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Effect of organic and mineral fertilizers and land management on soil enzyme activities

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Abstract. Sustainable and rational management of agrophytocenoses depends on various bio-indices and methods of application, particularly the development and protection of soil resources (Lai et al., 2002). Among other indices, enzyme activity is proposed as a universal index of soil fertility and contamination (Dilly et al., 2003). To ascertain and to make a comparison of bioactivity variation during the vegetation period, soil (*Endophypogleyi-Eutric Planasols-Ple-gln-w*, artificial drainage) samples were collected from rotation fields of different fertilizing and farming systems (intensive (IF) and organic (OF)) at the Training farm of the Lithuanian University of Agriculture during 2007–2008. N application stimulated urease and saccharase activity in different farming systems (OF and IF) and fertilizing management (manure and mineral fertilizers). When comparing mean soil bioactivity values of 2 years the highest manure effect was detected in the application year (winter wheat treatment) and conditioned the highest urease (8.21 mg NH_4^+–N g^{-1}) and saccharase (24.52 mg CG g^{-1} 24 h^{-1}) activity but gradually decreased later. The lowest mean of urease (3.62 mg NH_4^+–N g^{-1}) and saccharase (22.07 mg CG g^{-1} 24 h^{-1}) activity occurred in IF soil where mineral fertilizers were applied. Soil bioactivity properties (urease and saccharase activity) were positively correlated with soil nutrients (Corg., Ntotal). Urease and saccharase activity properties reflect changes of fertilizer type and management and thus can be used as bio-indicators of soil fertility.

Key words: urease, saccharase, fertilizer, farming system

INTRODUCTION

Soil fertility plays an important role in the sustainable development of the terrestrial ecosystem. Recently, concern regarding the long-term productivity and sustainability of agro-ecosystems is centered around various bio-indices and the application of biological methods, particularly the development and protection of soil resources (Svirskienė, 2003). Nowadays, characteristic of soil fertility is integrating vitality of biota responsive to environment changes (Dilly et al., 2007). Biota governs the soil role in metabolism processes of materials and energy in ecosystems and represents an integrated index of soil physical-chemical conditions (Schutter & Fuhrmann, 2001). Soil enzymes are produced by plants, animals and microorganisms, and may be present in dead cells and cell debris and also adsorbed by clay or incorporated into humic substances (Allison, 2005). Hydrolytic enzymes make nutrients available to plants and soil microorganisms from a wide range of complex substrates and are influenced by a wide range of soil properties such as pH, organic

matter and texture, and also by farming management and anthropogenic impacts (Joanisse et al., 2008; Li et al., 2008). Saccharase and urease are related to the C and N cycles, which are the fundamental factors in forming soil fertility (Dilly et al., 2007). Among other indices, enzyme activity is proposed as an index of soil fertility or contamination (Nannipieri et al., 2000; Li et al., 2008). These enzymes are non-cellular and persist for a long time in the soil matrix though they are sensitive to abiotic factors, especially to fertilizers (Zakarauskaitė at al., 2008). Some references pointed out an increase in the abundance of microorganisms and of some enzyme activity in case of applying of organic and mineral fertilizers (Li ir kt., 2008). Past studies have shown that large doses (> 120 kg ha^{-1}) of mineral fertilizers as well as cultivated crops change the composition and abundance of microorganisms (Monokrousos et al., 2006). Therefore, it is important to evaluate enzyme activity in specific soils and different farming systems.

The aim of this work was to ascertain the impact of manure and mineral NPK fertilizers on enzyme activity related to the C and N cycles urease and saccharase under the effect of applying of organic and intensive farming in short-term period on *Planasols*.

