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**INFLUENCE OF BLACK LOCUST (*ROBINIA PSEUDOACACIA* L.)
SHELTERBELTS ON FRACTIONAL HUMUS COMPOSITION
AND BIOCHEMICAL PROPERTIES OF ERODED LOESS SOIL**

**WPŁYW ZADRZEWIEŃ ROBINII AKACJOWEJ (*ROBINIA PSEUDOACACIA* L.)
NA SKŁAD FRAKCYJNY PRÓCHNICY I WŁAŚCIWOŚCI BIOCHEMICZNE
ZERODOWANEJ GLEBY LESSOWEJ**

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Streszczenie. Zbadano wpływ zadrzewień śródpolnych na skład frakcyjny próchnicy i właściwości biochemiczne gleby lessowej na terenie silnie zagrożonym erozją wodną. Prace badawcze prowadzono w trzech monokulturowych odcinkach liniowych zadrzewień śródpolnych, złożonych z 20-letnich nasadzeń robinii akacjowej (*Robinia pseudacacia* L.). Zadrzewienia założono na gruntach ornych zlokalizowanych na terenie lessowej mikrozelewni rolniczej. Zadrzewienia nr 1 i nr 3 leżą na płaskiej wierzchołku, a nr 2 wzdłuż zbocza o wystawie południowej i nachyleniu (w miejscu badań) około 15%. Zadrzewienia nr 1 i nr 2 biegną w kierunku N-S, a nr 3 rozciąga się w kierunku W-E. W rejonie każdego zadrzewienia wykonano po 3 odkrywki glebowe: w środku pasa zadrzewień oraz w odległości 2 i 20 m od krawędzi zadrzewień na gruntach ornych. Zadrzewienia wpływały istotnie na proces sekwestracji węgla organicznego w badanej glebie, jak również na zaawansowanie procesu humifikacji. Gleby w obrębie zadrzewień cechowały się wyższą zawartością węgla, substancji humusowych oraz kwasów fulwowych, a także większymi wartościami stosunku C kwasów huminowych do C kwasów fulwowych niż gleba orna. Zawartość węgla, substancji humusowych i kwasów fulwowych malała wraz z odległością od zadrzewień. Wzrost zawartości węgla organicznego w glebie w obrębie zadrzewień był stymulatorem korzystnych zmian aktywności badanych enzymów (dehydrogenaz, fosfataz, ureazy i proteaz) katalizujących najważniejsze procesy przemiany glebowej substancji organicznej. W obrębie zadrzewień aktywność analizowanych enzymów była istotnie wyższa, niż w glebie pola uprawnego, i malała istotnie wraz z odległością od pasa zadrzewienia. Potwierdza to pozytywny wpływ systemu agroforestry na stan biologiczny gleby.

Key words: soil, shelterbelt, humus fractional composition, enzymatic activity.

Słowa kluczowe: gleba, zadrzewienia śródpolne, skład frakcyjny próchnicy, aktywność enzymatyczną.

INTRODUCTION

Simplifications of agrosystem structures, which enable higher crop yields, lead to reducing their regulative and regenerative capabilities, as well as to lowering the degree of matter circulation cycle enclosure and a decrease in the system's storage capacity (Ryszkowski 1992; Bielińska and Węgorzek 2005). This causes agrosystems to become sources of intensive

area pollution. Sustainable systems of land use developed in world agriculture include agroforestry, which is the practice of integrating trees into agricultural landscape (Malezieux 2012). In some European countries, growing trees in conjunction with agriculture is a vestige of traditional land-management systems (Walter et al. 2003). As Ecological Focus Areas (EFA) vital for biodiversity and water management in arable soils, tree planting is an element of environmental policies in some EU countries (Biernat-Jarka 2012). By increasing the landscape's biological diversity, midfield afforestation enables optimisation of farming production combined with natural environment protection (Kajak et al. 2003; Palma et al. 2007).

