

## The changes of enzymatic activity of soil under eastern galega (*Galega orientalis* Lam.) after NPKCa fertilization

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### ABSTRACT

Changes in the enzymatic activity of soil variably fertilized with NPKCa were investigated in a field experiment carried out in 2005–2010. The study was conducted with a legume plant, i.e. eastern galega (*Galega orientalis* Lam.). The experiment was completely randomized and carried out in three replications with the following mineral fertilization: control, N, P, K, NPK, NP, NK, PK, NPKCa, PKCa, Ca, NKCa, and NPCa. Mineral fertilization was applied in kg/ha: (N-20, P-50, K-160, Ca-150). The soil samples collected from the Ap horizon (0–30 cm) of the rhizosphere in spring after the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> swathing had a  $\text{pH}_{\text{KCl}}$  in range from 6.55 to 6.93. The activity of acid phosphatase and alkaline phosphatase was at a low level. The highest activity of urease was recorded in the soil fertilized with NPK, whereas the highest activity of dehydrogenases was in the soil fertilized with PKCa.

**Keywords:** mineral fertilization; phosphatases; urease; dehydrogenases; biochemical index

The type of fertilization in plant cultivation affects enzymatic activity and thus the potential viability of plants to grow (Tabatabai 1994). The species and long-term fertilization can result in microbial community shifts in soils (Chu et al. 2007). Enzymes present in soil are similar to enzymes in other systems (Singh and Kumar 2008). Soil enzymes play a critical role in catalyzing reactions leading to organic matter decomposition and serve as bioindicators of biochemical and microbial soil activity (Koper et al. 2008). Salinity is the important environmental stress factor that affects the plant growth and nutrition of plant (Wang et al. 2012). In recent years, there has been a growing interest in new species of cultivated plants. They predominantly originate from other climatic zones and are termed 'alternative plants'. Multi-directional studies carried out in the Central-Eastern Poland (Symanowicz et al. 2013) on the adaptation of eastern galega (*Galega orientalis* Lam.) indicated the potential of its cultivation. Complete fertilization of eastern galega is poorly investigated and no studies on the impact of min-

eral fertilization on the enzymatic activity have been conducted, yet.

The objective of the studies was to determine the impact of varied mineral fertilization of eastern galega with NPKCa on the changes in the activity of selected soil enzymes and the level of biochemical soil fertility index.

### MATERIAL AND METHODS

The soil samples for laboratory analyses were collected in 2010 from the humus horizon (A<sub>0</sub>-loamy sand) of a field experiment carried out in 2005–2010 on research fields owned by the Siedlce University of Natural Sciences and Humanities, Poland (52°17'N, 22°28'E). The experiment was performed in a completely randomized method with three replications included one factor – thirteen mineral fertilization levels: 1. control (with no mineral fertilization); 2. N; 3. P; 4. K; 5. NPK; 6. NP; 7. NK; 8. PK; 9. NPKCa; 10. PKCa; 11. Ca; 12. NKCa; 13. NPCa. Mineral fertilization was applied

in kg/ha: (N-20, P-50, K-160, Ca-150). Nitrogen was applied in the early spring as ammonium nitrate, phosphorus – as triple superphosphate in autumn, potassium – as 60% potassium salt in two doses (I – in early spring; II – after the first swathing), and calcium – as carbonate calcium in the autumn. Experimental plot area was 3 m<sup>2</sup>. The activity of soil enzymes was determined four times (in spring, after the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> swathing of eastern galega which was harvested at budding) in soil samples from each mineral fertilization levels in three successive replications.

The phosphatases activity determination method consisted of incubation of reactive mixture containing soil and a substrate. The method of Tabatabai and Bremner (1969) used disodium 4-nitrophenyl phosphate hexahydrate in modified universal buffer (MUB) at pH 6.5 for acid phosphatase (AcP) and at pH 11 for alkaline phosphatase (ALP), as a substrate. The color intensity (yellow) due to released *p*-nitrophenol was then measured spectrophotometrically.

Urease activity was determined colorimetrically following incubation of soil with urea (aqueous solution) and citrate buffer according to modified method of Hoffmann and Teicher (1961).