MATERIALS AND METHODS

Bulk soil samples were collected from differently fertilized rotation fields and managed farming systems (intensive (IF) and organic (OF)) at Training Farm (Lithuanian University of Agriculture) during 2007–2008 to ascertain and compare changes of soil bioactivity. Medium amount of humus (2.44%), high amounts of P_2O_5 (214.9 mg kg^{-1}) and K_2O (172.6 mg kg^{-1}) were estimated in treated soil (*Endophypogleyi-Eutric Planasols*, artificial drainage) at Training Farm (FAO WRB, 2006; Pekarskas et al., 2008). Winter wheat (*Triticum aestivum*), spring cereals + undercropped clover (*Trifolium repens*); the 1st yr clover (*Trifolium repens*), the 2nd yr clover (*Trifolium repens*) + manure were the rotation crops during the experimental period in OF fields. Winter wheat (*Triticum aestivum*) was chosen in IF rotation (IF rotation composed of 8-rotation chains crops). Until 2006, after harvesting of the 2nd yr clover, the soil was manured with 40 t ha^{-1} in OF. As this dose is insufficient for crops, 80 t ha^{-1} manure was applied from 2007 in OF. $N_{120}P_{90}K_{90}$ rates of monomial fertilizers (ammonium saltpetre, (N – 34.4%); granulated super phosphate (P_2O_5 – 19%); potassium chloride (K_2O – 60%) were used in IF. P and K fertilizers were applied in early spring and N fertilizers – at the beginning of vegetation period.

Soil samples of Ap horizon 0–15(20) cm depth (2 replicates) were taken from each experimental site for estimation of enzyme activity on three occasions: in April (04), June (07) and August (08) in accordance with ISO 10 381–1: 2002. The soil was preconditioned for 15–20 d at laboratory temperature (approximately 22°C) before analysis. The content of organic C ($C_{org.}$) and total N (N_{total}) were determined as the main substrates for assayed enzymes. C_{org} was determined using the dry combustion method (ISO 13878) and N_{total} was measured as Kjeldahl nitrogen (ISO 10694).

Soil bioactivity was characterized by bioassay for hydrolytic enzyme activity in air-dried soil samples. All treatments and measurements were replicated three times. Saccharase (invertase) (EC 3.2.1.26) activity was measured according to the modified Hofmann and Seegerer method (Chundareva, 1973; Schinner et al., 1996). Urease (EC

3.5.1.5) activity was assayed according to the modified Hofmann and Schmidt spectrometric method. To determine urease activity, soil (5 g) was incubated for 24 h at 37°C with 1 ml of 0.08 M urea solution. In the controls, 1 ml deionised water was used instead of the urea solution. For the preparation of standards, 0, 0.25, 0.5, 1, 2.5, 4, 6, 8 and 10 ml of a 100-fold diluted 71.4 mM ammonium chloride solution were made up to 10 ml using 2 M KCl and then the NH_4^+ concentration was determined in aliquots of 0.5 ml, as reported above. Urease activity expressed in mg NH_4^+–N g^{-1} 24 h^{-1} and saccharase activity – in mg conventional glucose (CG) g^{-1} of air dried soil.

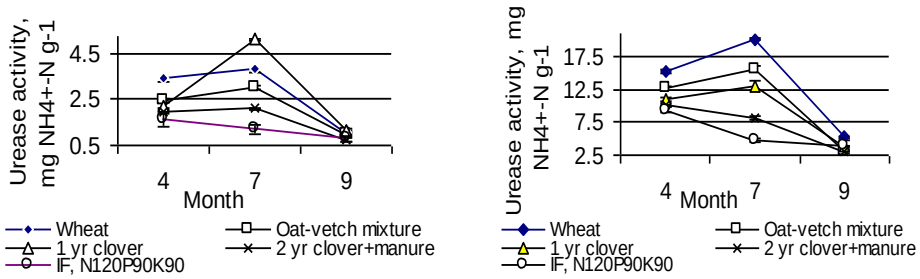
Statistical analysis

The confidence limits of the data were based on one–way analysis of variance by *ANOVA* (in case of significant interactions) followed by *post hoc Tukey theoretical criterion*. The least significant differences between treatment means were determined using Fisher's least significant differences (LSD_{05}). Standard deviation (*sd*) and correlation were calculated at level of statistical significance $P < 0.05$. Results of urease and saccharase activity are presented as a mean of 4 independent analyses at the 0.05 probability level.

