FORESTRY IDEAS, 2015, vol. 21, No 1 (49): 55–66

ENZYME ACTIVITY OF FOREST FIRE-INFLUENCED SOILS FROM NORTH SLOPES OF RILA MOUNTAIN (REGION OF DOLNA BANIA)

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Received: 07 July 2014 Accepted: 20 April 2014

Abstract

Enzyme activity 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 (region of Dolna Bania) have been analysed in samples, collected 7 days after wildfire occurrence. Results on catalase, cellulase and protease enzyme activities in fire-affected soils show a considerable increase in comparison with the same enzyme activities of the control (fire-unaffected) site. The activities of these three enzymes can be used as indicators of the on-going biological processes in post fire-affected soils. The values of catalase and cellulase were lower in the forest litter, and higher in the subsequent layers of soil. Protease activity increases in depth in three of analyzed fire-influenced soils. In the control soil the activity of this enzyme remains constant in depth of the soil profile. Higher pH values and lower soil humidity were established in fire-affected soils compared to the control soil. A higher content of organic carbon and nitrogen in the soil of surface fire as compared to those with crown fire and control soil was established.

Key words: fire-influenced forest soils, catalase activity, cellulase activity, protease activity.

Introduction

The fires are a major cause of forests degradation. They cause transformation of habitats, which affects their natural biological functions. The impact of fire on soil microorganisms is direct through heating and/or indirect by modifying the properties of the soil.

The most important factor that determines the number, composition and activity of microorganisms in soil after a fire is the severity of burning, which is determined by its intensity and duration. The adverse effects of fire on the soil depend on its intensity and the properties of impacted soil. The combustion of organic matter leads to an increase in the content of mineral compounds in the soil. According to the studies of Bogdanov (2008) magnesium and calcium are the elements, which amounts are changed most significantly. The content of the total nitrogen is an important indicator of forest recovery processes after fire. Changes are found in the particle size distribution of fire-influenced soils, which are mainly in the sand/silt ratio. The clay fraction is changed to a relatively less extent (Bogdanov 2012). The changes which occur in composition, physico-chemical properties and structure of the soil after fire usually lead to a decrease in the number of specific microbial groups (Wells et al. 1979). The activity of soil microbiota also decreases due the changes in content and composition of soil organic matter. In a short term, the number of heterotrophic bacteria and their activity increases, mainly due to an increase in dissolved carbon content and availability of nutrients in the fire-influenced soils. After depletion of easily mineralized organic compounds, the initial increase of microbes count is reduced and the remaining carbon and nitrogen forms become more resistant to microbial degradation (Mataix-Solera 2009).

Studies of Hernández et al. (1997) show that the activity of the enzymes dehydrogenase, urease, protease, arylsulfatase, phosphatase, β-glucosidase are inhibited in soils for 9 months after the fire is extinguished. In such soils they established a high content of nitrate, ammonium ions, digestible forms of phosphorus and potassium, whereas the organic carbon content was lower than that in unaffected soils. A reduction of nitrogen mineralization and nitrification for the first year after the fire and increase during the third year in fire-influenced soils compared to the control were registered by Miesel et al. (2011). They documented a consistent reduction in the activities of acidic phosphatase, chitinase and phenoloxidase. The reduction of chitinase activity reveals a decrease in the bacterial abundance, reduction of the importance of chitin as a source of C and N or an increase in the availability of more labile forms of organic matter (Hanzlikova and Jandera 1993). The reduction in enzyme activities after a fire can be a consequence of a high intensive fire (Fioretto et al. 2005), increase in soil pH (Acosta-Martínez and Tabatabai 2000) and reduction of soil microbial biomass (Kandeler and Eder 1993). Reduction of nitrogen mineralization in soils after fire is also noted by other authors (Monleon et al. 1997, DeLuca and Zouhar 2000, Durán et al. 2009). Phosphatases (acidic and alkaline) can clearly demonstrate the extent of fire impact on the soil, even one year after it (Pourezza et al. 2014). Staddon et al. (1998) found that the acidic phosphatase activity remains suppressed four years after the fire. The above mentioned and other studies demonstrate that soil biochemical properties, and particularly soil enzymatic activity are sensitive to stress on ecosystems and have the potential to serve as an indicator of the sustainability of managed ecosystems (Bergstrom et al. 1998, Dick 1994). Thus, the activity of soil enzymes can be used as a bio-indicator of ongoing biological processes in fire-influenced soils and the recovery process occurring in them.

