ISSN 1392-3196 / e-ISSN 2335-8947
Zemdirbyste-Agriculture, vol. 101, No. 2 (2014), p. 139–146
DOI 10.13080/z-a.2014.101.018

Changes in enzyme activities as affected by green-manure catch crops and mineral nitrogen fertilization

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Abstract

The estimation of soil microbial activity has been an important research issue because of the significant role that microorganisms play in nutrient cycling in soil. In a 3-year (August 2005 – August 2008) experiment, the effect of catch crops: oilseed radish (*Raphanus sativus* var. *olifera* L.) and field pea (*Pisum sativum* L.) *vs.* plots without a catch crop, and inorganic nitrogen (N) fertilization at 0, 40, 80, 120, 160 kg ha^{-1} $year^{-1}$ on soil dehydrogenase (DH), catalase (CAT) and fluorescein diacetate hydrolysis (FDAH) activities were investigated on albic, cutanic *Luvisols* (*LVab*, *LVct*). The catch crops were sown at the beginning of August and ploughed in autumn in 2005, 2006 and 2007. Then the main crop, spring wheat (*Triticum aestivum* L.), was grown in 2006, 2007 and 2008. Soil samples were taken twice a year, before spring wheat sowing (March or April) and after harvesting (August). Generally, catch crops significantly influenced DH and CAT activities in all of the years of the investigation, while FDAH activity was affected only in 2007. There were higher DH and FDAH activities in catch crop treatment versus the control and only in some cases in field pea than in oilseed radish when both catch crops treatments were compared. DH and CAT activities were always higher in August than in March or April. Nitrogen fertilization influenced DH and FDAH activities only in 2007, while CAT activity was influenced during the entire experimental period (2006–2008). Generally, the highest CAT activity was noted at N rates of 80 or 120 kg ha^{-1} $year^{-1}$. Chemical properties (organic carbon, total nitrogen, pH 1 M KCl) were not significantly affected by catch crops and inorganic N fertilization treatments. The studied indices of microbial biomass were significantly correlated with chemical soil properties.

Key words: catalase, dehydrogenase, fluorescein diacetate hydrolysis, green manure, *Luvisol*, mineral N fertilization.

Introduction

A significant decrease in the application of organic fertilizers has been observed due to the present economic circumstances of Poland, which has caused a decrease in the stock of farm animals (Statistical yearbook..., 2011). Many farmers have given up animal production and plants are now fertilized mostly with mineral fertilizers. Consequently, the depletion of soil organic matter and a decrease in soil fertility has occurred. Another consequence of the low animal stock is the small area planted with fodder plants and the domination of cereals and oilseed rape in the crop rotation, which have a low coefficient of soil organic matter reproduction. One of the management practices aimed at limiting the worsening of soil properties in simplified crop rotation is catch crops treatment and the use of their biomass as green manure. The benefits of crop rotation and green manuring in maintaining soil organic matter level have been established (Constantin et al., 2010). Some researchers have studied the influence of catch crops cultivated for green manure on soil biological properties (Kosteckas, Marcinkevičienė, 2009; Arlauskienė, Maikštėnienė, 2010). A rapid increase in soil microorganisms content and activity occurs after a green manure crop is incorporated in the soil thereby increasing soil biological activity (Melero et al., 2006). Although a large number of researchers have examined how nitrogen affects microbial biomass content and activity (e.g., Treseder, 2008; Ramirez et al., 2010), we still know very little about how different sources and forms of N may impact microbial biomass, since contradictory results have been obtained. Adding N to soil can affect microbial activity directly by increasing N availability or as a result of indirect effects of the fertilizer inputs on other soil chemical properties (e.g., changes in pH or concentration of ions other than N) (Treseder, 2008). The positive or negative effect of fertilization on microbial biomass depends on many factors such as the composition of the microbial community, soil type and fertility, soil physico-chemical properties, the form and the rate of the fertilizer and the time it is applied.