A good indicator of transformations occurring in the soil under the effects of natural and anthropogenic factors is the activity of enzymes responsible for biogeochemical transformations in the circulation of elements (Bielińska et al. 2014). The enzymatic tests are considered to be one of the more sensitive indicators of ecosystem functioning (Utobo and Tewari 2015). Changes in the composition of humic substances and enzyme activities in soil under agroforestry systems may have an influence on the global soil fertility status and, consequently, on crop quantity and quality (Udawatta et al. 2008; Trevisan et al. 2010). The purpose of the study was to evaluate the effect of black locust (*Robinia pseudoacacia* L.) shelterbelts on fractional humus composition and enzymatic activity in the area highly susceptible to water erosion.

MATERIAL AND METHODS

The research work was undertaken along three transects located within field shelterbelts planted with 50 trees of black locust (*Robinia pseudacacia* L.) each. The shelterbelts are situated on a field where spring wheat of the Izera variety was cultivated. The 5-metre wide shelterbelts were planted in autumn 1996 as a component of anti-erosion amelioration system on arable land. The area is located in the north-eastern part of the Nałęczów Plateau, a meso-region of the Lublin Upland, in the valley of the Ciemięga River. Ciemięga River catchment is an area predominantly (90%) under agricultural land use, with diverse loess relief and steep slopes ranging from 5–10%, descending into a narrow river valley. The slopes are covered with Cambisols developed from loess. Due to its highly productive soil, the area has been intensively used for agricultural purposes. Shelterbelts 1 and 3 are located on the flat hilltop and run north-south whereas shelterbelt 2 is situated along the north-facing slope of 15% at the study site and runs west-east. Overall, 9 soil pits were dug: (i) 3 pits in the central part of each 5 m wide tree strip, and (ii) 6 pits in the adjacent arable fields, with 2 pits at a distance of 2 m and 20 m made for each of 3 plots, perpendicularly to the shelterbelt edges, west of shelterbelts 1 and 2, and south of shelterbelt 3.

The sampling was performed in September 2015, at a depth of 0–20 cm, after spring wheat harvest. In the collected soil samples, organic carbon (C_{org}) and total nitrogen (N_t) contents were determined using a LECO TCNS Analyzer as well as pH in 1 mol · dm⁻³ KCl (ISO 10390). In order to analyze the fractional humus composition, carbon distribution was determined in the main fractions of humus compounds (C_{HS}) and in humic acids (C_{HA}) as described by Stevenson (1994), while carbon content of fulvic acids (C_{FA}) was calculated as the difference: $C_{FA} = C_{HS} - C_{HA}$. Activities of four enzymes were also determined, namely:

of dehydrogenases (Thalmann 1968), phosphatases (Tabatabai and Bremner 1969), proteases (Ladd and Butler 1972), and urease (Zantua and Bremner 1975). These enzymes are involved directly in mineralization and biogeochemical cycles of carbon, nitrogen and phosphorus in the soil and are highly responsive to environmental factors. The differences between mean values were verified with a t-test, and the significance of – with the variance analysis method (ANOVA).

RESULTS AND DISCUSSION

Increased distance from the shelterbelts belt led to accumulation of organic carbon and total nitrogen in the soils of all observed areas. The content of these components in soils within the shelterbelts belt was within 12.92–14.89 g · kg⁻¹ and 1.19–1.46 g · kg⁻¹ ranges, respectively, and was statistically markedly higher than in soils located at a distance of 20 m from the shelterbelts (Table 1). Similar relations were shown in studies by e.g. Mazurek (2006), Ferrari and Wall (2007) oraz Piotrowska and Mazurek (2009). Black locust used in agroforestry systems may contribute to an increase in soil organic carbon as a result of decomposition of leaves and other plant parts (Wang et al. 2012). The C_{org} : N_t ratio was within 9.28 to 12.41. The noted differences were generally statistically insignificant (Table 1). Hilltop soils (shelterbelt 1 and 3) were characterised by a reaction from slightly acidic to acidic while the soil along the hill slope (shelterbelt 2) exhibited a neutral reaction.