Soil dehydrogenases activity in soil was determined colorimetrically method (Casida et al. 1964) using TTC (2,3,5-triphenyl tetrazolium chloride) as a substrate which was reduced to TPF (triphenyl formazan) during incubation. All colorimetric data was determined with a spectrophotometer UV-VIS Lambda 25 (Perkin Elmer, Waltham, USA).

Organic carbon was recorded applying oxidation-titrimetric method by Kalembasa (1991). In this method soil and potassium dichromate and acid mixture (sulphuric acid and phosphoric acid – 5:1) was added. Assuming the green colour was corrected, indicator (N-phenyl antranilic acid) was added and the suspension in the flask was titrated with Mohr's salt.

Results from chemical determinations were subjected to statistical analysis using the analysis of variance (Statistica 10 PL; Statsoft, Oklahoma, USA) and significant differences determined by the Tukey's test. The criterion for significance was set at  $P < 0.05$ . In order to find the correlations between organic carbon, yield and activity of soil enzymes (AcP, ALP, dehydrogenase (Deh), urease (Ure)). Assessment of soil fertility growth potential based upon a single enzyme may be biased and thus several enzymes were examined in present study. Potential biochemical index of soil fertility

(Mw) was calculated to include activities of AcP, ALP, Ure, Deh as well as organic carbon content (Kucharski et al. 2009):

$$Mw = (AcP + ALP + Deh + Ure \times 10^{-1}) \times \%C$$

## RESULTS AND DISCUSSION

The analysis of variance indicated a significant impact of the applied mineral fertilization on changes in the acid phosphatase activity (Table 1). Significant differences in the acid phosphatase activity were observed among the treatments fertilized without P, Ca (NK), without N (PKCa) and without P (NKCa). The highest average activity of acid phosphatase (0.17 mmol pNP/kg dry matter (dm)/h) was determined in the soil fertilized without nitrogen (PKCa) and without P (NKCa). The soil collected in spring after the application of full mineral fertilization (NPKCa) had the highest acid phosphatase activity (0.21 mmol pNP/kg dm/h).

The soil sampled after the 1<sup>st</sup> and 2<sup>nd</sup> swathing of eastern galega (*Galega orientalis* Lam.) had a lower acid phosphatase activity compared to the soil collected at other time points. In studies by Koper and Lemanowicz (2008b), Radulov et al. (2011), the highest amount of acid phosphatase (1.53 mmol pNP/kg/h) was determined in soil with  $pH_{KCl} = 5.3-5.5$  fertilized with N-120 kg/ha. The applied mineral fertilization caused a significant difference between treatments fertilized without P, Ca (NK), with full mineral fertilization (NPKCa) and without K (NPCa) in the average activity of alkaline phosphatase (Table 1). The level of alkaline phosphatase activity was higher by 60% than the activity of acid phosphatase and approximated a comparable level in the soil sampled in spring after the 1<sup>st</sup> and 2<sup>nd</sup> swathing (0.22–0.23 mmol pNP/kg dm/h). The highest activity of alkaline phosphatase (0.27 mmol pNP/kg dm/h) was measured in the soil sampled after the 3<sup>rd</sup> swathing of the test plant. In studies by Lemanowicz (2013) the highest amount of alkaline phosphatase was determined in soil collected from the control treatment (with no nitrogen fertilization). The values of correlation coefficients between the activity of acid phosphatase and alkaline phosphatase ( $r = 0.85$ ) were significant in the soil collected from the rhizosphere zone of eastern galega. The analysed soil had a  $pH_{KCl}$  in range from 6.55 to 6.93. The soil pH in the conducted studies did not favour a high activity of phosphatases. Soil enzymes AcP and ALP are a sensitive indicator of

Table 1. Activities of acid (AcP) and alkaline (AlP) phosphatases (mmol pNP/kg dry matter/h)