The microbial biomass is considered to be an important and labile fraction of soil organic matter that is involved in energy and nutrient cycling (Li et al., 2009). Soil biota size and activity are directly related to the amount and quality of carbon and other nutrients that are available from plant residues and organic amendments (Okur et al., 2008). Some criteria for estimating overall microbial activity in soil have been proposed. Direct cell counting, soil microbial biomass carbon and nitrogen content, dehydrogenase and catalase activity, soil respiration, fluorescein diacetate hydrolysis activity and adenosine triphosphate content have become those studied most often (Benedetti, Dilly, 2006; Green et al., 2006). Dehydrogenase and catalase are intracellular enzymes that are involved in the microbial, oxidoreductase metabolism (Gianfreda, Ruggiero, 2006). Dehydrogenase activity (EC 1.1.) is known to oxidize soil organic matter by transferring protons and electrons from substrates to acceptors. Since dehydrogenase activity reflects the total range of oxidative activity of soil microflora, it can be used as a good indicator of total microbiological activity (Kızılkaya, Hepşen, 2007). Catalase (hydrogen-peroxide oxidoreductase, EC 1.11.1.6.) is found mainly in all aerobic bacteria and most facultative anaerobes, although it remains active outside of a cell due to its association with organic matter and/or sorption on clay minerals (Nannipieri et al., 2002). Catalase activity is considered to be an indicator of aerobic microbial activity and soil fertility (Włodarczyk et al., 2001). The soil global hydrolase activity is evaluated by measuring the hydrolysis rate of fluorescein diacetate hydrolysis (FDAH). This method is considered to be a suitable index of the overall enzyme and microbial activity in soil because the hydrolysis of FDA is carried out by active cells with a variety of enzymes, including proteases, lipases and esterases (Green et al., 2006). It has been stated that the properties related to microbial biomass size and activity are more dynamic and respond more quickly to changes in crop management practices or environmental conditions than do properties such as total soil organic matter. Therefore, they are often used as sensitive indicators of organic matter turnover (Tejada et al., 2008).

The aim of the study was to assess the influence of catch crops grown for green manure and increasing mineral N fertilization rates on the activity of the soil microbial biomass measured by DH, CAT and FDAH activities. We hypothesized that: 1) biochemical variables related to microbial activity such as DH, CAT and FDAH activities would be improved by field pea (*Pisum sativum* L.) and oilseed radish (*Raphanus sativus* var. *olifera* L.) grown as catch crops, compared to the control, 2) the activities of the DH, CAT and FDAH would increase with increasing rates of N fertilization and 3) significant relationships between the studied enzymes and chemical properties of soil could be expected.

Material and methods

Site description and experimental design. The effect of catch crops and inorganic nitrogen (N) fertilization treatments on soil biological activity indices was investigated in a 3-year (2005–2008) experiment. The two-factor, split-plot design experiment with four replications was carried out at the Experimental Station in Mochełek near Bydgoszcz, Midwestern Poland. The soil of the field experiment was albic, cutanic *Luvisols* (*LVab*, *LVct*), that have the following pattern: Ap-Eet-Bt-C (IUSS Working Group WRB, 2007), with a fine sandy loam texture (clay 5%, sand 70%, silt 23%). The first factor was the N fertilization with NH_4NO_3 that was applied before and during the spring wheat growth period: the control (without N fertilization), 40 kg ha^{-1} N (before sowing), 80 kg ha^{-1} N (40 kg before sowing and 40 kg in the phase of the 1–2 nodes), 120 kg ha^{-1} N (60 kg before sowing and 60 kg in the phase of the 1–2 nodes), 160 kg ha^{-1} N (60 kg before sowing, 60 kg in the phase of the 1–2 nodes and 40 kg before heading). The following catch crops treatment: field pea (*Pisum sativum* L.), oilseed radish (*Raphanus sativus* var. *olifera* L.), and the control without a catch crop was the second factor. The forecrop for plants grown as catch crops was spring barley (*Hordeum vulgare* L.). After the harvesting of spring barley, the straw was removed from the field and the soil was ploughed. Before the catch crops were sown the soil was cultivated using a cultivator with a roller. Catch crops were sown within the period of 3–11 of August in 2005–2007. No fertilization was applied before and during catch crops growing. The catch crops were harvested at the beginning of October and November every year (2005–2007). After harvesting, the green biomass of catch crops was chopped, spread on the soil surface and ploughed in. Spring wheat (*Triticum aestivum* L.) was sown between 30th of March and 11th of April of the following years (2006–2008) in the fields with incorporated catch crops biomass as well as in the control fields. Nitrogen fertilization was applied according to the experimental design. Moreover, phosphorus and potassium were broadcast before spring wheat sowing at the doses of 28.4 kg ha^{-1} P (as $(NH_4)_3PO_4$) and 74.7 kg ha^{-1} K (as KCl, 60% of K) and mixed with soil by means of a cultivator with a crumbler roller. Soil samples were collected twice a year, before spring wheat sowing (at the beginning of April in 2006 and at the end of March in 2007 and 2008) and after spring wheat harvesting (August 2006–2008). Soil samples were collected from a depth of 0–30 cm with an Egner's sampling stick. On each plot, 10 samples were collected randomly and bulked to provide one representative sample per plot. The site is characterized by a moderate climate with an average annual temperature over the study period of about 8.6°C and an average annual rainfall of 527 mm.

