

SOIL β -GLUCOSIDASE ACTIVITY UNDER WINTER WHEAT CULTIVATED IN CROP ROTATION SYSTEMS DEPLETING AND ENRICHING THE SOIL IN ORGANIC MATTER

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Abstract

β -glucosidase (E.C. 3.2.1.21), an enzyme involved in cellulose degradation, plays an important role in the soil organic carbon cycle. Cellulose is the most abundant organic compound in the biosphere so a product of its enzymatic hydrolysis is important as an energy source for soil microorganisms. Since β -glucosidase is very sensitive to different factors, determination of its activity might be helpful in soil quality monitoring. The objective of the study was to assess the effect of various doses of farmyard manure (FYM) and mineral nitrogen on β -glucosidase activity in soil samples taken under winter wheat cultivated in crop rotation systems depleting soil from organic matter (A) and enriching soil in organic matter (B). Soil samples were taken in 2002 from a two-factor fertilization experiment carried out as randomized sub-blocks cropped with winter wheat cultivated on lessivé soil. The experiment was located at the Experimental Station of the Institute of Tillage and Soil Science in Grabowo on the Vistula River. All fertilization combinations included FYM (0, 20, 40, 60 and 80 t ha⁻¹) and nitrogen fertilization (0, 40, 80 and 120 kg ha⁻¹). The activity of β -glucosidase was determined according to Eivazi, Tabatabai (1988). The enzyme activity ranged 3.604-7.041 mM pNP g⁻¹ h⁻¹ in soil samples taken from crop rotation A and between 4.931-7.445 mM pNP g⁻¹ h⁻¹ in those collected from the crop rotation enriching the soil in organic matter. These data were closely related to the applied FYM and nitrogen fertilization doses. Moreover, β -glucosidase activity depended significantly on sampling dates. Enzyme activity was closely connected with soil organic carbon and total nitrogen content, which was confirmed by highly significant correlation coefficients between these parameters ($r=0.611-0.770$ for C_{org}, and $r=0.844-0.912$ for N_{og}; $p<0.01$ and $p<0.001$).

Key words: β -glucosidase activity, farmyard manure, nitrogen fertilization, lessivé soil.

AKTYWNOŚĆ β -GLUKOZYDAZY GLEBOWEJ SPOD PSZENICY OZIMEJ W ZMIANOWANIU ZUBOŻAJĄCYM I WZBOGACAJĄCYM GLEBĘ W MATERIEŃ ORGANICZNĄ

Abstrakt

Biorąca udział w rozkładzie celulozy β -glukozydaza (E.C. 3.2.1.21) odgrywa ważną rolę w obiegu węgla w glebie. Jak wiadomo, celuloza to jeden z najobficiej występujących związków organicznych w biosferze, a produkt jego hydrolizy enzymatycznej stanowi cenne źródło energii dla mikroorganizmów glebowych. Ponieważ β -glukozydaza jest bardzo czuła na działanie różnorodnych czynników, oznaczenie jej aktywności może być pomocne w monitorowaniu jakości gleby. Celem pracy było określenie wpływu zróżnicowanych dawek obornika i nawożenia azotowego na aktywność β -glukozydazy w próbkach gleby spod pszenicy ozimej uprawianej w zamianowaniu zubożającym (A) i wzbogacającym (B) glebę w materię organiczną. Próbki gleby do badań pobrano w 2002 r. spod pszenicy ozimej uprawianej na glebie płowej w dwuczynnikowym doświadczeniu nawozowym założonym metodą losowych podbloków. Doświadczenie zlokalizowano w RZD w Grabowie nad Wisłą. W każdym z bloków zastosowano kombinacje nawozowe obornika (0, 20, 40, 60 i 80 t ha⁻¹) oraz nawożenia azotowego (0, 40, 80 i 120 kg ha⁻¹).

Aktywność β -glukozydazy oznaczono wg metody EIVAZI, TABATABAI (1988). Kształtowała się ona w zakresie 3,604-7,041 mM pNP g⁻¹ h⁻¹ w próbkach gleby z doświadczenia A oraz 4,931-7,445 mM pNP g⁻¹ h⁻¹ w próbkach z doświadczenia B i pozostawała w ścisłej zależności od zastosowanych dawek obornika i azotu mineralnego. Aktywność β -glukozydazy zależała także istotnie od terminu pobrania próbek glebowych. Ponadto aktywność oznaczonego enzymu była ściśle powiązana z zawartością C_{org} i N_{og}, o czym świadczą uzyskane wysokie współczynniki korelacji ($r = 0,611-0,770$ – dla C_{org}, oraz $r = 0,844-0,912$ – dla N_{og}; $p < 0,01$ i $p < 0,001$).