Mineral fertilization	Terms								Mean	
	spring		after 1 <sup>st</sup> cut		after 2 <sup>nd</sup> cut		after 3 <sup>rd</sup> cut			
	AcP	AlP	AcP	AlP	AcP	AlP	AcP	AlP	AcP	AlP
Control	0.18	0.24	0.18	0.24	0.11	0.23	0.16	0.23	0.16	0.23
N	0.16 <sup>b</sup>	0.23 <sup>b</sup>	0.14 <sup>ab</sup>	0.22 <sup>b</sup>	0.10 <sup>b</sup>	0.22	0.15 <sup>b</sup>	0.25 <sup>b</sup>	0.14	0.23
P	0.17 <sup>b</sup>	0.20 <sup>b</sup>	0.12 <sup>ab</sup>	0.21 <sup>b</sup>	0.12	0.20	0.16 <sup>b</sup>	0.26 <sup>b</sup>	0.14	0.22
K	0.19 <sup>b</sup>	0.22 <sup>b</sup>	0.16 <sup>b</sup>	0.24 <sup>b</sup>	0.12	0.20	0.17 <sup>b</sup>	0.25 <sup>b</sup>	0.16	0.23
NPK	0.20 <sup>b</sup>	0.25 <sup>b</sup>	0.14 <sup>a</sup>	0.23 <sup>b</sup>	0.12	0.20	0.16 <sup>b</sup>	0.26 <sup>b</sup>	0.15	0.23
NP	0.16 <sup>b</sup>	0.24 <sup>b</sup>	0.17 <sup>b</sup>	0.26 <sup>b</sup>	0.13	0.23	0.17 <sup>b</sup>	0.25 <sup>b</sup>	0.16	0.24
NK	0.10 <sup>ab</sup>	0.15 <sup>ab</sup>	0.14 <sup>ab</sup>	0.25 <sup>b</sup>	0.13	0.22	0.17 <sup>b</sup>	0.29 <sup>ab</sup>	0.13 <sup>b</sup>	0.20 <sup>b</sup>
PK	0.15 <sup>b</sup>	0.21 <sup>b</sup>	0.15 <sup>ab</sup>	0.22 <sup>b</sup>	0.10 <sup>b</sup>	0.19 <sup>b</sup>	0.16 <sup>b</sup>	0.27 <sup>ab</sup>	0.14	0.22
NPKCa	0.21 <sup>b</sup>	0.23 <sup>b</sup>	0.16 <sup>b</sup>	0.26 <sup>b</sup>	0.13	0.22	0.15 <sup>b</sup>	0.28 <sup>ab</sup>	0.16	0.25 <sup>b</sup>
PKCa	0.18 <sup>b</sup>	0.22 <sup>b</sup>	0.16 <sup>b</sup>	0.22 <sup>b</sup>	0.14	0.24	0.21 <sup>ab</sup>	0.30 <sup>ab</sup>	0.17 <sup>b</sup>	0.24
Ca	0.16 <sup>b</sup>	0.22 <sup>b</sup>	0.16 <sup>b</sup>	0.22 <sup>b</sup>	0.12	0.21	0.18 <sup>b</sup>	0.28 <sup>ab</sup>	0.15	0.23
NKCa	0.18 <sup>b</sup>	0.26 <sup>b</sup>	0.15 <sup>ab</sup>	0.19 <sup>ab</sup>	0.15 <sup>b</sup>	0.26 <sup>b</sup>	0.20 <sup>ab</sup>	0.30 <sup>ab</sup>	0.17 <sup>b</sup>	0.25 <sup>b</sup>
NPCa	0.17 <sup>b</sup>	0.26 <sup>b</sup>	0.16 <sup>b</sup>	0.22 <sup>b</sup>	0.13	0.24	0.20 <sup>ab</sup>	0.28 <sup>ab</sup>	0.16	0.25 <sup>b</sup>
Mean	0.17	0.23	0.15	0.23	0.12	0.22	0.17	0.27	0.15	0.24
<i>LSD</i> <sub>0.05</sub>	0.06	0.05	0.03	0.04	0.05	0.06	0.03	0.04	0.04	0.05

Explanations are in Material and Methods. The data in the table are means ( $n = 3$ ). <sup>a</sup>letters indicate significant differences only with respect to control; <sup>b</sup>letters indicate significant differences among mineral fertilization levels

soil transformation (Koper and Lemanowicz 2008a). The enzymatic reaction speed is mainly determined by the enzyme concentration involved in reaction, substrate concentration, temperature, the presence of activators and inhibitors and pH (Koper et al. 2008).

