated, thanks to endospores, cysts and conidial spores, among others, or could have been deposited from the regenerating flora and/or leaves brought in from other areas by air movements.

Rodriguez et al. (2018) found that the restoration of organic matter and bacterial and fungal populations in areas burnt out by a strong fire occurred 2-3 years after the fire. Prieto-Fernandez et al. (1998) had found that the regeneration of microbial communities lasted at least 4 years. Also in the study presented here, the communities of microorganisms, three years after the fires, were found to be at a highly advanced stage of regeneration.

The hight abundance of soil mesofauna in the soil organic layer is not unusual because springtails and mites inhabit mainly the top soil layers and forest litter (e.g. Bardgett and Cook 1998).

Rutigiano et al. (2013) had found that the abundance of mesofauna was affected by the availability of food. Although springtails are considered to be food generalist, their diet consists predominantly of fungi (e.g. Rusek 1989, Petersen 2002), which may be an explanation for the strong correlation with the fungi observed in the study, in soil organic layer Mites include various trophic groups. The moss mites (Oribatida), which were only recorded in the studied areas, similarly to the springtails can feed on fungi, but also on bacteria (Schneider et al. 2004). The eating-up of fungi by springtails and moss mites was undoubtedly conducive to the development of bacteria. So, the strong correlation between bacteria and mites, observed in the forest areas examined, may be the result of not only food availability but also competition. The results of the PCA indicate the possibility of competing springtails with mites for food that would be fungi. Abiotic factors such as soil pH, moisture and temperature affect the abundance of mesofauna (e.g. Hågvar 1984, Huhta and Häninen 2001). Hence the correlation between the abundance of mesofauna and moisture, or the degree of wetting and soil pH, observed in the tested forest areas. It is believed that the restoration of communities of soil organisms takes 2-7 years (e.g Huta et al. 1967, Malmström et al. 2008, Kim and Jung 2008, Saifutdinov et al. 2018). The similarity in the abundance of mesofauna on burnt-out sites (irrespective of fire intensity) and sites unaffected by fire may be evidence that three years after the fire those invertebrate communities had probably been restored (in terms of numbers). This is within the range of results obtained by other researchers. For example, Metz and Farrier (1971), investigating the impact of the incidence of fires on the restoration of mesofauna communities, had found that springtail and mite communities would become restored when fires occurred every 4 years. While, Malmstrom (2010) found that the rate at which the abundance of mesofauna became restored depended on the strength of the fire and the restoration could still continue 5 years after the fire. Saifutdinov et al. (2018) found that the restoration of springtail communities in boreal forests lasted 5-6 years after a surface fire of moderate intensity.

The restoration of springtail and mite communities on burnt-out sites undoubtedly depended on their chances of surviving the fire (e.g. individuals escaping into deeper layers of soil) (e.g. Gongalsky et al. 2012). Life strategies and the ability to spread around were obviously of vital importance in the restoration of the mesofauna (e.g. Petersen 1995). Springtails can recolonize burnt-out areas by actively migrating from nearby unaffected areas or unburnt parts of the fire site (e.g. Shaw 1997). These invertebrates have great migration abilities and can cover large distances in a short time (even more than a dozen centimetres in a week) (Hågvar 1995). However, what is also very important in the recolonization of fire-affected areas, and thus in the possibility of restoring communities of springtails and mites, the latter, unlike the former, having limited migration abilities, is passive spreading, i.e. by wind or water, or thanks to a phoretic relationship (e.g. Dighton et al. 1997, Ouarner et al. 2010). Wherein, the presence of unburnt areas, the so-called migration corridors is important in colonization the burnt areas by mesofauna, (e.g. Zeitsev et al. 2014).

In summary, three years after the fire, the communities of soil mesofauna are seen as becoming restored in the burnt-out areas, at least in terms of numbers. Ecosystem restoration research re-quires a long-term, interdisciplinary commitment and special attention based on an understanding of the environmental conditions and processes that shaped the evolution of species structure of the soil organism communities.

5. Conclusions

1. The communities of microorganisms, three years after the fires, were found to be at a highly advanced stage of regeneration.
2. The restoration of soil organisms in terms of numbers is to a large extent advanced, especially true for soil mesofauna.
3. The presented research confirmed the hypothesis that the restoration of microbial communities and the restoration of mesofauna are interrelated, which is also affected by the environmental conditions of the soils after a fire.
4. The hypothesis that the restoration of soil organisms occurs similarly regardless of the intensity of the fire was partially confirmed in the presented study. It was true for mesofauna and only for the soil microorganisms inhabiting soil organic layer. It seems that the reconstruction of microorganisms inhabiting the mineral soil layer proceeds with a fire strength gradient.

