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ACTIVITY OF SOIL ENZYMES IN RHIZOSPHERE OF SCOTS PINE AS A MARKER OF THE QUALITY OF WOODED, FORMERLY ARABLE, SOILS

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Summary: The purpose of this paper is the assessment of the influence of rhizosphere on the enzymatic activity of formerly arable soils afforested with Scots Pine (*Pinus sylvestris* L.). A comparison was made between the enzyme activity of formerly arable soils under 15-year-old and 51-year-old stands, and the enzyme activity of forest soils under 4-year-old cultivation and 104-year-old stands. It has been shown that the rhizosphere in formerly arable soils has considerable influence on the increase of enzyme activity catalysing the most important processes of organic substance change.

Key words: afforestation, post-arable soils, enzymatic activity, rhizosphere

1. INTRODUCTION

Management of soil resources as part of balanced development, considering the social, economic and ecologic purposes, requires an annual donation of certain arable areas for forestation, particularly those less agriculturally useful [Project 2000, "II Environmental Policy"]. It will allow the reasonable formation of agricultural and forest border as well as protection (under the ligneous plants communities of natural character) of soils being in danger of degradation [Sklodowski 2002; Olszewska, Smal 2008].

Afforestation of agricultural lands radically changes their physical, chemical and biological characteristics [Kahle i in. 2005; Wall, Hytönen 2005; Olszewska, Smal 2008]. Soil enzyme activity reflects the transformation of the soil environment taking place due to a change in the way the land is exploited [Clarholm 1993; Gorzelak 1996; Bielińska, Mocek-Płóciniak 2009].

Biochemical processes in the rhizosphere soil play an important structural and functional part in the dynamics of the nutritional cycle of plants and they can

significantly influence their growth and development. Changes in soil enzyme activity within the rhizosphere allow the determination of those environment disturbances influencing both the soil and the plants [Baran, Bielinska 2008].
The purpose of this paper is the assessment of the influence of the rhizosphere on the enzymatic activity of formerly arable soils afforested with Scots Pine (*Pinus sylvestris* L.).

2. MATERIALS AND METHODS

The study was located in the eastern part of Lubelskie province, in the Sobibor Forest Division, where, at 73%, Scots Pine constitutes the main forest generating species [Okruch 2004].
In the paper, formerly arable soils under 15-year-old stands in the area of Dubeczno in unit 314a and 51-year-old stands in the area of Sobibor in units 256a and 235d were compared with forest soils under 4-year-old cultivation (the restoration of a clearing of a 104-year-old pine stand) – the area of Sobibor, unit 314i. All the stands come from the planting.
The description of the stands is shown in Table 1. The 51-year-old area (research areas 2 and 3) was characterised with the loosening of occlusion due to numerous tree drop-outs destroyed by parasitic fungi. The loosening of tree stands on research area 2 was even, whereas research area 3 took the form of visible gaps. Habitats of research areas 2-5 are in a fresh forest while the habitat of research area 1 is potentially mixed fresh forest. There appear rusty soils (Dystric Arenosol) of grain composition from loose to weak clay sands.
Soil samples were collected for laboratory analysis in September 2009. The end parts of roots together with the adhesive soil were cut off and pulled out of the soil humus level (the depth of 2-7 cm) on each of the research areas from five randomly chosen plants. A soil sample was collected from the roots through shaking [Tarafdar, Jungk 1987]. The soil collected within the roots was considered as rhizosphere zone soil (R). Small roots were meticulously removed from the samples. Simultaneously, soil not overgrown with roots was collected from the same level – it was considered as non-rhizosphere zone soil (N). Individual samples were averaged within respective research areas and enzymatic as well as chemical analyses were performed in three repetitions.
Within the framework of enzymatic analyses the following activity was defined: dehydrogenases [Thalmann 1968], acid and alkaline phosphatase [Tabatabai, Bremner 1969], urease [Zantua, Bremner 1975], and protease [Ladd, Butler 1972]. Chemical analyses considered for marking of: pH in 1 mol $KCl \cdot dm^{-3}$ [ISO 10390], organic karbon [ISO 14235] and total nitrogen [ISO 13878] content altogether.
The significance of differences between particular enzymatic marking values has been assessed by means of a Tukey test as $p < 0.05$.