In the presented studies, the activity of urease in the soil significantly increased under the influence of varied mineral fertilization: (P; NPK; NP; NK; PK; NPKCa; PKCa; NPCa) (Table 2). According to Yang et al. (2008) and Zhao et al. (2009), urease activity predominantly depends on the type of mineral fertilization. The highest mean level of urease activity (35.21 mg N-NH<sub>4</sub><sup>+</sup>/kg dm/h) was determined in the soil fertilized with no Ca (NPK). The soil samples after the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> swathing of eastern galega had the highest activity of urease (which was twice as high compared to the spring). The activity of urease in soil increased at subsequent sampling points, which was associated with actual soil temperature which has a substantial impact on the activity level of this enzyme. The activity of urease in the soil sampled after the 1<sup>st</sup> swathing was positively

correlated with the content of carbon in organic compounds ( $r = 0.81$ ). According to Shi et al. (2008) urease is sensitive to the changes of soil organic matter. In the present studies, the highest average level of dehydrogenases activity in relation to the control treatments was recorded with K, PKCa, NKCa and NPCa fertilization (Table 2). The soil sampled after the 1<sup>st</sup> and 2<sup>nd</sup> swathing of eastern galega had a lower dehydrogenases activity compared to the soil collected at other time points. The average activity of dehydrogenases was positively correlated with the biochemical soil fertility index ( $r = 0.82$ ). The highest correlation coefficient was recorded between the activity of dehydrogenases in the soil sampled after the 2<sup>nd</sup> and 3<sup>rd</sup> swathing and the biochemical soil fertility index ( $r = 0.90$ ,  $r = 0.70$ ). In studies by Martyniuk et al. (2010) the highest activity of dehydrogenases was determined in soil collected under pasture mixtures with nitrogen fertilization.

The average carbon content in organic compounds in the analysed soil ranged from 2.39% to 2.94% (Table 3). The statistical calculations dem-

Table 2. Activities of urease (Ure) (mg N-NH<sub>4</sub><sup>+</sup>/kg dry matter/h) and dehydrogenases (Deh) (cm<sup>3</sup> H<sub>2</sub>/kg dm/h)