Analysis of soil properties. Dehydrogenase (DH) was determined according to Thalmann (1968) after soil incubation with 2,3,5-triphenyl-tetrazolium chloride and a measurement of triphenylformazan (TPF) absorbance at 546 nm and was expressed as mg of TPF kg^{-1} 24 h^{-1}. Catalase (CAT) activity was measured by manganometric titration of the surplus of H_2O_2 under acidic conditions according to the procedure of Johnson and Temple (1964). The CAT activity was expressed as mg H_2O_2 g^{-1} h^{-1}. Total soil hydrolytic activity was evaluated by measuring the activity of fluorescein diacetate hydrolysis (FDAH) as described by Adam and Duncan (2001). One unit of FDAH activity was defined as mg of fluorescein (F) produced at 37°C for 1 hour by 1 kg of dried soil (mg F kg^{-1} h^{-1}). The microbial activity assays were performed with field-moist soil and calculated on the basis of the oven-dry (105°C) soil weight. Triplicates were performed for each activity assay. Total nitrogen (N_{tot}) in the soil was determined by the Kjeldahl method. Soil organic carbon (C_{org}) content was determined using the dichromate oxidation procedure, while soil pH (1 M KCl) was measured using the potentiometric method in 1:2.5 soil:solution suspensions.

Statistical analysis. An *ANOVA* test was performed on the results and analyses were carried out using *Statistica 8.1* for *Windows* software. All studied properties data follow normal distribution according to the Shapiro-Wilk test. A two-way analysis of variance was performed to examine the main effect of N fertilization rates and the type of catch crop on the studied enzyme activities. The first factor (N fertilization) was applied at five levels, while the second factor (catch crops) was given in three levels. When significant treatment effects were found, Tukey's test was used to compare treatment means. Means were considered significantly different at $P < 0.05$. Relations between the enzymatic activity and the chemical parameters were estimated by correlation analysis based on Pearson's correlation coefficients ($P < 0.05$).

Results and discussion

Chemical properties. Generally, neither catch crop nor N fertilization treatments influenced chemical properties (total nitrogen, organic carbon, pH_{KCl}) significantly ($P < 0.05$). That is why we did not present the data in a table. Total N content ranged from 0.63 to 0.75 g kg^{-1}, with a mean value of 0.68 g kg^{-1}, while C_{org} ranged from 6.28 to 8.0 g kg^{-1}, with a mean value of 7.07 g kg^{-1} (mean values of the period 2006–2008). The average pH_{KCl} in 2006 was 6.7 in March and 6.3 in August, while in 2007 and 2008 pH_{KCl} values were similar in spring and summer samples, and the mean values were 6.1 and 5.5, respectively.

Temporal changes of studied enzymes. The properties studied displayed clear temporal changes. All of the studied parameters were generally higher in the summer samples as compared to the spring samples for each of the catch crop treatments (Tables 1–3). They also differed significantly in each year of the study (2006–2008) (FDAH data presented only for 2007). The seasonal changes can be explained by the enhanced rhizosphere microbial population that accompanies the rapid development of the crop root system during the growing period (Weisskopf et al., 2008). The release of nutrients into the soil from decaying organic matter with a high contribution of catch crops biomass would increase microbial activity even further and could account for the continuing increase in enzymatic activity (Rodríguez-Kábana, Truelove, 1982). Moreover, it is commonly known that weather conditions, especially rainfall and temperature, have a great impact on soil biological properties (Niu et al., 2012). All of the properties studied showed a higher activity in August when the temperature was higher than in March or April. However, we did not find any marked interaction between the rainfall level and microbial biomass data in the subsequent years of the experiment (data not presented).