The aim of this study was to investigate the soil protease (actual), cellulase and catalase (potential) activities in order to identify the most powerful enzyme activity as an indicator of ongoing biological processes in burned soils.

Materials and Methods

Representative sampling sites (RSS) have been chosen within the coniferous forests on the territory of Dolna Bania Government Forestry Enterprises (GFE). They were situated on the north slopes of Rila Mountain, affected by a forest fire in August 2012. The soil samples were collected 7 days after wildfire occurrence in tree replications at each RSS and at both depths – 0–5 and 5–20 cm. The altitude of the studied area is 650 m a.s.l. The plantations studied are twenty-five years old. Their main characteristics are presented in Table 1.

The soil from the studied region is Haplic Luvisols, LVh (WRBSR 2006). The control sampling site was established at a 20 m distance from the fire-affected ones. The samples were taken with a sterile instrument from 0–5 cm and 5–20 cm depths of the soil profile, as well as forest litter from fire-affected sampling sites, in sterile paper bags. Four more soil samples from the surface mineral soil have been collected randomly at each RSS in order to ensure homogenous and representative samples for analyses. Sampling and sample preparation processing have been performed following the contemporary methodological approach, described in details in ICP Manual (Cools and De Vos 2010). The samples were transported and analyzed within 48 h. Prior the incubation, they were stored in a refrigerator at 4–10 °C.

The enzymatic activities of fire-affected soils were studied through measuring the activity of three enzymes – cellulase, catalase (potential activity) and protease (actual activity).

The catalase activity was determined in triplicate by manganese-metric method (Khaziev 1976).

To determine the cellulose-degradation activity the laboratory method of Khaziev (1976) was used. The three sterilised filter paper strips were applied to the soil samples in Petri dishes. The incubation was performed at 60 % humidity of soil. Every 15th day the percentage of the degraded paper area was accounted.

The actual protease activity was investigated through setting-up of three

Table 1. Studied areas parameters.

RSS, No	Studied cases	Dominant tree species	Area, ha	Geographical positions, latitude and longitude
1	Crown fire	*Pinus sylvestris* L.	0.2	42°19’23.78” N 23°46’37.09” E
2	Crown fire	*Larix desidua* Mill.	9.3	42°19’23.77” N 23°46’37.10” E
3	Surface fire	*Pinus sylvestris* L.	4.4	42°19’23.75” N 23°46’37.11” E
4	Surface fire	*Pinus sylvestris* L. *Quercus cerris* L.	0.9	42°19’23.58” N 23°46’36.89” E
5	Unburned	*Pinus sylvestris* L.	0.6	42°19’25.25” N 23°46’36.40” E

Table 2. Chemical characteristics of studied soils.

RSS, No	Soil depth, cm/forest litter	Soil moisture content* (W, %) Mean	pH (H_2O) Mean ± SD	C, % Mean ± SD	N, % Mean ± SD
1	FL	4.8	7.44±0.22	41.55±2.15	0.4485±0.10
	0–5	8.7	6.06±0.13	1.656±0.25	0.0417±0.02
	5–20	10.7	5.53±0.11	1.380±0.19	0.0313±0.01
2	FL	4.1	8.01±0.16	30.60±2.17	0.3691±0.08
	0–5	8.4	6.42±0.10	1.932±0.19	0.0849±0.02
	5–20	8.5	6.12±0.08	1.242±0.08	0.0700±0.02
3	FL	3.8	7.28±0.20	54.45±3.14	0.6578±0.11
	0–5	8.6	5.81±0.09	2.346±0.21	0.0954±0.03
	5–20	7.7	5.40±0.08	1.794±0.09	0.0819±0.02
4	FL	7.1	6.80±0.10	40.65±3.55	0.2915±0.06
	0–5	9.3	6.12±0.11	3.450±0.13	0.1298±0.04
	5–20	9.3	5.85±0.07	2.208±0.11	0.0981±0.02
5	FL–L	6.14	5.06±0.08	59.70±3.45	1.5647±0.06
	FL–F	7.67	5.08±0.06	45.90±2.21	0.6877±0.03
	FL–H	9.4	5.37±0.07	22.20±2.03	0.1495±0.03
	0–5	16.1	5.47±0.10	2.070±0.11	0.0932±0.02
	5–20	23.13	5.26±0.11	1.104±0.06	0.0521± 0.02

