MICROBIAL ACTIVITY IN FOREST SOIL UNDER BEECH, SPRUCE, DOUGLAS FIR AND FIR

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Summary: *The aim of this research was to investigate the microbial activity in forest soil from different sites under deciduous and coniferous trees in Serbia. One site on Stara planina was under beech trees (Fagus sp.) while another under mixture of spruce (Picea sp.) and Douglas fir (Pseudotsuga sp.). The site on Kopaonik was under mixture of beech (Fagus sp.) and spruce (Picea sp.) trees. The site on Tara was dominantly under fir (Abies sp.), beech (Fagus sp.) and spruce (Picea sp.). The total number of bacteria, the number of actinobacteria, fungi and microorganisms involved in N and C cycles were determined using standard method of agar plates. The activities of dehydrogenase and ß-glucosidase enzymes were measured by spectrophotometric methods. The microbial activity was affected by tree species and sampling time. The highest dehydrogenase activity, total number of bacteria, number of actinobacteria, aminoheterotrophs, amylolytic and cellulolytic microorganisms were determined in soil under beech trees. The highest total number of fungi and number of pectinolytic microorganisms were determined in soil under spruce and Douglas fir trees. The correlation analyses proved the existence of statistically significant interdependency among investigated parameters.*

Key words: microorganisms, forest soil, dehydrogenase, ß-glucosidase activity.

INTRODUCTION

Soil chemical and microbiological characteristics are directly as well as indirectly affected by the dominant tree species (Priha et al., 2001). Forest soils are known for its constant and high inputs of plant materials, therefore those soils are rich in organic carbon (C) compounds as well as in microbial biomass. Plant litter C together with the C from plant roots represents the dominant sources of C in temperate forest soils. Microbial decomposition of soil organic matter (SOM) represents a key driver of the forest's C and N cycle. In temperate forest ecosystems a seasonal pattern of microbial processes is related to a seasonal variation in availability of different substrates, as well as variation in soil temperature and moisture (Kaiser et al., 2011). Many studies have focused on the quantification of microbial biomass and enzyme activities in forest soils (Šnajdr et al., 2008). However, studies of forest soils microbial activities in Serbia are in its infancy, since quantitative and qualitative determination of microbial populations and enzymatic activity starts in the last decade.

The aim of this work was to quantify the microbiological activity in the soil under beech (*Fagus* sp.), spruce (*Picea* sp.), Douglas-fir (*Pseudotsuga* sp.) and fir (*Abies* sp.).

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MATERIAL AND METHODS

One site on Stara planina (SP1) was under beech trees (*Fagus* sp.) while another (SP2) under mixture of spruce (*Picea* sp.) and Douglas fir (*Pseudotsuga* sp.). The site on Kopaonik (K) was under mixture of beech (*Fagus* sp.) and spruce (*Picea* sp.) trees. The site on Tara (T) was dominantly under fir (*Abies* sp.), beech (*Fagus* sp.) and spruce (*Picea* sp.). The soil chemical and physical characteristics are presented in Table 1. At all sites, the composition of the plant communities is considered to be natural, which means that it has not been changed by human interference.

Table 1. Soil chemical and physical characteristics

Site	Depth (cm)	$CaCO_3$ (%)	Humus (%)	pH in H_2O	Nitrogen (%)	P_2O_5 (mg/kg)	K_2O (mg/kg)	Total sand (%)	Total clay (%)	Texture
SP1	0-30	0,42	4,00	6,41	0,25	9,5	7,8	39,80	60,20	Clay loam
SP2	0-30	0,00	3,60	6,15	0,10	8,0	6,4	66,00	34,00	Sandy clay
K	0-30	0,00	3,00	4,83	0,15	6,5	5,4	77,00	23,00	Sandy clay
T	0-30	2,10	10,17	4,76	0,39	6,9	5,5	72,00	28,00	Sandy clay

Soil samples were taken from the depth of 0-30 cm, after the removal of plant litter performed in June and August in 2012. At each site, four replicate bulk samples were taken, consisting of 10 randomly collected sub-samples. Soil samples were places in sterile polyurethane bags and kept on temperature of 4^0C. After transportation to the laboratory, the samples were sieved through a 2 mm sieve and stored until used.

Microbiological measurements included the determination of total number of bacteria, actinobacteria, fungi, aminoheterotrophs, amylolytic, cellulolytic and pectinolytic microorganisms. Activity of enzymes dehydrogenase and β-glucosidase were also measured. The number of microorganisms were determined by standard agar plate method (Trolldenier, 1996) preparing a ten-fold dilution series, and applying the appropriate dilutions on nutrient plates. After incubation, plates were inspected. The number of microorganisms was calculated to one gram absolutely dry soil and presented as CFU/g soil. Dehydrogenase activity of soil was determined spectrophotometrically (Lenhard, 1956; Thalmann, 1968). The activity of enzyme β-glucosidase was measured by method of Hayano, (1973).

Statistical data analyses were performed using STATISTICA 12 software (Hamburg, Germany). Data were treated with two-way analysis of variance. Means were compared using Fisher test (p <0.05). Correlation analysis was performed to determine whether there were any linear relationships among treatments.