Tab. 1. List of research stands [Bielińska, Węgorek 2010]

Object	Locality	Soil	Tree age [years]	Stand density
1	area of Dubeczno, unit 314a	post- arable	15	full
2	area of Sobibor, unit 256a	post- arable	51	moderate
3	area of Sobibor, unit 235d	post- arable	51	under-stock
4	area of Sobibor, unit 314d	forest	4	-
5	area of Sobibor, unit 314i	forest	104	moderate

3. RESULTS AND DISCUSSION

The examined soils were characterized by a very acidic reaction (Table 2). The lowest pH values in 1 mol $KCl \cdot dm^{-3}$: from 2.90 (R) to 3.37 (N) were stated in forest soil (research area 5). A significant share in the acidification of forest soils (created from formations poor in alkaline cations) is left to physical and chemical processes which participate in the weathering of minerals, as well as biological processes connected with the circulation of C and N [Kurek 2002; Marcinek i in. 2008]. Vegetation cover is a factor which has a significant influence on the forest soils reaction [Kabala 1995]. During the intake of mineral components by tree roots, protons of hydrogen are released which increase acidification. Kurek [2002] observed that natural processes of soil acidification in natural forest ecosystems are not so much intensive so as to produce an amount of H^+ protons exceeding the buffer potential of soils. Higher values of pH_{KCl} in the case of the formerly arable soils (research areas 1-3) and forest soil under the restoration (research area 4) than in forest soil under the 104-year-old stand (research area 5) could be related to the diversity of the stands' age. Anderson and Domsh [1993] showed that age and stand type in forests, thus qualitative differences in primary production, have a significant influence on the development of soil pH.

Values of pH_{KCl} in the rhizosphere in the examined soils were lower than those in the non-rhizosphere zone within the range of 0.22-0.90 pH unit in 1 mol $KCl \cdot dm^{-3}$ (Table 2). Many rhizosphere bacteria produce low-molecule organic acids such as: citric, oxalic, malic, succinic, salicylic, gall, and asparagine [Trudgill 1988; Kurek 2002]. Low-molecule organic acids, which are synthesized by rhizosphere bacteria, are water soluble and they can contribute significantly to soil acidification through the release of hydrogen protons to soil solution after dissociation [Kurek 2002].

Formerly arable soils (research areas 1-3) were characterised by a significantly lower organic carbon and nitrogen content altogether than the forest soils (research areas 4 and 5) – Table 2. The lower content of C_{org} and N_{org} in the formerly arable soils could be connected with a huge loss of these components from the soil during intensive tree growth in the first years after afforestation, as demonstrated by the results obtained from many researchers [Jug i in. 1999; Vesterdal i in. 2002; Smal, Olszewska 2008]. Moreover, the supply of after-harvest remainders in he formerly arable soils is reduced while the amount of organic matter reaching the soil from the forest bed is too small to fill in the loss of soil components [Smal, Olszewska 2008]. The highest content of C_{org} and N_{og} was observed in the soil of the 104-year-old stand (research area 5), whereas the lowest one in the soil of the 15-year-old stand (research area 1) – Table 2. According to Zwolinski [1998], a significant movement of organic compounds of carbon into the soil mineral layer and the creation of the humus level in forest soils only happen after 30 years.
Within the 51-year-old stands the content of C_{org} and N_{og} in the samples of the soil from area 2 was higher than that in the soil of area 3 where the stands were less dense (they were gapped); however, the differences were not statistically relevant (Table 2).

Tab. 2. Selected chemical properties of soils [Bielińska, Węgorek 2010] (values in the column followed by the same letter are not significantly at $p < 0.05$, „t”- test)

Object	Soil zone	pH	C	N	C:N
		KCl	[$g \cdot kg^{-1}$]		
1	R	3.12	5.19b	0.37d	14.0b
	N	4.02	4.06a	0.23a	17.6c
2	R	3.41	6.89f	0.49f	14.0b
	N	3.81	5.47c	0.31b	17.6c
3	R	3.41	6.75f	0.47f	14.3b
	N	3.65	5.26c	0.29b	18.1c
4	R	3.32	7.09g	0.56g	12.6a
	N	3.54	5.93d	0.34c	17.4c
5	R	2.90	7.75h	0.62h	12.5a
	N	3.37	6.14e	0.40e	15.3b

R – rhizosphere; N – non-rhizosphere

Rhizosphere zone soil in all research areas was characterised by a substantially higher organic carbon and nitrogen content altogether than the non-rhizosphere zone soil (Table 2). Many research papers [Lynch, Whips 1990; Priha et al. 1999; Baran, Bielinska 2008] indicate that rhizosphere zone soils contain higher concentrations of soluble carbon than non-rhizosphere zone soils. Lynch and

Whips [1990] proved that the amount of C_{org} released by plants to the rhizosphere can amount to 40% of the total dry mass produced by the plant.