Table 1. Dehydrogenase (DH) activity (mg TPF kg^{-1} 24 h^{-1}) as influenced by the catch crop and N fertilization (kg ha^{-1}) treatments

Year	Month	N fertilization (1st factor)	Catch crop (2nd factor)			
			field pea	oilseed radish	control	mean
2006	March		5.2 a	5.0 a	5.1 a	5.1
	August	0	7.8 aA	7.5 aA	7.2 aA	7.5 A
		40	8.5 aA	8.1 aA	7.2 aA	7.9 A
		80	9.1 aA	8.4 aA	9.2 aA	8.9 A
		120	9.9 aA	8.6 abA	7.4 bA	8.6 A
		160	10.2 aA	9.1 abA	7.3 bA	8.9 A
		mean	9.1 a	8.3 ab	7.7 b	8.4
2007	March		20.1 a	12.1 b	10.3 b	14.2
	August	0	28.7 bAB	43.0 aA	16.5 cA	29.4 A
		40	38.5 aA	33.7 aAB	16.8 bA	29.7 A
		80	30.0 aAB	32.5 aAB	22.6 bA	28.4 A
		120	26.7 aAB	20.0 bBC	18.2 bA	21.6 B
		160	23.0 aB	22.8 aB	14.0 bA	19.9 B
		mean	29.4 a	30.4 a	17.6 b	25.8
2008	March		30.7 a	27.5 ab	23.5 b	27.2
	August	0	40.0 aA	38.3 aA	28.7 bA	35.7 A
		40	42.9 aA	40.2 aA	33.9 bA	39.0 A
		80	50.4 aA	43.4 abA	39.4 bA	44.4 A
		120	37.7 aA	34.2 abA	29.2 bA	33.7 A
		160	32.3 aA	34.2 aA	28.4 aA	31.6 A
		mean	40.7 a	38.1 a	31.9 b	36.9

Notes. TPF – triphenylformazan. Lowercase letters indicate comparison among catch crops treatment (within the same N fertilization rate in August); values followed by the same lowercase letters within each row do not differ significantly at $P < 0.05$. Uppercase letters indicate comparison among N fertilization rates within the same catch crop; values followed by the same uppercase letters within each column do not differ significantly at $P < 0.05$.

The influence of catch crops and N fertilization on the studied enzymes. The results showed that catch crops applied as green manure and N fertilization may greatly alter soil microbial biomass activity as measured by DH and CAT activities (Tables 1–2 and 4–5). Incorporation of catch crops did not affect DH activity in March 2006 compared to the control. There was a significantly higher DH activity in March 2007 in field pea as compared to oilseed radish and control (Table 1). In the last year of the investigation (March 2008), DH activity in field pea was significantly higher than in the control. In August 2006, the soil DH activity was significantly affected by catch crops treatment with 120 and 160 kg ha^{-1} $year^{-1}$ N only. In 2007 and 2008, DH activity was usually significantly higher in both catch crops treatments than in the control, except for the soil fertilized with 160 kg ha^{-1} $year^{-1}$ N in 2008 (Table 1). DH activity appeared to be more sensitive to the catch crops treatment studied here than the FDAH activity, probably due to its being associated with a viable microbial population (Nannipieri et al., 2002). The results of our study confirm that the microbial biomass was more active in the plots with catch crops, mainly because of the contribution of plant residues incorporated into the soil.

Table 2. Catalase (CAT) activity (mg H_2O_2 g^{-1} h^{-1}) as influenced by the catch crop and N fertilization (kg ha^{-1}) treatments

Year	Month	N fertilization (1st factor)	Catch crop (2nd factor)			
			field pea	oilseed radish	control	mean
2006	March		15.4 a	16.6 a	15.7 a	15.9
	August	0	21.5 aB	18.4 bC	20.6 aA	20.2 A
		40	23.4 aB	21.5 bB	21.3 bA	22.1 A
		80	26.3 aA	24.3 bA	22.3 cA	24.3 A
		120	23.6 aB	21.1 bB	21.5 bA	22.1 A
		160	23.8 aB	21.5 bB	21.1 bA	22.1 A
		mean	23.7 a	21.4 b	21.4 b	22.2
2007	March		21.2 ab	22.2 a	20.5 b	21.3
	August	0	28.9 aC	24.5 bC	22.9 bC	25.4 C
		40	31.9 aB	27.7 bB	25.2 cB	28.3 A
		80	34.2 aA	28.9 bA	25.8 cB	29.6 A
		120	31.6 aB	29.6 bA	27.3 cA	29.5 A
		160	28.3 aC	24.2 bC	25.8 bB	26.1 B
		mean	31.0 a	27.0 b	25.4 b	27.8
2008	March		27.3 a	25.0 a	24.0 a	25.9
	August	0	32.6 aC	27.7 bC	24.3 cB	28.2 C
		40	35.3 aBC	31.8 bAB	30.6 bA	32.6 B
		80	40.7 aA	43.6 bA	32.4 cA	38.9 A
		120	37.1 aA	34.4 bA	31.5 cA	34.3 A
		160	32.0 aC	30.6 abBC	28.9 bA	30.5 BC
		mean	35.5 a	33.6 a	29.5 b	32.9