undeveloped film reel strips with size 5×3.5 cm in each soil layer from RSS and subsequent extraction and recognition the percent of decomposed area (30 days after the setting) (Khaziev 1976). Main soil characteristics are given in Table 2.

Statistical data processing includes calculation of the mean values of triplicated analyses for each parameter and standard deviation (± SD), bivariate correlation (Pearson) to analyse correlation among soil enzyme activities and soil chemical properties. Statistica 7 program for Windows and Excel 2010 were used for calculations.

Results and Discussion

Soil physicochemical properties differed between fire affected and control RSS (Table 2). Soil moisture content (W, %) was lower in forest litter layer from fire affected variants. Compared to the control sites, pH after forest fire was increased in all experimental variants especially in that, affected by crown fire. Soil organic carbon content was lower in soil affected by crown fire at 0–5 cm, compared to the unburned control, while the surface fire, provoked an increase in soil organic carbon amount.

Changes in enzyme activities between fire affected and unburned variants are

Table 3. Catalase activity of soil microorganisms.

RSS, No	Variants	Forest litter layer (*FL*)/Soil depth, cm	Catalase, mL $O_2 \cdot 30\ min^{-1}$ Mean ± SD
1	Crown fire	Forest litter	0.47±0.06
		0–5	1.85±0.09
		5–20	1.87±0.06
2	Crown fire	Forest litter	1.10±0.10
		0–5	1.87±0.06
		5–20	1.97±0.06
3	Surface fire	Forest litter	0.73±0.03
		0–5	1.73±0.06
		5–20	1.62±0.06
4	Surface fire	Forest litter	1.33±0.03
		0–5	1.73±0.08
		5–20	1.63±0.06
5	Control sampling site	*L* – layer of forest litter (*FL* – *L*)	1.63±0.08
		F – fermentative layer of forest litter (*FL* – *F*)	0.63±0.03
		H – humified layer of forest litter (*FL* – *H*)	0.98±0.03
		0–5	1.65±0.05
		5–20	1.82±0.03

presented in Table 3, 4 and Fig. 1. The correlation matrices of the physicochemical indexes of forest litter/soil and catalase and protease enzymes activities is presented in Table 5. The activity of cellulase was assayed in relation to time course changes.

Catalase enzyme activity plays an important role in soil solution chemistry and can change oxidation-reduction reactions in soil (Wang et al. 2012). Results show that low catalase activity was observed in the litter of the fire-influenced coniferous forest (RSS 1, 2 and 3) and in *F* and *H* layers of the control site (Table 3). The activity of litter enzyme was higher for mixed forests (RSS4) and lowest for that of the Scots pine (RSS1). For control site forest litter the catalase activity had its highest value in *L* layer, followed by that in humified *H* layer and was lowest in the fermentation *F* layer.

The metabolism of microbes, including synthesis of enzymes is activated mainly by external factors, one of which is the forest fire. Higher catalase activities with approximately equal values were found in the

Table 4. Protease activity of soil microorganisms.