The value of the proportion of C:N in rhizosphere zone soils was in the range 12.5-14.3, whereas that in the non-rhizosphere zone soil it was from 15.3 (research area 5) to 17.4-18.1 in the remaining areas (Table 2). Narrower values of the proportion of C:N in the rhizosphere zone than in the non-rhizosphere zone validate the positive influence on the pace of humus synthesis and its reserve renewal.

The enzymatic activity of the examined soils was significantly varied depending on the environment (Table 3). The direction and intensification of the examined biochemical processes depended on the enzyme type, which was connected with the enzyme immunity to environmental factors as well as the content of specific substrates for the enzymatic reactions in soil [Kieliszewska-Rokicka 2001].

The formerly arable soils (research areas 1-3) were characterised by a significantly lower activity of dehydrogenase, acid and alkaline phosphatase as well as protease than the forest soils (research areas 4 and 5) – Table 3. The highest activity of these enzymes was noted under the 104-year-old stand. Despite a very acidic reaction, the observed stimulation of enzyme activity was accompanied by a higher C_{org} and N_{og} content than in the soils from the remaining (1-4) research areas (Table 2). Many studies [Pennanen et al. 1998; Januszek 1999; Kurek 2002; Domżał, Bielińska 2007] state that the main factor determining enzyme activity in acidic soils is the content of C_{org} and N_{og} as microorganism (found, for example, in the forest bed) adaptation to the lowered pH takes place with time. According to Januszek [1999] the increase in enzymatic soil activity together with the increase in hydrogen proton charge can result from the supply of enzymes from dead microorganisms in the soil, as well as desorption of enzymes from soil colloids connected to sorption exchange and the change in the cationic content in soil colloids due to the acidity of environment. The soil under the 15-year-old tree stands (area 1), where the content of C_{org} and N_{og} was the lowest (Table 2), was characterised by the lowest activity of dehydrogenase, the researched phosphatase and protheasis (Table 3). Kieliszewska-Rokicka [2001] underlines the fact that the content of organic coal determines the development and activity of the soil microflora which constitutes the main source of many soil enzymes.

The activity of the analysed enzymes in the soil under the 51-year-old stands was higher in research area 2 than in area 3 (of smaller occlusion); albeit statistically relevant differences were noted in the case of dehydrogenases in the rhizosphere zone (Table 3).

In the case of urease, the formerly arable soils under the 15-year-old stands (research area 1) were characterised with a higher activity of this enzyme while the lowest activity was noted under the 104-year-old stands (Table 3), which could be connected with the diverse urea (the substrate of urease) content. Urease is immune to external factors, and under stress conditions its activity increases. The only factor that limits its activity is substrate accessibility – the urea, as it is an extracellular enzyme that is synthesized only in its presence [Domzal, Bielinska 2007].

Tab. 2. Enzymatic activity of soils [Bielińska, Węgorek 2010] (Dh – dehydrogenases in $cm^3\ H_2 \cdot kg^{-1} \cdot d^{-1}$, Pac – acid phosphatase and Pal – alkaline phosphatase in mmol $PNP \cdot kg^{-1} \cdot h^{-1}$, U – urease in mg $N\text{-}NH_4^+ \cdot kg^{-1} \cdot h^{-1}$, P – protease in mg $tyrozyny \cdot kg^{-1} \cdot h^{-1}$; values in the column followed by the same letter are not significantly at $p < 0.05$, „t”- test)

Object	Soil zone	Dh	Pac	Pal	U	P
1	R	1.17b	29.36e	8.94d	8.16c	5.21c
	N	0.72a	14.82a	4.59a	5.14b	3.24a
2	R	1.51d	32.51f	11.43e	5.25b	5.85c
	N	0.94a	17.03b	6.28b	3.47a	3.72a
3	R	1.41c	30.72e	9.12d	4.92b	5.38c
	N	0.89a	16.41b	5.96b	3.19a	3.31a
4	R	1.69e	36.15g	13.72f	4.85b	6.17d
	N	1.04b	18.73c	7.53c	3.21a	3.89b
5	R	2.41f	44.89h	16.05g	4.49b	8.95e
	N	1.38c	22.96d	8.47d	3.01a	5.48c