Słowa kluczowe: aktywność β -glukozydazy, obornik, nawożenie azotowe, gleba płowa.

INTRODUCTION

A group of cellulolytic microorganisms producing a specific enzyme complex is responsible for cellulose degradation, the most abundant organic compound in the biosphere (RUSSEL et al. 2005). This complex of enzymes hydrolyzing cellulose consists of endocellulases (endo- β -1,4-glucanase), which randomly cleave β -1,4-glucosidic linkages in the cellulose chain, exocellulases, which release cellobiose (and other celloligosaccharides) from non-reducing ends of cellulose molecules and β -glucosidase (EC 3.2.1.21), which catalyses the degradation of cellobiose to two molecules of glucose and releases glucose from non-reducing ends of celloligosaccharides (RAPA, BEERMANN 1991). Thus, β -glucosidase activity plays a crucial role in the C cycle of soils and the product of its enzymatic hydrolysis is important as an energy source for soil microorganisms (TABATABAI 1994, BANDICK, DICK 1999, JIMČNEZ et al. 2007).

One of the main anthropogenic factors affecting soil enzymatic activity is fertilization, both organic and mineral. Organic fertilization, mainly with farmyard manure, beneficially increases organic carbon and nitrogen con-

centrations in soil and affects the quality and quantity of organic matter (JARECKI, KRZYWY 1991). Thus, fertilization has crucial influence on soil biological status and the enzymatic activity. Since β -glucosidases are proteins which are very sensitive to different natural and anthropogenic factors (BANDICK, DICK 1999, GIANFREDA, RUGGIERO 2006), determination of their activity might be helpful in monitoring soil quality, especially soil subject to differentiated organic and mineral fertilization in various crop rotations.

The objective of the study was to assess the effect of various doses of farmyard manure (FYM) and mineral nitrogen on β -glucosidase activity in soil samples taken under winter wheat cultivated in crop rotation systems depleting and enriching the soil in organic matter.

MATERIAL AND METHODS

Soil samples were taken in 2002 from a two-factor fertilization experiment carried out as randomized sub-blocks located at the Experimental Station of the Institute of Tillage and Soil Science in Grabowo-on-the-Vistula, cropped with winter wheat cultivated on lessivé soil. Soil material was collected from the surface horizon of soil from crop rotation systems depleting (A) and enriching (B) the soil in organic matter. Soil was sampled three times in each vegetation season of winter wheat (at the end of March – date I, mid-May – date II, mid-July – date III) and after harvest (the beginning of September – date IV). The crop rotation systems applied in both experiments are given below:

Year	Crop rotation A	Crop rotation B
2001	potato	potato
2002	winter wheat	winter wheat + white mustard spring
2003	spring barley	barley + red clover
2004	corn	red clover + grasses

The fertilization combinations of every sub-block included FYM (0, 20, 40, 60 and 80 t ha⁻¹) and nitrogen fertilization (0, 40, 80 and 120 kg ha⁻¹). β -glucosidase activity was determined according to EIVAZI and TABATABAI (1988). The method is based on the spectrophotometrical measure of *p*-nitrophenyl (*p*NP) released after 1 hour of incubation of soil samples at 37°C with *p*-nitrophenyl- β -D-glucopyranoside (*p*NPG) in modified universal buffer (pH 6.0) as the substrate. The enzyme activity was expressed as mmoles *p*NP released kg⁻¹ dry soil per 1 hour (mM *p*NP kg⁻¹ h⁻¹). Soil chemical properties, such as organic carbon content (C_{ORG}), total nitrogen content (N_{TOT}) and pH in 1 mol KCl dm³, were determined according to standard methods accepted in soil science.

RESULTS AND DISCUSSION

Soil samples collected from both crop rotations had acid to slight acid reaction. The pH values measured in 1 mol KCl:dm³ ranged 4.5-6.1 for soil samples from crop rotation A, while the corresponding values for experiment B ranged 4.9-6.1. Total nitrogen content reached 0.889-1.005 g kg⁻¹ in soil material collected from the experiment enriching soil with organic matter, and 0.826-1.029 g kg⁻¹ in soil samples taken from crop rotation A (mean values for FYM and nitrogen fertilization doses). Increasing FYM doses significantly increased the total nitrogen content in the soil samples collected from both investigated crop rotations. However, no significant influence of nitrogen fertilization on total nitrogen concentration was shown in soil samples from either crop rotation.