Explanations under Table 1

Table 3. Fluorescein diacetate hydrolysis (FDAH) activity (mg F kg^{-1} h^{-1}) as influenced by the catch crop and N fertilization (kg ha^{-1}) treatments

Year	Month	N fertilization (1st factor)	Catch crop (2nd factor)			
			field pea	oilseed radish	control	mean
2007	March		23.5 a	25.2 a	29.2 a	26.0
	August	0	27.7 aAB	27.9 aB	24.0 bB	26.5 B
		40	32.5 aA	31.3 abA	28.6 bAB	30.8 AB
		80	35.1 aA	34.2 aA	32.7 aA	34.0 A
		120	31.0 aA	30.3 abAB	27.2 bAB	29.5 B
		160	23.8 aB	27.1 aB	23.9 aB	24.9 B
		mean	30.0 a	30.2 a	27.3 b	29.2

Explanations under Table 1

Table 4. Statistical differences (*F*-values and significance level) between means of variables by one-way *ANOVA* with factor catch crops (d.f. = 2)

Enzymes	Years (March)		
	2006	2007	2008
Dehydrogenase	0.166 n.s.	7.995*	1.423 n.s.
Calatase	1.214 n.s.	5.542*	2.093 n.s.
Fluorescein diacetate hydrolysis	1.386 n.s.	1.723 n.s.	0.483 n.s.

d.f. – degree of freedom, n.s. – not significant; * – $P < 0.05$

Generally, the fertilization treatment had a weaker impact on DH compared to the catch crops (Tables 1 and 5). The activity was affected only in the plots with field pea and oilseed radish catch crops in 2007. The results of some authors suggest that DH activity is sensitive to the activatory and/or inhibitory effects associated with an increasing amount of applied N fertilizers (Goyal et al., 1992; Klikocka et al., 2012), which is in disagreement with our results, which showed that DH was poorly influenced by mineral N fertilization rates. In other studies, the DH activity was also poorly influenced by mineral N fertilization. Thus, in two different regimes of organic manuring on a sandy arable soil (Kautz et al.,

Table 5. Statistical differences (F-values and significance level) between means of variables by two-way *ANOVA* with factors catch crops and N fertilization rates (August 2006–2008)

Enzymes	Year	N fertilization (d.f. = 4)	Catch crops (d.f. = 2)	Interactions: catch crops × N fertilization (d.f. = 8)
Dehydrogenase	2006	3.571 n.s.	5.157*	1.116 n.s.
	2007	7.684**	31.669***	3.988**
	2008	3.130 n.s.	4.110*	0.164 n.s.
Calatase	2006	9.388**	16.554***	1.062 n.s.
	2007	33.789***	29.326***	1.199 n.s.
	2008	14.810***	28.694***	0.992 n.s.
Fluorescein diacetate hydrolysis	2006	1.562 n.s.	3.276 n.s.	1.116 n.s.
	2007	60.000***	12.301***	1.071 n.s.
	2008	2.825 n.s.	3.488 n.s.	1.408 n.s.

d.f. – degree of freedom, n.s. – not significant; * – $P < 0.05$, ** – $P < 0.01$, *** – $P < 0.001$

2004) and in sandy loam under maize (Marinari et al., 2000), mineral N fertilizer had weaker effects on DH activity than organic manuring. According to Marinari et al. (2000), this might be due to the fact that mineral N additions are rapidly dispersed into the soil organic matter or are lost by leaching without affecting soil biological activity. As stated by Melero et al. (2006), no effect of different N fertilization rates (0, 50 and 150 kg ha^{-1} year^{-1} N) was found on DH activity. According to Goyal et al. (1992), the low response of DH activity to N doses may be due to the interference of nitrates, which serve as an alternate electron acceptor, resulting in low activities at higher rates of N fertilizer application. As a consequence, using DH activity as a microbial indicator in soil with high doses of N fertilizers is not recommended by some authors (Okur et al., 2008).