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Abstract

Fires, on account of their emotional, economic and cognitive aspects, are of interest to numerous researchers, and their impact is often unpredictable and difficult to investigate. They are among some of the most dynamic factors shaping terrestrial ecosystems. They destroy vegetation and alter the physicochemical and biological properties of the soil. Fires significantly influence the abundance and biodiversity of soil microorganisms and soil mesofauna, which are important elements of soils of every ecosystem, especially forest soils. Restoration of communities of soil organisms takes place at different rates and depends on, among other things, the intensity of the fire. The aim of the research was to determine the degree of restoration of the abundance of microorganisms and mesofauna in areas burnt out by anthropogenic fires of different intensity in an old pine forest. The research was conducted in a two-hundred-year-old pine stand (Peucedano-Pinetum), in the Kampinos National Park (near Warsaw, central Poland). In August 2018, three years after the fires, 3 test areas (10×10 m) were designated on each burnt-out site: after a weak fire (W) and after a strong fire (S), and also in adjacent unburnt (control, C) areas. In each test area, 6 samples were taken both from the organic layer (0-5 cm) and the mineral layer (5-25 cm – for microorganisms, and 5-10 cm for mesofauna) of the soil using standard methods for microorganisms and mesofauna. It was found that three years after the fires, the restoration of communities of soil organisms in terms of numbers was at an advanced stage (this was especially true for soil mesofauna). Based on the PCA analysis, it was found that the restoration of microbial communities and of the communities of mesofauna were interrelated, which was also influenced by the environmental conditions of the soils after the fires, in particular the physicochemical soil properties resulting from the intensity of the fire.

Keywords:
soil microorganisms, soil mesofauna, fire, Brunic Arenosol, forest soil

Wybrane właściwości biologiczne gleby na wypalonym obszarze pod starodrzewami sosny trzy lata po pożarze

Streszczenie

Pożary, ze względu na emocjonalne, ekonomiczne i poznawcze aspekty, są przedmiotem zainteresowań licznych badaczy, a ich wpływ często jest nieprzewidywalny i trudny do zbadania. Należą do jednych z najbardziej dynamicznych czynników kształtujących ekosystemy lądowe. Niszczą roślinność, zmieniają fizykochemiczne i biologiczne właściwości gleby. Pożary w istotny sposób kształtują liczebność i bioróżnorodność mikroorganizmów glebowych i mezofauny glebowej, będących istotnymi elementami gleb każdego ekosystemu, zwłaszcza gleb leśnych. Odbudowa zespołów organizmów glebowych zachodzi w różnym tempie i zależy między innymi od siły pożaru. Celem badań było ustalenie stopnia odbudowy liczebności mikroorganizmów i mezofauny w obszarach wypalonych po pożarach antropogenicznych o różnej sile w starodrzewie sosnowym. Badania prowadzono w dwustuletnim drzewostanie sosnowym (Peucedano-Pinetum), w Kampinoskim Parku Narodowym (koło Warszawy, centralna Polska). W sierpniu 2018, trzy lata po pożarach wyznaczono po 3 powierzchnie badawcze (10x10 m) na pożarzyskach: po słabym pożarze (W) i mocnym pożarze (S) oraz przyległych obszarach niewypalonych (kontrolnych, C). Na każdej powierzchni badawczej pobierano po 6 prób w warstwie organicznej (0-5 cm) i mineralnej gleby (5-25 cm – w przypadku mikroorganizmów i 5-10 cm w przypadku mezofauny) stosując standardowe metody dla mikroorganizmów i mezofauny. Stwierdzono, że po trzech latach po pożarze odbudowa zespołów organizmów glebowych pod względem liczebności jest w dużym stopniu zaawansowana (dotyczy to zwłaszcza mezofauny glebowej). Na podstawie analizy PCA stwierdzono, że odbudowa zespołów mezofauny i mikroorganizmów są powiązane ze sobą, na co mają wpływ także warunki środowiskowe gleb po pożarze, zwłaszcza właściwości fizykochemiczne gleby, wynikające z siły pożaru.

Słowa kluczowe:
mikroorganizmy glebowe, mezofauna glebowa, pożar, Brunic Arenosol, gleba leśna