Table 1. Total organic carbon (C_{org}) and total nitrogen (N_t) contents in soil [g · kg⁻¹], and pH values in 1 mol · dm⁻³ KCl of the soil

Tabela 1. Zawartość węgla organicznego i azotu ogółem w glebie [g · kg⁻¹] oraz pH w 1 mol · dm⁻³ KCl

Shelterbelt No. Zadrzewienie nr	Distance from shelterbelt Odległość od zadrzewienia [m]	C _{org}	N _t	C _{org} : N _t	pH
1	0	13.83	1.29	10.72	5.08
	2	11.41	1.23	9.28	5.14
	20	10.18	0.82	12.41	4.78
2	0	14.89	1.46	10.20	6.91
	2	12.97	1.27	10.21	7.06
	20	12.05	1.11	10.86	7.17
3	0	12.92	1.19	10.86	5.32
	2	11.01	1.04	10.59	5.68
	20	9.87	0.84	11.75	5.51
LSD _{0,05} – NIR _{0,05}		2.04	0.29	1.62	–

Share of humic substances, humic and fulvic acids carbon in C_{org} content in soils within the shelterbelts belt was within: 34.71–48.95%; 20.72–33.16% and 12.43–20.03% ranges, respectively, and was statistically markedly decreased with increasing distance from the tree-planted sites (Table 2). The analysis of fractional humus composition in the soil under black locus trees in the study by Dinghua et al. (2001) and Mazurek and Piotrowska (2010) showed similar connections. Higher carbon content of humic acids in the soil under the tree strips than from the arable field indicates slow change in fractional humus composition under the black locust stands (Mazurek and Bejger 2014). The highest values for the discussed parameters were noted for shelterbelt 2. The discrepancies of soil properties observed

between the study sections may be linked with water erosion and the stage of tree stand development. Values of C_{HA} to C_{FA} ratios were higher in the soil collected within and in the immediate vicinity of the shelterbelts compared to the soil taken at a distance of 20 m from their edges (Table 2).

Table 2. Share of humic substances, humic and fulvic acids carbon in C_{org} content (% C_{org}), and $C_{HS} : C_{FA}$ ratio in soil

Tabela 2. Udział substancji humusowych, kwasów humusowych i fulwowych w C_{org} (% C_{org}) oraz stosunek $C_{HS} : C_{FA}$ w glebie

Shelterbelt No. Zadrzewienie nr	Distance from shelterbelt Odległość od zadrzewienia [m]	C_{HS}	C_{HA}	C_{FA}	$C_{HA} : C_{FA}$
1	0	48.95	31.32	17.63	1.78
	2	41.79	28.64	13.15	2.18
	20	36.02	23.59	12.43	1.90
2	0	53.19	33.16	20.03	1.66
	2	47.34	30.73	16.61	1.85
	20	37.92	22.78	15.14	1.50
3	0	47.65	29.45	18.20	1.62
	2	40.87	25.99	14.88	1.75
	20	34.71	20.72	13.99	1.48
LSD _{0,05} – NIR _{0,05}		4.57	3.21	4.62	0.17

The activity of the analysed enzymes in the soil of the tested sites was within: dehydrogenase from 2.21 to 5.24 $\text{cm}^3 \text{H}_2 \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, phosphatases from 3.11 to 10.35 $\text{mmol PNP} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, urease from 8.07 to 25.11 $\text{mg N-NH}_4^+ \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ and protease from 7.76 to 17.59 $\text{mg tyrosine} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$. Regardless of their topographic location and geographic direction, the shelterbelts substantially stimulated the activity of all the enzymes (Table 3).

Table 3. The enzymatic activity of the soil

Tabela 3. Aktywność enzymatyczna gleby

Shelterbelt No. Zadrzewienie nr	Distance from shelterbelt Odległość od zadrzewienia [m]	DhA	PhA	UA	PA
1	0	4.17	6.81	16.02	14.54
	2	3.31	5.26	11.67	12.72
	20	2.29	3.11	8.07	8.45
2	0	5.24	10.35	25.11	17.59
	2	4.36	9.08	18.32	14.41
	20	2.79	7.42	13.08	8.99
3	0	4.54	8.97	23.94	14.27
	2	3.47	5.41	11.76	11.23
	20	2.21	4.32	8.89	7.76
LSD _{0,05} – NIR _{0,05}		0.84	2.11	4.79	3.37

DhA – dehydrogenases activity in $\text{cm}^3 \text{H}_2 \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ – aktywność dehydrogenaz w $\text{cm}^3 \text{H}_2 \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, PhA – phosphatases activity in $\text{mmol PNP} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ – aktywność fosfataz w $\text{mmol PNP} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, UA – urease activity in $\text{mg N-NH}_4^+ \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ – aktywność ureazy w $\text{mg N-NH}_4^+ \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, PA – proteases activity in $\text{mg tyrosine} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ – aktywność proteazy w $\text{mg tyrozyny} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$.