RESULTS

The number of the investigated groups of microorganisms and enzymatic activity of forest soils were influenced by tree species and soil sampling time. The results of this study are shown in Table 2.

Table 2. Microbiological activity in forest soil

	(*Fagus* sp.)	(*Picea* sp. + *Pseudotsuga* sp.)	(*Fagus* sp. + *Picea* sp.)	(*Abies* sp.+ *Fagus* sp. + *Picea* sp.)
TNB (10^5 CFU/g soil)				
I sampling	92,47[a]	25,50[d]	20,94[d]	16,70[d]
II sampling	71,88[ab]	39,25[cd]	61,57[bc]	42,72[abc]
ACT (10^4 CFU /g soil)				
I sampling	14,30[a]	1,58[b]	0,94[b]	0[b]
II sampling	0,56[b]	0,63[b]	0,42[b]	0,17[b]
FNG (10^3 CFU /g soil)				
I sampling	14,95[b]	31,75[a]	0,91[e]	6,63[cd]
II sampling	8,76[c]	19,56[b]	1,67[de]	0,78[e]

AMH (10^5 CFU /g soil)				
I sampling	30,40^b	10,32^c	5,52^c	4,82^c
II sampling	74,53^a	9,60^c	3,11^c	1,40^c
AML(10^5 CFU /g soil)				
I sampling	20,76^a	8,74^b	1,02^{cd}	3,65^{bcd}
II sampling	6,29^{bc}	1,88^{cd}	0,75^{cd}	0,15^d
CEL(10^4 CFU /g soil)				
I sampling	29,91^a	20,63^{ab}	33,14^a	1,82^c
II sampling	9,19^b	10,17^{bc}	2,51^c	2,34^c
PEC(10^2 CFU /g soil)				
I sampling	168,08^a	165,19^a	11,07^b	18,07^b
II sampling	11,39^b	49,38^b	1,67^b	3,90^b
DHA (μg TPF/10g soil)				
I sampling	676,2^b	196,2^c	240,0^c	92,4^c
II sampling	1115,8^a	149,5^c	172,4^c	30,5^c
GLU (μmol p-NP/5g soil)				
I sampling	48,07^a	34,33^b	49,10^a	49,27^a
II sampling	22,80^b	22,33^b	26,37^b	30,07^b

Note: same letter in superscript indicates no statistically significant difference between mean values on the basis of LSD test (p< 0.05); TNB-total number of bacteria, ACT-actinobacteria, FNG-fungi, AMH-aminoheterotrophs, AML-amylolytic mo, CEL-celullolytic mo, PEC-pectinolytic mo, DHA-dehydrogenases, GLU-β-glucosidases

Microbial abundance was more influenced by tree species. The highest total number of bacteria, actinobacteria, bacteria from C and N cycle were determined in soil under beech. The dehydrogenase activity was also much higher under beech stands. Sites under dominantly coniferous trees were characterized by decreased microbial number in soil but increased enzymatic activity. The dehydrogenase activity in soil under fir, beech and spruce was more than ten times less in comparison with its activity on site under beech trees. However, β-glucosidase activity was higher under spruce and fir, beech, spruce, respectively. Some general observations were made concerning the relations between investigated parameters of soil microbial activities. Correlation analysis proved the existence of statistically significant interdependency among treatments (Table 3).

Table 3. Spearman Rank Order Correlations

	TNB	ACT	FNG	AMH	AML	CEL	PEC	DHA
ACT	*0,433**							
FNG	*0,416**	*0,710**						
AMH	*0,482**	*0,567**	*0,444**					
AML	0,078	*0,615**	*0,686**	0,401				
CEL	0,085	*0,560**	*0,480**	*0,475**	0,361			
PEC	0,086	*0,594**	*0,542*	*0,541**	*0,858**	*0,542**		
DHA	0,396	*0,825**	*0,560*	*0,493**	0,307	*0,639**	0,336	
GLU	-0,148	-0,006	0,179	0,031	-0,089	*0,455**	0,161	0,072

Note: * in superscript: correlations are statistically significant at p <,05000;
TNB-total number of bacteria, ACT-actinobacteria, FNG-fungi, AMH-aminoheterotrophs, AML-amylolytic mo, CEL-cellulolytic mo, PEC-pectinolytic mo, DHA-dehydrogenase activity, GLU-β glucosidase activity

DISCUSSION

Nutrient cycles and ecosystem functioning is heavily influenced by seasonal changes of soil microbial biomass (Lipson and Schmidt, 2004). This research showed that the number of most investigated groups of microorganisms was highest in soil under beech stands (SP1). Soil on that site has good chemical and physical characteristics, as mentioned. This suggests the existance of favorable conditions in soil for microbial growth. The high pH of the beech plots would favor the growth of bacteria (Killham, 1994), and may also account for the lower fungal biomass associated with this tree species. This is consistent with the findings of Frostegard and Baath (1996). They found higher fungal: bacterial ratios in more acid coniferous forest soils than in beech forest soils of higher pH, based on PLFA profiles. The high bacterial:fungal ratio in beech forest soil might also be related to their higher moisture content. Gram-negative bacteria, including pseudomonads are particularly sensitive to water stress (Killham, 1994). Gram-positive bacteria, in particular actinomycetes, are amongst the most water stress tolerant of all soil organisms.