β -glucosidase activity (Table 1) was higher in soil samples taken from plots of the experiment enriching the soil with organic matter as compared with the activity found in the soil material taken from the crop rotation system depleting the soil from organic matter. The results indicated the importance of incorporating plants of the fabae family in a crop rotation system and the influence of plant residues left behind in the soil as a result of intercrop cultivation on the soil's biological activity status.

The enzyme activity ranged from 3.604 to 7.041 mM pNP g⁻¹ h⁻¹ in the soil samples taken from crop rotation A and from 4.931 to 7.445 mM pNP g⁻¹ h⁻¹ in those collected from the crop rotation enriching the soil with organic matter. These data were closely related with the FYM and nitrogen fertilization doses applied (Table 1). The enzyme activity was modified by farmyard manure fertilization to a higher degree than by mineral nitrogen. Organic fertilization caused an increase in β -glucosidase activity by enhancing the abundance of a microbial population, which is the main source of the enzyme in soil (MARCOTE et al. 2001, BÖHME, BÖHME 2006).

For most of the soil samples, it was shown that higher FYM doses were accompanied by higher β -glucosidase activities. The biggest difference between the enzyme activity in the control soil sample and samples from the plots fertilized with the maximum FYM dose (80 t ha⁻¹) was noted in experiment A (an increase from 41% to 46%, as dependent on N doses) in comparison with the corresponding samples from crop rotation B (an increase from 22% to 43%, as dependent on N doses).

No clear influence of the applied nitrogen doses on soil β -glucosidase was found. However, when FYM was not applied, increasing doses of nitrogen fertilization clearly elevated the enzyme activity in the soil samples from both crop rotations.

In the soil samples from both experiments, whenever FYM was applied, increasing N fertilization doses did not cause any increase in β -glucosidase

Table 1
Soil β -glucosidase activity and organic carbon content in soil samples taken from crop rotation systems A and B
(mean values for sampling dates)

Parameters	FYM* (t ha ⁻¹)	Crop rotation system A						Crop rotation system B					
		Nitrogen fertilization** (kg ha ⁻¹)											
β -glucosidase	0	3.604	3.825	4.011	3.913	3.838	4.931	5.425	5.311	5.232	5.225		
	20	4.513	4.432	4.445	4.625	4.504	6.554	5.911	6.173	6.811	6.362		
	40	5.000	4.741	5.227	5.279	5.062	5.912	6.410	6.342	6.713	6.344		
	60	7.041	6.910	6.713	6.532	6.799	7.337	6.931	6.968	7.248	7.121		
	80	6.710	6.919	6.924	6.625	6.795	7.445	7.175	7.212	6.731	7.141		
	mean	5.374	5.365	5.464	5.395	5.400	6.436	6.370	6.401	6.547	6.439		
	LSD _{0,05}		factor I: 0.286 ; factor II: 0.139						factor I: 0.284 ; factor II: 0.187				
Organic carbon (g kg ⁻¹)	0	7.812	6.519	6.625	7.839	7.199	8.823	8.345	9.221	9.212	8.900		
	20	6.241	6.909	6.421	7.279	6.713	9.554	9.615	9.832	10.34	9.835		
	40	7.606	7.291	7.120	7.465	7.371	9.314	9.721	9.681	10.00	9.679		
	60	8.715	8.204	7.820	8.125	8.216	9.991	10.12	9.665	9.441	9.804		
	80	7.404	7.821	8.045	8.578	7.962	10.10	10.23	10.95	10.34	10.41		
	mean	7.556	7.349	7.206	7.857	7.492	9.556	9.606	9.870	9.867	9.725		
	LSD _{0,05}		factor I: 0.518 ; factor II: n.s.						factor I: 0.428 ; factor II: n.s				

*FYM – farmyard manure – factor I; **nitrogen fertilization – factor II; n.s. – differences not significant

activity, which in some cases even decreased significantly, especially when FYM was applied in the doses of 60 and 80 t ha⁻¹.