RSS, No	Soil depth, cm	Protease, % degraded area 30th day Mean ± SD
1	0–5	2.87±0.06
	5–20	1.42±0.03
2	0–5	8.57±0.08
	5–20	25.67±0.06
3	0–5	7.10±0.10
	5–20	42.85±0.09
4	0–5	8.57±0.06
	5–20	11.67±0.06
5	0–5	1.41±0.05
	5–20	1.44±0.05

fire-influenced soils compared to those in the control one. The catalase activity and physicochemical properties of forest litter varied considerably within studied variants. However, positive correlation between catalase activity and moisture content of the forest litter was found (Table 5). The lowest moisture in forest litter from fire affected sites is probably due to greater losses of water through transpiration from disturbed stands (Gömöryová et al. 2008). The enzyme activity of the mineral soil from RSS, affected by surface fire (No 3 and 4) and that in the soil from control sampling site (No 5) is lower in comparison with that influenced by crown fire. The established values for surface 0–5 cm soil layer of the RSS3, RSS4 and RSS5 were equal to 1.7 ml $O_2 \cdot 30$ min^{-1}. The similar values 1.6 ml $O_2 \cdot 30$ min^{-1} were found for catalase activity in soil from 5–20 cm layer of RSS3 and RSS4. This result is consistent with those of Eivazi and Bayan (1996) who studied the effects of long-term prescribed burning on enzyme activities in a forest ecosystem and found that burning greatly reduced enzyme activities, mainly due to the destruction of forest floor vegetation.

The catalase activity in soil influenced by crown fire (RSS1 and RSS2) is higher 1.9–2.0. In both burned and unburned forests, the distribution trend of enzymes activities of spruce (*Picea balfouriana* var. *rubescens* Rehder & E.H.Wilson) forest was similar (Yong-Mei et al. 2005). The increased activity of catalase in the top 20 cm soil of the crown fire affected forests may be due to the changed forms of organic compounds caused by burning. In our study, the catalase activity showed rather strong positive correlation to soil pH, whereas it correlates negatively with soil moisture, organic matter and total nitrogen content (Table 5). Gömöryová (2004) found that catalase activity exhibits highly significant correlations (P > 0.999) with soil reaction. The negative correlation of catalase with the soil moisture

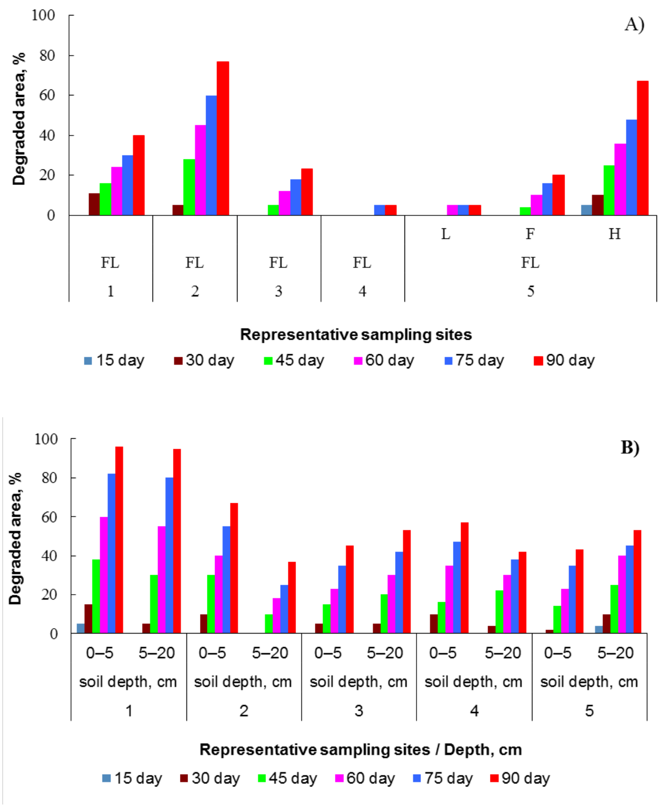

Fig. 1. Cellulase activity of soil microorganisms: A) in forest litter; B) in mineral soil layer 0–5 cm and 5–20 cm.

content (W, %) in 0–5 cm of mineral soil could be interpreted in terms of differences in soil oxidation status (Gömöryová et al. 2008). High significant negative correlations occurred between catalase and organic matter of soil. Because of increased soil enzyme activities in surface soil horizons, the transformation of organic matter was faster than that in deeper horizons and the rapid release of inorganic nutrients in soil during this process could be the reason for negative correlation.