The relative abundance of bacteria increased with pH (Högberg et al., 2007). Soils under beech-spruce (K) and fir-beech-spruce (T) were less favorable for microorganisms. Many systematic and physiological groups of microorganisms, as well as dehydrogenase activity were decreased in comparison with soils under beech (SP1) and spruce-Douglas fir (SP2). This can be explained by lower soil pH, moisture content, nutrient availability and specificity of forest litter. As mentioned, coniferous trees are dominating on these two sites. Many researchers stated that Norway spruce is a tree species which gradually change soil fertility in an unfavorable direction by lowering the soil pH, decomposition rates and concentration of exchangeable nutrients (Priha and Smolander, 1997; Menyailo et al., 2002). However, β-glucosidase activity was higher in acidic soils. This finding is in accordance with the results of Das et al. (2011). The authors found that the cellulase activity decreases with the increase in pH values. The highest mean value was found at pH 5.0 and the lowest at pH 8.0. In the temperate zone, soil moisture content does not represent a limitation of enzymatic activity because it does not drop to low values for long times (Baldrian et al., 2013). Differences between tree species may also be due to differences in ground vegetation, since herbs and grasses were more abundant in the deciduous stand than in the coniferous stands (Nieminen and Smolander, 2006). On the other hand, moss litter has a lower pH and decomposes more slowly than the dead parts of most herbs and grasses (Mikola, 1954). Furthermore, coniferous trees produce numerous secondary metabolites, e.g. phenols and tannins (Manninen et al., 2002). Phenols and especially tannins affect the mineralization of organic C and N compounds, they have a toxic effect on microorganisms and inhibit soil enzymatic activity (Kanerva et al., 2006).

In this research the counts of actinobacteria, fungi, aminoheterotrophs and cellulolytic microorganisms positively correlated with dehydrogenase activity while the cellulolytic enzyme activity correlated only with the number of cellulolytic microorganisms. It means that the microbial community, largely composed of bacteria, is metabolically active. Fungi are also known to be responsible for the production of many enzymes in soils (Theuerl and Buscot, 2010), although they presence in the forest soil does not always result in elevated enzyme activity. Often saprotrophic fungi in the forest soil are metabolically inactive.

CONCLUSION

In conclusion, result of this study gave us an insight about the current picture regarding microbial activity in forest soils on several locations. This research was based on analyses of culturable bacteria and fungi, hence the findings were created according to these parameters. In average, bacteria were more abundant in soil and the most favorable conditions for microbial growth were in soil under beech trees. Of course, one year data are not sufficient or reliable enough to create a trend or make a conclusion because of huge heterogenicity and variability of numerous factors (soil conditions, tree species, climatic factors, sampling time and place among the most important). The implementation of few other techniques (e.g. methods of molecular determination, analyses of microbial C, N and P biomass, biochemical tests) are essential to gain a better insight in forest ecosystem functioning.

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MIKROBIOLOŠKA AKTIVNOST U ŠUMSKOM ZEMLJIŠTU POD SASTOJINAMA BUKVE, JELE, SMRČE I DUGLAZIJE

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Izvod: Cilj ovog istraživanja je bio da se ispita mikrobiološka aktivnosti u zemljištu sa različitih lokaliteta pod listopadnim i četinarskim šumama u Srbiji. Jedan lokalitet na Staroj planini (SP1) je pod sastojinom bukve (*Fagus* sp.), dok je drugi (SP2) pod sastojinom smrče (*Picea* sp.) i duglazije (*Pseudotsuga* sp.). Lokalitet na Kopaoniku (K) je pod sastojinom bukve (*Fagus* sp.) i smrče (*Picea* sp.). Lokalitet na Tari (T) je dominantno pod sastojinama jele (*Abies* sp.), bukve (*Fagus* sp.) i smrče (*Picea* sp.). Ukupan broj bakterija, broj aktinomiceta, gljiva i mikroorganizama uključenih u cikluse azota (N) i ugljenika (C) su određene standardnom metodom agarnih ploča. Aktivnosti enzima dehidrogenaze i β-glukozidaze su merene spektrofotometrijski. Mikrobna aktivnost je zavisila od vrste šumskih sastojina kao i od vremena uzorkovanja zemljišta. Najveća dehidrogenaza aktivnosti, ukupan broj bakterija, broj aktinomiceta, aminoheterotrofa, amilolitskih i celulolitskih mikroorganizama su određene u zemljištu pod bukovim stablima. Najveći ukupan broj gljiva i brojnost pektinolitskih mikroorganizama su utvrđene u zemljištu pod smrčom i duglazijom. Korelaciona analiza je dokazala postojanje statistički značajne međuzavisnosti između ispitivanih parametara.

Ključne reči: *mikroorganizmi, šumsko zemljište, dehidrogenaza, β-glukozidaza*