The lowest CAT activity was noted in 2006 and in the succeeding years the activity was higher (Table 2). In 2006 and 2007, higher CAT activity in August was found with the field pea catch crop, lower with the oilseed radish catch crop and in the control. In 2008, CAT activity in the control was significantly lower than after both catch crops. Soil CAT activity changed significantly as a function of N fertilization treatments and in the entire experiment period (2006–2008). Increasing rates from 0 to 80 or 120 kg ha^{-1} year^{-1} N gradually increased the CAT activity in catch crop treatments. The highest CAT activity was noted at the rate of 80 kg ha^{-1} year^{-1} N in 2006, while in 2007 and 2008, it was recorded at a dose of 80–120 kg ha^{-1} year^{-1} N. The highest N fertilization rate (160 kg ha^{-1} year^{-1} N) decreased the CAT activity in all of the years and for both catch crops, compared to the lower (80–120 kg ha^{-1} year^{-1} N) N fertilization rates. In the control, the influence of N fertilization on CAT activity in 2007 was similar to that after catch crops, while in 2006 this parameter was unaffected by N rates. As stated by Rodríguez-Kábana and Truelove (1982), the CAT activity was less sensitive to added N fertilization than to legume rotation components. The addition of inorganic N (67 kg ha^{-1}) to the plots with winter legumes and PK fertilization resulted in a small but significant reduction in CAT activity. Furthermore, the same authors stated that the elimination of a winter legume from the otherwise complete (NPK) fertilization regime resulted in a drastic reduction in CAT activity. This suggested that the major component that influences CAT activity was the plant biomass incorporated into the soil and not the fertilization regime.

FDAH activity was significantly influenced by the rate of N fertilization and catch crops treatments only in August 2007 (Tables 3–5). The small variations in FDAH activity displayed in two of the three studied years were expected. This activity is, after all, a measure of the overall hydrolytic ability of a soil and as such, it reflects the positive and negative contributions due to the different hydrolytic enzyme activities (Piotrowska et al., 2006). Earlier Bandick and Dick (1999) reported that FDAH activity is relatively stable between seasons and averaged across the sampling period did not show any statistically significant differences. Similar data was found in this research, where the results obtained in March or April were almost at the same level as in August (data presented only for 2007). The highest FDAH activity in 2007 was noted with 40–120 kg ha^{-1} year^{-1} N in plots with field pea and with 80 kg ha^{-1} year^{-1} N in oilseed radish and the control. Significantly higher FDAH rates were obtained with field pea catch crops versus the control with the 0, 40 and 120 kg ha^{-1} year^{-1} N, while at rates of 80 and 160 kg ha^{-1} year^{-1} N, there were no significant differences between field pea, oilseed radish and the control.

To summarize, the highest activity of the microbial properties studied was noted in the treatments where catch crops were applied as green manure together with moderate mineral N fertilization (80–120 kg ha^{-1} year^{-1} N). Such conditions were also optimal for yielding of spring wheat (Wilczewski, 2013). This suggested the great significance of joint organic and mineral fertilization for optimal soil biological activity and consequently for better conditions for plant growth and yield. In fact, as stated by Shah et al. (2009), the application of combined mineral (75% of manurial components mass) and organic (25% of manurial components mass) fertilization significantly increased the yield of wheat grain and straw compared to 100% of organic or mineral fertilizers applied separately. Microbial biomass is considered to be a better indicator of any of soil alteration than organic matter (C_{org}), as it responds to changes in soil management more rapidly and sensitively (Okur et al., 2008). In fact, both catch crop and N fertilization treatments, in contrast to microbial biomass activity indices, did not significantly influence N_{tot} and C_{org} concentrations in this experiment.

Correlation among the studied properties. According to the linear regression analysis, the indices of soil biomass activity were significantly correlated with some chemical properties (Table 6).

Table 6. Correlation matrix between soil enzyme activities and chemical properties in experimental period (2006–2008)

Month		C_{org}	N_{tot}	pH_{KCl}
March (April 2006) (n = 36)	dehydrogenase calatase	–	–	0.534
	fluorescein	0.566	0.356	–
	diacetate hydrolysis	–	0.555	–
August (n = 90)	dehydrogenase calatase	0.696	0.378	0.567
	fluorescein	0.756	0.324	0.458
	diacetate hydrolysis	0.624	0.430	0.539

C_{org} – organic carbon, N_{tot} – total nitrogen; only coefficients significant at $P < 0.05$ are presented

In March (April 2006) CAT activity was significantly correlated with C_{org} and N_{tot}, while FDAH activity was significantly correlated with N_{tot} content ($P < 0.05$). All enzyme activities in August were significantly correlated with C_{org}, N_{tot}, and pH_{KCl} values. In addition, the indices of soil microbial biomass activity were significantly and positively correlated with each other ($r^2 = 0.598–0.629$) (Fig.).