The slight catalase increase in depth of the soil under European larch (RSS2), and in the 5–20 cm soil layer of the control site (Scots pine – RSS5) could be probably in result of its presence of vegetation origin. The negative correlation of catalase activity with organic matter and soil nitrogen content was kept within 20 cm soil depth.

Cellulase is an enzyme that breaks down cellulose. Through its activity in the soil, the direction and the rate of mineralization processes could be characterized. It was found that the activity of cellulase is increased after day 30

Table 5. Correlation coefficients (*R*-value) between enzyme activities and chemical properties of forest litter and mineral soil at 0–5 cm and 5–20 cm depth (significance at *P* < 0.05 level).

Variable	W, %	pH (H_2O)	C, %	N, %	Enzymes
Forest litter					
W, %	1.00	-0.82	0.34	0.71	0.66
pH		1.00	-0.78	-0.88	-0.3
C, %			1.00	0.82	-0.14
N, %				1.00	0.05
Catalase					1.00
Soil depth 0–5 cm					
W, %	1.00	-0.35	0.98	0.86	-0.89
pH		1.00	-0.14	-0.02	0.69*
C,%			1.00	0.92	-0.79*
N, %				1.00	-0.73*
Catalase					1.00
Soil depth 5–20 cm					
W, %	1.00	-0.53	-0.39	-0.16	0.02
pH		1.00	0.19	0.39	0.35
C, %			1.00	0.77	-0.82*
N, %				1.00	-0.64*
Catalase					1.00
Soil depth 0–5 cm					
W, %	1.00	-0.35	0.98	0.86	0.48
pH		1.00	-0.14	-0.02	0.33
C, %			1.00	0.92	0.62*
N, %				1.00	0.86*
Protease					1.00
Soil depth 5–20 cm					
W, %	1.00	-0.53	-0.39	-0.16	-0.50
pH		1.00	0.19	0.39	0.18
C, %			1.00	0.77	0.30
N, %				1.00	0.56
Protease					1.00

Note: *correlation is significant $p < 0.05$.

of fire occurrence in the soil from all fire-affected sites. In the forest litter of the control site, cellulase activity increases gradually from *L* through *F* and reaches maximum values in *H* layer, which is determined by higher mineralization rate in the humified layer (Fig. 1).

In the sites affected by the fire, the enzyme activity of forest litter is lower than that in the soil depths, except European larch (RSS2). In general, cellulose activity is lower in recently burned soils than in non-burned ones (Fioretto et al. 2002) due to the high heat sensitivity of fungi which are the main cellulose degradators in soil. Moreover, cellulose is one of the most heat-affected organic fractions in soil during forest fire occurrence (Fernández et al. 2001).

The highest cellulase activity was observed in both mineral soil and litter of RSS1 site at 90th day of observation, whereas the lowest one was recorded for the surface (0–5 cm) and deeper (5–20 cm) soil layers of the control (RSS5) and RSS3, respectively. All these are covered with Scots pine forest. An increased cellulase activity, especially in the surface soil layer, was observed in the soils affected by the fire in comparison to that of the control.

The crown fire affects more significantly the cellulase activity in comparison with a surface fire, probably, as a result of organic matter and ash accumulation after fire. Higher levels of cellulase activity were found in variants of crown fire in comparison to those in variants of surface fire. Wang et al. (2012) showed that the increase in organic matter accumulation improved cellulase activity, thus increasing the decomposition rate of cellulose in plant litter.

The cellulase activity decreases in depth of the fire-influenced soils (except RSS3) in a contrast to the control soil (RSS5), where the opposite trend is recorded. Cellulose activity in RSS5 is relatively lower as that in RSS3.

The protease activity of the soil microorganisms is much higher in the fire-influenced soils in comparison with that of the control soil (Table 4).

The proteolytic enzymes in soil catalyze the hydrolysis of proteins to peptides and amino acids, thus affecting soil N cycling. The protease activity of fire-affected surface soil layer (0–5 m) was suppressed in a comparison to the activity of the enzyme in the deeper soil layer (except RSS1). The highest protease activity in the 5–20 cm soil layer was observed in RSS3 site (Scots pine), and the lowest one – in RSS1 and RSS5.