R – rhizosphere; N – non-rhizosphere

Another factor that modifies the activity of enzymes in the analysed soils could be the diverse age of the pine stands [Kieliszewska-Rokicka 2001; Domzal, Bielinska 2007]. Kieliszewska-Rokicka [2001] observed the increase of dehydrogenase activity in soil with age and size of pine seedlings growing in tree nurseries. By significantly influencing the concentration of soluble carbon in soil, the age of a tree determines changes in soil enzymes activity [Domzal, Bielinska 2007]. How strongly related is the enzyme activity to the development of a plant root system can be observed in many research papers [Januszek 1999; Priha i in. 1999; Domżał, Bielińska 2007].

In the soils of all research areas the activity of the examined enzymes in rhizosphere was several times higher than in the non-rhizosphere zone (Table 2 and 3). It is related to the dynamic development of microorganisms in the root area due to the abundance of easy-to-reach energy substances [Januszek 1999; Priha i in. 1999; Kieliszewska-Rokicka 2001; Baran, Bielińska 2008]. Dahm [1998] mentions the high biological activity in the rhizosphere soil in tree nurseries and young stands, mainly pines.

Tab. 3. The value of the ratio (R:P) of the activity of dehydrogenase (Dh), acid phosphatase (Pac), alkaline phosphatase (Pal), urease (U) and protease (P) in rhizosphere soil (R) and non-rhizosphere soil (N)[Bielińska, Węgorek 2010]

Object	Dh	Pac	Pal	U	P
1	1.6	1.9	1.9	1.6	1.6
2	1.6	1.9	1.8	1.5	1.6
3	1.6	1.8	1.5	1.5	1.6
4	1.5	1.9	1.8	1.5	1.5
5	1.7	1.9	1.9	1.5	1.6

The highest values in the ratio of activity of the analysed enzymes in the rhizosphere soil to their activity in the non-rhizosphere soil were noted mainly in the case of acid phosphatase (1.9) and alkaline phosphatase (1.8-1.9) with the exception of research area 3 where the value of this ratio was 1.8 for acid phosphatase and 1.5 for alkaline phosphatase (Table 4). This may indicate disturbances in the soil environment and reflect the condition of the stands. Strong diminishing of stands were observed in research area 3 caused by parasitic fungi. Generally, high phosphatase activity in the rhizosphere zone soil demonstrates the concentration of phospholitic microorganisms in this area. Phosphatase activity in the rhizosphere soil increases with the rise of assimilable forms of inorganic phosphorus deficiency caused by intensive growth of the tree root mass [Januszek 1999].

4. CONCLUSIONS

- In the analysed soil the rhizosphere significantly influenced the increase of the enzymes activity which catalyses the most important processes of organic substance change.
- The observed biological activation of soils in the rhizosphere zone indicates the usefulness of the research on enzymatic activity in the rhizosphere zone as a sensitive indicator of soil environment reaction to afforestation.
- The values in the ratio of activity of the analysed enzymes in the rhizosphere soil to their activity in the non-rhizosphere soil reflected the environment disturbances influencing both the soil and the plants.
- Narrower values of C:N in the rhizosphere than in the non-rhizosphere soil confirms the positive influence of the rhizosphere on the pace of humus synthesis and its resources renewal.
- The utilization of enzymatic tests for the analysis of the functioning of the soil system allows the assessment of the effectiveness of the recommendations concerning the shaping of forest ecosystems for formerly arable soils.

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AKTYWNOŚĆ ENZYMÓW GLEBOWYCH W RYZOSFERZE SOSNY ZWYCZAJNEJ JAKO WSKAŹNIK JAKOŚCI ZALESIONYCH GLEB POROLNYCH

Streszczenie

Celem pracy jest ocena wpływu ryzosfery na aktywność enzymatyczną gleb porolnych zalesionych sosną zwyczajną (*Pinus sylvestris* L.). W pracy porównywano aktywność enzymów gleb porolnych pod drzewostanami 15-letnimi oraz 51-letnimi z aktywnością enzymów gleb leśnych pod 4-letnią uprawą i pod drzewostanami 104-letnimi. Wykazano, że w glebach porolnych ryzosfera w istotny sposób wpływa na wzrost aktywności enzymów katalizujących najważniejsze procesy przemiany substancji organicznej.