RESULTS AND DISCUSSION

Urease responses to fertilizing management

Providing soil with organic N stimulates heterotrophic organisms, which are decomposing organic substrates, and the activity of hydrolytic enzymes (Dilly et al., 2007). Therefore manure fertilization in 2007 (OF, 40 t ha^{-1}) significantly increased urease activity (1.0–3.84 mg NH_4^+–N g^{-1}). This response confirms the results of Lai et al. (2002), Smith & Powlson (2003), who showed that the presence of readily-available organic N (manure) stimulated urease activity. $N_{120}P_{90}K_{90}$ application in IF affected significantly lower urease activity (1.65–0.86 mg NH_4^+–N g^{-1}) over the whole vegetation period as compared with all treatments (Fig. 1a).



a b

Fig. 1. Fluctuation of urease activity during 2007 (a) and 2008 (b) vegetation period (mean ± SD, $P < 0.05$)

Urease activity was higher at the beginning of vegetation than in autumn due to untapped resources of substrates and humidity in soil and increasing temperature. With the exception of IF, the peak value of urease activity was achieved in summer in other treatments (OF management) presumably due to the stimulatory effect of the improved vegetation conditions for enzyme activity (Sowerby et al., 2005). Urease activity was

significantly lower in autumn and ranged between 0.78–1.08 mg NH_4^+–N g^{-1}, in agreement with Sowerby et al. (2005) due to decreasing N resources and poorer vegetation conditions.

Urease activity was higher in 2008 than in 2007 due to the increase in the manure doze up to 80 t ha^{-1} and ranged widely between 4.78 and 20.25 mg NH_4^+–N g^{-1} (Fig. 1b). Tendencies of urease fluctuation during vegetation period were quite similar to fluctuation in 2007 in all treatments. Significantly higher urease activity (with exception of IF treatment) was recorded in summer and ranged between 4.78–20.25 mg NH_4^+–N g^{-1} as compared with that in spring and autumn. The effect of intensive mineralization of both organic and mineral N resources in the soil influenced the decrease in urease activity in the autumn; only 3.80–5.24 mg NH_4^+–N g^{-1} urease activity occurred in this period of vegetation.

In agreement with reports (Li et al., 2008), organic N application indicated a stimulatory effect on urease activity in OF system comparing with mineral fertilizing management in IF (Fig. 2). In comparison with 2-year mean values data the highest manure effect was detected in the year following its application (after the 2nd yr clover in winter wheat treatment) and conditioned the highest urease activity (2 year mean–8.21 mg NH_4^+–N g^{-1}), which gradually decreased later. Significantly lower mean urease activity (3.62 mg NH_4^+–N g^{-1}) occurred in IF soil where mineral fertilizers were applied. Urease activity variation confirmed that it could be used as an indicator reflecting differences in soil quality between organically and conventionally managed crop fields.