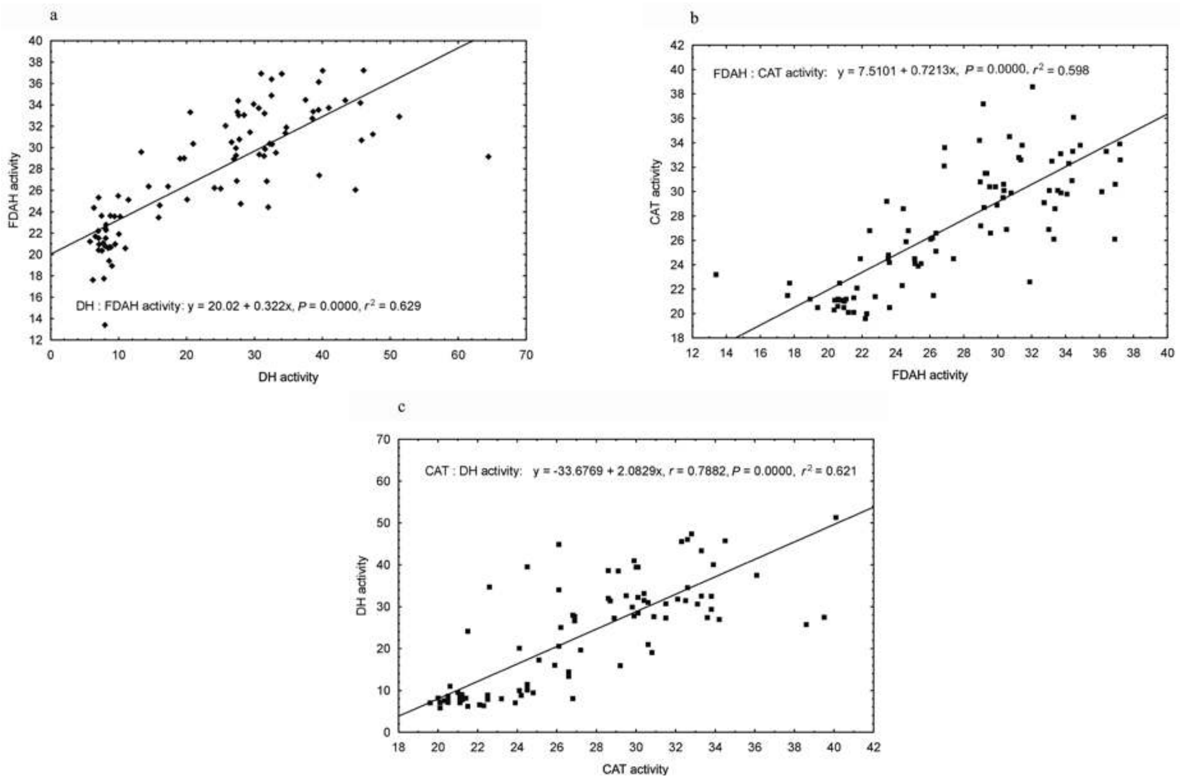

Figure. The relationship between dehydrogenase (DH) and fluorescein diacetate hydrolysis (FDAH) activities (A), FDAH and catalase (CAT) activities (B) and CAT and DH activities (C) (average values for August 2006–2008)

Soil C_{org} and N are among the most important soil properties that may considerably influence the activities of soil enzymes since they reflect the level of organic matter (Gianfreda, Ruggiero, 2006). That is why soil enzyme activities are expected to be positively related to soil organic matter. Catch crops and N fertilization treatments can enhance soil microbial biomass content and activity by increasing the organic matter content in soil. The other expected correlations were those between pH_{KCl} and microbial biomass activity since each enzyme shows a characteristic pH-dependent activity profile, an optimum pH for its maximum activity and a specific stability, which is related to soil pH (Gianfreda, Ruggiero, 2006).