No clear tendency describing the influence of nitrogen fertilization on soil enzymatic activity has been reported in earlier papers. The way nitrogen fertilization affects soil enzymes depends, among other factors, on the kind of fertilization, dose of the fertilizer, the time of its application and the individual character of an enzyme (GIANFREDA, RUGGIERO 2006). For instance EIVAZI and TABATABAI (1990) showed that application of N-NO₃ in various doses partly inhibited β -glucosidase activity, while in a greenhouse experiment with corn, the enzymatic activity was directly proportional to the N-NH₄NO₃ amount applied over 164 days of the experiment (FAUCI, DICK 1994). According to DICK et al. (1988) enzymatic activity is not directly related to the N cycle (as the β -glucosidase activity). In their experiment, it did not correlate with nitrogen fertilizers used in various doses.

Moreover, β -glucosidase activity depended significantly on the sampling dates (Figure 1). The enzyme was less active in the soil samples taken on sampling dates I and II in the experiment where soil was enriched with organic matter as compared with the soil samples collected in the summer and autumn. The opposite was noted for the soil samples taken from crop rotation B, where the activity was the lowest in the samples collected on sampling dates III and IV.

No significant influence of nitrogen fertilization on organic carbon content was proved, although it was essentially modified by FYM doses in soil samples from both experiments (Table 1). In most soil samples, along with an increase in the FYM dose, the organic carbon content increased as well. Positive influence of farmyard manure fertilization on the organic carbon content, as well as the accumulation, mineralization and humification of

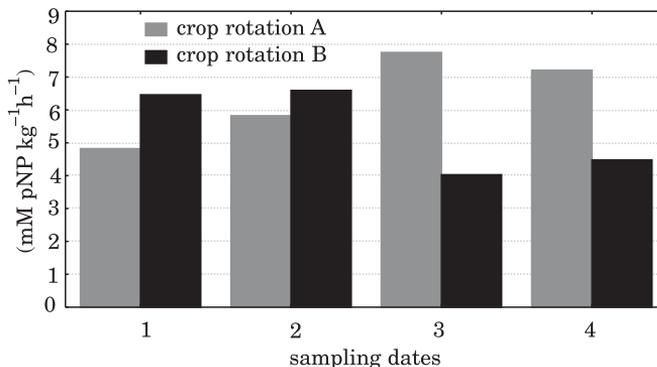


Fig. 1. β -glucosidase activity in soil samples taken from crop rotation systems A and B as dependent on sampling dates

organic matter, has been shown by many researchers (JARECKI, KRZYWY 1991, MAĆKOWIAK, ŻEBROWSKI 1999, MERCIK et al. 2004).

β -glucosidase activity, which plays a key role in the soil carbon cycling, was closely connected with the organic carbon content in the soil samples taken from both experiments on all sampling dates. Correlation coefficients between the discussed parameters ranged between $r = 0.611$ and $r = 0.770$, with $p < 0.05$ and $p < 0.001$, respectively. SIMILARLY, BÖHME and BÖHME (2006) obtained significant and positive correlation coefficients between β -glucosidase activity and organic carbon content in soil samples under spring barley and sugar beets cultivated in a long-term fertilization experiment ($r = 0.965$ and 0.954 with $p < 0.001$, respectively). Some other trials have also produced evidence for a significant relationship between soil β -glucosidase activity and organic carbon content (EIVAZI, TABATABAI 1990, BANDICK, DICK 1999). According to LANDGRAF and KLOSE (2002), soil β -glucosidase activity is closely related to the content of easily soluble organic carbon. Moreover, soil β -glucosidase activity was significantly and positively correlated with total nitrogen ($r = 0.844-0.912$, $p < 0.001$) and pH in KCl ($r = 0.632 = 0.773$; $p < 0.05$ and $p < 0.001$), but only in soil samples collected from systems depleting the soil from organic matter (B).

CONCLUSIONS

1. Higher β -glucosidase activity observed in the soil samples taken from the systems enriching the soil with organic matter as compared to the values measured in the crop rotation depleting the soil from organic matter indicated the importance of plant residues introduced into soil as a result of intercrop cultivation as well as the significance of cultivation of the fabae plants in the soil enzymatic activity status.

2. Organic carbon and total nitrogen content as well as soil β -glucosidase activity in soil samples taken from both crop rotations coincided with increasing FYM doses.

3. No clear tendency was observed in the β -glucosidase activity as influenced by increasing nitrogen fertilization doses. Moreover, nitrogen fertilization did not influence significantly organic carbon and total nitrogen content in the soil samples collected from both crop rotation systems.

4. While soil β -glucosidase activity was characterized by seasonal variability, there was no clear direction in the enzyme activity when the soil samples from both experiments were compared.

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