Similar results were obtained by Bielińska and Węgorzek (2005) oraz Bielińska et al. (2004; 2008), among others. The soil enzymatic activity decreased markedly with increasing distance from the edges of shelterbelts, which confirms a positive impact of agroforestry system on soil biology. The differences in soil enzymatic activities between the sampled sections (Table 3) were associated with abiotic habitat-forming factors (temperature, humidity, insolation). Enzyme activity depends largely on soil moisture and oxygenation (Bielińska et al. 2014). The highest activity of the tested enzymes was observed in soil located near Shelterbelt No. 2 along the southern hill slope. Another factor that positively affected the enzymatic activity of the soil along the slope was its pH (Table 1). Soil reaction is of great importance to the synthesis of microbial biomass and microbial biomass carbon (C_{mic}) in the total content of soil organic carbon (Kurek 2002). The $C_{mic} : C_{org}$ ratio serves as an indicator of relative accessibility of substrates to enzymatic reactions. The presence of carbon substrates induces and stimulates the biosynthesis of enzymes by soil microorganisms (Kieliszewska-Rokicka 2001). Other studies (Krämer et al. 2000; Landmeyer 2001) indicate that the positive effects of shelterbelts on the enzymatic activity of soil results from an increase in biomass of enzyme-producing microbes, and from the release of root-secreted substances that support the metabolism of microorganisms.

CONCLUSIONS

1. The shelterbelts had a significant influence on soil organic carbon sequestration and humification progress.
2. Carbon contents of humic substances and fulvic acids as well as values of humic to fulvic acid ratios were higher in the soil under the locust trees than in the soil from the arable field.
3. The carbon content of humic substances and fulvic acids decreased with increasing distance from the tree-planted sites.
4. The observed significant increase in organic carbon content in the soil within the shelterbelts areas was a stimulator of changes in the activity of enzymes that catalyse the most important processes of soil organic matter transformation. This indicates that mid-field shelterbelts increased the self-regulation, resistance and buffer potential of the studied farmland ecosystem
5. Compared to the arable soil, the soil under *Robinia pseudacacia* had higher enzyme activities decreasing significantly with distance from the tree strips, which confirms a positive impact of agroforestry system on soil biology.
6. The obtained research results show that mid-field shelterbelts participate significantly in the regulation of essential natural processes, which include matter circulation and energy flow between the components of an agricultural ecosystem.

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Abstract. The purpose of the study was to investigate the impact of field shelterbelts on fractional humus composition and biochemical properties of loess soil in the area highly threatened by water erosion. To this end, soil samples were collected along three transects situated within 20-year-old black locust (*Robinia pseudoacacia* L.) shelterbelts. The plantings were established in a loess micro-basin located on arable land. Shelterbelts 1 and 3 are located on the flat hilltop and run north-south whereas shelterbelt 2 is situated along the north-facing slope of 15% at the study site and runs west-east. Overall, 9 soil pits were dug: (i) 3 pits in the central part of each 5 m wide tree strip, and (ii) 6 pits in the adjacent arable fields, with 2 pits at a distance of 2 m and 20 m made for each of 3 plots, perpendicularly to the shelterbelt edges, west of shelterbelts 1 and 2, and south of shelterbelt 3. The shelterbelts had a significant influence on soil organic carbon sequestration and humification progress. Carbon contents of humic substances and fulvic acids as well as values of humic to fulvic acid ratios were higher in the soil under the locust trees than in the soil from the arable field. The carbon content of humic substances and fulvic acids decreased with increasing distance from the tree-planted sites. The increase in soil organic carbon levels in the soil from the shelterbelts led to positive changes in the activity of the enzymes studied (i.e. dehydrogenases, phosphatases, proteases, and urease) catalyzing the most important processes of soil organic matter transformations. Compared to the arable soil, the soil under *Robinia pseudoacacia* had higher enzyme activities decreasing significantly with distance from the tree strips. This confirms a positive impact of agroforestry system on soil biology.