Mineral fertilization	Terms								Mean	
	spring		after 1 <sup>st</sup> cut		after 2 <sup>nd</sup> cut		after 3 <sup>rd</sup> cut			
	Ure	Deh	Ure	Deh	Ure	Deh	Ure	Deh	Ure	Deh
Control	14.50	5.99	42.33	6.57	31.84	5.49	31.33	9.32	30.00	6.84
N	17.75 <sup>ab</sup>	8.23	44.66 <sup>ab</sup>	7.99	39.66 <sup>ab</sup>	9.65 <sup>ab</sup>	40.16 <sup>a</sup>	10.90	33.35	9.19
P	17.66 <sup>ab</sup>	7.24	44.00	9.32 <sup>ab</sup>	38.66 <sup>ab</sup>	8.07 <sup>b</sup>	36.33 <sup>b</sup>	11.65 <sup>b</sup>	35.12 <sup>a</sup>	9.07
K	17.67 <sup>ab</sup>	8.82	42.99 <sup>b</sup>	9.49 <sup>ab</sup>	37.66 <sup>ab</sup>	10.99 <sup>a</sup>	39.33 <sup>a</sup>	10.15	33.66	9.86 <sup>a</sup>
NPK	18.91 <sup>ab</sup>	10.57 <sup>ab</sup>	42.99 <sup>b</sup>	8.49	39.63 <sup>ab</sup>	11.35 <sup>ab</sup>	41.33 <sup>a</sup>	8.40 <sup>b</sup>	35.21 <sup>a</sup>	9.70
NP	16.67 <sup>b</sup>	7.49	45.67 <sup>ab</sup>	9.16 <sup>a</sup>	36.66 <sup>ab</sup>	10.49 <sup>a</sup>	37.33 <sup>a</sup>	10.48	34.08 <sup>a</sup>	9.40
NK	14.50 <sup>b</sup>	7.41	46.17 <sup>ab</sup>	7.16	37.33 <sup>ab</sup>	13.32 <sup>ab</sup>	41.33 <sup>a</sup>	9.66 <sup>b</sup>	34.83 <sup>a</sup>	9.39
PK	15.16 <sup>b</sup>	6.49 <sup>b</sup>	45.17 <sup>ab</sup>	5.99 <sup>b</sup>	36.00	8.24 <sup>b</sup>	40.83 <sup>a</sup>	10.16	34.29 <sup>a</sup>	7.72
NPKCa	14.50 <sup>b</sup>	8.49	45.67 <sup>ab</sup>	7.49	35.33	13.73 <sup>ab</sup>	41.83 <sup>ab</sup>	8.49 <sup>b</sup>	34.33 <sup>a</sup>	9.55
PKCa	16.16 <sup>b</sup>	10.81 <sup>ab</sup>	44.66 <sup>ab</sup>	9.07 <sup>b</sup>	35.67	13.82 <sup>ab</sup>	39.31 <sup>a</sup>	12.65 <sup>ab</sup>	33.95 <sup>a</sup>	10.55 <sup>a</sup>
Ca	16.16 <sup>b</sup>	9.41 <sup>a</sup>	42.33 <sup>b</sup>	8.18	35.66	8.49 <sup>b</sup>	37.33 <sup>a</sup>	8.99 <sup>b</sup>	32.87	9.68
NKCa	15.67 <sup>b</sup>	9.49 <sup>a</sup>	44.66 <sup>ab</sup>	9.57 <sup>ab</sup>	31.66 <sup>b</sup>	12.23 <sup>ab</sup>	37.00 <sup>a</sup>	8.49 <sup>b</sup>	32.25	9.94 <sup>a</sup>
NPCa	19.83 <sup>ab</sup>	6.66 <sup>b</sup>	44.33 <sup>b</sup>	8.49	31.50 <sup>b</sup>	13.65 <sup>ab</sup>	41.16 <sup>a</sup>	10.66	34.20 <sup>a</sup>	9.86 <sup>a</sup>
Mean	16.55	8.24	44.28	8.23	35.94	10.73	38.82	10.00	33.90	9.30
LSD <sub>0.05</sub>	3.09	3.10	2.31	2.54	4.60	3.17	5.17	2.77	3.79	2.89

Explanations are in Material and Methods. The data in the table are means ( $n = 3$ ). <sup>a</sup>letters indicate significant differences only with respect to control; <sup>b</sup>letters indicate significant differences among mineral fertilization levels

onstrated significant differences in the content of carbon in organic compounds in the soil sampled from treatments fertilized with phosphorus at 50 kg P/ha (2.89%) and carbonate calcium at 150 kg Ca/ha (2.39%). The applied calcium fertilizer increased the rate of mineralization of organic carbon compounds.

The same tendency of changes in the content of carbon in organic complexes was recorded at the spring sampling time point and after the 1<sup>st</sup> swathing of the test plant. The analysis of soil samples after the 2<sup>nd</sup> swathing, indicated the significant impact of NPKCa fertilization on the reduction of organic carbon for treatments which had a single mineral fertilizers applied. The final effect of the presented studies was the calculated biochemical soil fertility index (Table 3) which was at a high level (ranging on average from 26.47 to 41.90). The results are significantly higher than those reported by Kalembasa and Symanowicz (2012) and Kucharski et al. (2009) who carried out the studies under laboratory conditions. A lack of possibility for comparing the results from field studies and

laboratory experiments was also indicated by other authors (Hu and Cao 2007, Iovieno et al. 2009). The applied mineral fertilization without nitrogen (PKCa) exerted the highest impact on the increase in the biochemical soil fertility index. The same tendency in the changes of biochemical soil fertility index was found at spring sampling and after the 2<sup>nd</sup> and 3<sup>rd</sup> swathing. The time point of soil sampling (soil temperature) had a decisive impact on the value of biochemical soil fertility index. Moreover, a significant correlation was observed between the soil biochemical fertility index sampled after the 3<sup>rd</sup> swathing of the test plant and the content of organic carbon in the soil ( $r = 0.84$ ). A significant correlation between the enzyme activity and organic matter was also reported by Krzyżaniak and Lemanowicz (2013).