Conclusions

1. Catch crops grown for green manure significantly increased soil biological activity during the growing period of spring wheat within the three experimental years, whereas the impact of field pea appeared to be not much better than that of oilseed radish. Therefore, it can be concluded that the cultivation of both

catch crops studied may be considered a good practice in helping to increase soil biological activity, which is a measure of soil fertility.

2. The catalase (CAT) activity was the only property clearly influenced by nitrogen (N) fertilization, while dehydrogenase (DH) and fluorescein diacetate hydrolysis (FDAH) activities were changed significantly only in one of the three experimental years. Generally in the treatments with catch crops, the CAT activity increased significantly with increasing N fertilization rates up to 80 or 120 kg ha^{-1} year^{-1} N and then decreased markedly. We can therefore conclude that the combined effect of catch crops applied as green manure and moderate mineral N fertilization was better for soil microbial biomass activity than that of only green manure or N fertilization and that this can be recommended as a means of increasing soil biological activity.

3. As compared to FDAH, both oxidoreductase activities were more sensitive to the presence of catch crops, while only CAT activity reacted significantly to the N fertilization rates within the entire study period. That is why the DH and CAT activities appeared to be better indicators of the influence of the factors studied on soil biological activity compared to the FDAH activity.

Acknowledgments

The authors thank the University of Technology and Life Sciences in Bydgoszcz for supporting this work. Much gratitude is due to Michele Simmons for proofreading the article.

Received 22 05 2013
Accepted 31 10 2013

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ISSN 1392-3196 / e-ISSN 2335-8947
Zemdirbyste-Agriculture, vol. 101, No. 2 (2014), p. 139–146
DOI 10.13080/z-a.2014.101.018

Fermentų veiklos pokyčiai dirvožemyje, priklausomai nuo žaliajai trąšai auginamų augalų ir tręšimo mineraliniu azotu

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Santrauka

Mikroorganizmai turi didelės reikšmės dirvožemio maisto medžiagų apykaitai, todėl jie yra svarbus tyrimų objektas. Tyrimas buvo atliekamas trejus metus (nuo 2005 m. rugpjūčio iki 2008 m. rugpjūčio mėn.). Jo metu vertinta tarpinių augalų aliejinių ridikų (*Raphanus sativus* var. *olifera* L.) bei sėjamųjų žirnių (*Pisum sativum* L.) ir tręšimo neorganiniu azotu (N) 0, 40, 80, 120 bei 160 kg ha^{-1} metai^{-1} įtaka dirvožemio dehidrogenazės, katalazės ir fluoresceino diacetato hidrolizės veiklai pajaurėjusiame išplautžemyje (IDe). Tarpiniai augalai buvo pasėti rugpjūčio pradžioje ir užarti 2005, 2006 bei 2007 m. rudenį. Pagrindiniai augalai vasariniai kviečiai (*Triticum aestivum* L.) auginti 2006, 2007 ir 2008 m. Dirvožemio ėminiai imti du kartus per metus prieš vasarinių kviečių sėją (kovo arba balandžio mėn.) ir po derliaus nuėmimo (rugpjūčio mėn.). Tarpiniai augalai turėjo didelės įtakos dehidrogenazės ir katalazės veiklai visais tyrimų metais, o fluoresceino diacetato hidrolizės veiklai – tik 2007 m. Didesnė dehidrogenazės ir fluoresceino diacetato hidrolizės įtaka buvo nustatyta laukeliuose su tarpiniais augalais, palyginus su kontroliniu variantu, ir tik kai kuriais atvejais – žirnių laukelyje, palyginus su ridikų. Dehidrogenazės ir katalazės veikla visuomet buvo intensyvesnė rugpjūčio mėnesį, palyginus su kovo arba balandžio. Tręšimas azotu dehidrogenazės ir fluoresceino diacetato hidrolizės veiklai turėjo įtakos tik 2007 m., o katalazės – visą tyrimų laikotarpį (2006–2008 m.). Didžiausia katalazės veikla nustatyta patręšus 80 arba 120 kg ha^{-1} metai^{-1} azoto. Dirvožemio cheminėms savybėms (organinei angliai, suminiam azotui, pH_{KCl}) tarpiniai augalai ir tręšimas neorganiniu azotu didelės įtakos neturėjo. Tirti mikrobų biomasės rodikliai esmingai koreliavo su dirvožemio cheminėmis savybėmis.

Reikšminiai žodžiai: dehidrogenazė, išplautžemis, fluorescino diacetato hidrolizė, katalazė, tręšimas mineraliniu azotu, žalioji trąša.