In the surface soil layer, the highest protease activity was found in both – RSS2 (crown fire) with European larch and RSS4 (surface fire) with a mixed forest of Scots pine and Turkey oak. The lowest protease activity was observed in the soil of RSS1 (crown fire) and in the control soil (RSS5), both covered with Scots pine.

The reason for this trend may be indirect effect of fire (especially of the crown one) on the enzymes' activities from the surface soil layers, enhancing migration and accumulation of proteins (highly mobile) along soil profile to the deeper soil layers. Soil proteins are more mobile in soil than other carbon sources (Buyer et al. 2001).

In our opinion, there is a relation of the variation of enzyme activity to soil moisture, pH, organic carbon and nitrogen contents – the most important soil characteristics controlling survival and growth of many microbes (Table 5). In our study, catalase activity showed a strong positive correlation to soil pH, whereas the correlation with soil moisture, organic matter and nitrogen content were negative. Neutral-alkaline pH optima have been reported for soil proteases (Kamimura and Hayano 2000). In many studies, the strong dependence of catalase activity on organic C and pH was observed (Müllebner 1984, Gömöryová 2004, Shi et al. 2008). The increase in soil catalase activity affects soil solution chemistry trough changes of soil redox conditions. This change of soil solution has the potential to dissociate bonds within complexes of organic matter and therefore alter soil carbon storage. Hence, the high value of catalase activity in mineral soil indicated an accelerating transformation of organic matter.

High positive correlation of the protease enzyme activity with nitrogen and carbon content in the surface 5 cm of the mineral soil layer indicated the activation of soil microorganisms by addition organic materials in result of burning (Dick 1994, Nannipieri et al. 1983). Organic residues added to soil promote microbial and soil enzyme activities (Masciandaro et al. 1997).

The processes of soil organic matter decomposition/mineralization result in the release of NH_4^+ and CO_2 as end-products. Factors governing organic matter decomposition also influence on N mineralization. Among them, soil moisture and pH are here mentioned as important factors affecting soil organic matter decomposition, and therefore also for N mineralization.

Among the processes of soil organic matter decomposition/mineralization resulting in the release of NH_4^+ and CO_2 as end-products are soil moisture and pH. Our results show low values of correlation coefficients for protease activity and soil moisture and pH, which suppose stable enzyme activity at soil pH range from 5.06 to 6.42 and soil moisture from 6.1 % to 7.7 %. In dry soil, enzymes may have become adsorbed to clays and tannins, rendering them less susceptible to proteolytic breakdown but able to maintain reactivity (Ensminger and Gieseking 1942, Kandeler 1990).

Conclusions

The activities of catalase, cellulase and protease are higher in the fire-influenced soils compared to the control ones, which indicate the high sensitivity of microorganisms to fire. Higher temperature reduces enzymes' activities compare with that in the mineral deeper soil layers.

Catalase activity was highest in *L* layer of the forest litter from the control sampling site in comparison to the other two layers of forest litter, while cellulase activity reached maximum in both studied mineral soil layers of soil from RSS 1. There was no changes in catalase activity along soil profile of control sampling site. The protease activity depends on fire intensity and the lowest in the topsoil layer, which is the one most affected by the fire. Along the soil depth of fire affected soils the protease activity increases in the soil of the control sampling site. The activity of this enzyme remains low and constant in depth of the soil profile.

There is no clear relationship between the enzyme activities and the type of vegetation. The crown fire provokes the higher catalase and cellulase activity in comparison to those, influenced by surface fire. This trend was not observed for the protease activity.

The catalase activity showed a strong positive correlation to soil pH, whereas the correlation with soil moisture, organic matter and nitrogen content were negative. High positive correlation of protease activity was found with nitrogen and carbon content in the surface 5 cm of the mineral soil layer indicated.

The activity of the investigated enzymes can be used as a bioindicator of ongoing biological processes in fire-influenced soils and the recovery processes occurring therein.

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