However, data on eastern galega yields were not the subject of the article, for full knowledge some information is attached. The cumulative yield of fresh mass from three swathings of eastern galega (*Galega orientalis* Lam.) was 57.1 t/ha. The highest yield of green mass was generated where no

Table 3. Total organic carbon (TOC, %) and biochemical index of potential soil fertility (Mw)

Mineral fertilization	Terms								Mean	
	spring		after 1 <sup>st</sup> cut		after 2 <sup>nd</sup> cut		after 3 <sup>rd</sup> cut		TOC	Mw
	TOC	Mw	TOC	Mw	TOC	Mw	TOC	Mw		
Control	2.84	22.32	2.57	28.84	2.44	21.99	2.55	32.75	2.60	26.47
N	2.83	29.42	3.05 <sup>b</sup>	39.09	2.82 <sup>b</sup>	39.30	2.49	38.14	2.80	36.49
P-	3.59 <sup>ab</sup>	33.66	2.75	34.98	2.43 <sup>b</sup>	29.78	2.79 <sup>b</sup>	43.81	2.94 <sup>b</sup>	35.56
K	2.35 <sup>b</sup>	25.84	2.49 <sup>b</sup>	35.33	2.90 <sup>ab</sup>	43.72	2.55	36.98	2.57	35.47
NPK	2.63	33.95	2.37 <sup>b</sup>	31.19	2.36 <sup>b</sup>	36.89	2.53	32.77	2.47	33.70
NP	2.48	23.70	2.98 <sup>b</sup>	42.19	2.98 <sup>ab</sup>	43.26	2.41	35.26	2.71	36.10
NK	2.75	25.05	2.87	34.92	2.46 <sup>b</sup>	42.81	2.67	38.05	2.69	35.21
PK	2.69	22.50	2.84	30.89	2.70	32.75	2.02 <sup>b</sup>	29.64	2.56	28.94
NPKCa	2.39 <sup>b</sup>	24.80	3.07 <sup>ab</sup>	38.30	2.36 <sup>b</sup>	41.57	3.02 <sup>b</sup>	39.57	2.71	36.06
PKCa	2.65	33.99	2.65	36.88	2.54 <sup>b</sup>	45.13	3.02 <sup>b</sup>	51.61	2.71	41.90
Ca	2.34 <sup>b</sup>	26.69	2.53 <sup>b</sup>	32.37	2.62	32.45	2.06 <sup>b</sup>	27.16	2.39 <sup>b</sup>	29.67
NKCa	2.83	32.54	2.97 <sup>b</sup>	42.70	2.75	43.47	2.80 <sup>b</sup>	35.53	2.84	38.56
NPCa	2.64	23.95	2.89 <sup>b</sup>	38.44	2.91 <sup>ab</sup>	49.96	2.92 <sup>b</sup>	44.55	2.84	39.22
Mean	2.62	27.75	2.77	35.85	2.64	38.69	2.63	37.37	2.66	34.87
<i>LSD</i> <sub>0.05</sub>	0.58	–	0.50	–	0.43	–	0.71	–	0.55	–

Explanations are in Material and Methods. The data in the table are means ( $n = 3$ ). <sup>a</sup>letters indicate significant differences only with respect to control; <sup>b</sup>letters indicate significant differences among mineral fertilization levels

phosphorus fertilization was applied (N-KCa). The significant correlation between the biochemical fertility index of tested soil and total yield of eastern galega indicates a close correlation between the enzymatic soil activity and the yield volume.

The present study demonstrated a significant influence of increased soil enzyme activity and carbon in organic compounds on the biochemical soil fertility index. A high biochemical soil fertility index value indicates the possibility of generating high perennial legume cultivation yields and maintaining good soil culture.

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