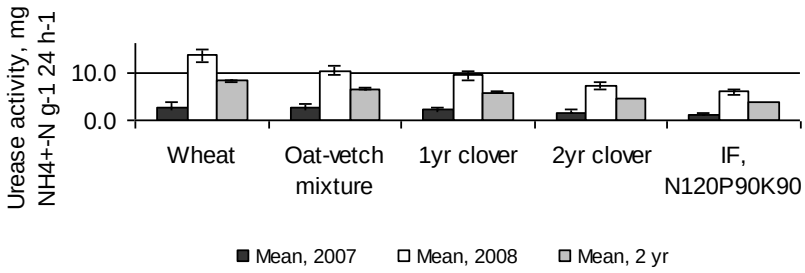
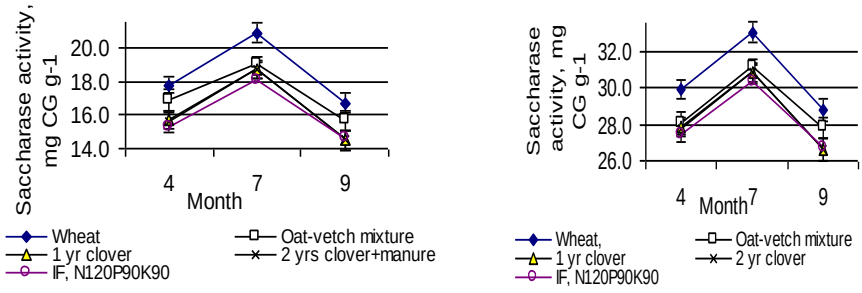


Fig. 2. Urease activity response on rotation and fertilizers (mean ± SD, $P < 0.05$).

Saccharase responses to fertilizing management

As reported by Marschner et al. (2003), a positive response in saccharase activity followed manure application (Fig. 3). Application of 40 t per ha manure in 2007 caused less saccharase activity (14.5–20.9 mg CG g^{-1} 24 h^{-1}) as compared with elevated manure application (80 t ha^{-1}) in 2008 (26.7–33.0 mg CG g^{-1} 24 h^{-1}). Values of saccharase activity showed high soil enrichment with enzyme in both experiment years due to high $C_{org.}$ content (1.70–1.72%). During vegetation, saccharase activity varied in the same way as urease.



a b

Fig. 3. Fluctuation of saccharase activity during 2007(a) and 2008 (b) vegetation period (mean ± SD, $P < 0.05$).

Univariate analysis of variance of 2007–2008 year data revealed significant differences of the negative impact of climate conditions in April (7.1-8.8°C; 22.2–32.1 mm precipitation) and September (12.1–12.8°C; 41.5–43.0 mm precipitation) on saccharase activity (respectively 15.3–29.9 and 14.5–29.9 mg CG g^{-1} 24 h^{-1}). Sufficient temperature (17.1–18.1°C) and humidity (147.1–43.0 mm) resulted the highest enzyme activity (18.1–33.0 mg CG g^{-1} 24 h^{-1}) in mid-summer in both experimental years. Significantly higher saccharase activity was recorded in wheat treatment after autumn application of manure. In all terms saccharase activity decreased according to gradient of increasing years after fertilizing with manure (Dodor & Tabatabai, 2003). In both years, the lowest enzyme activity was recorded in IF with mineral fertilizing. The correlation between saccharase activity and soil N_{total} and C_{org} was not significant.

When comparing 2-year mean values, the highest manuring effect was detected in the application year (winter wheat treatment) and conditioned the highest saccharase activity (2 year mean –24.52 mg CG g^{-1} 24 h^{-1}) but gradually decreased later (Fig. 4).

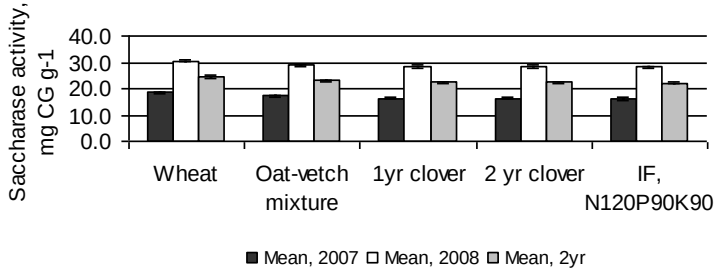


Fig. 4. Response of saccharase activity to rotation and fertilizing (mean ± SD, $P< 0.05$).

Significantly lower saccharase mean activity (22.07 mg CG g^{-1} 24 h^{-1}) occurred in IF soil, where only mineral fertilizers were applied, than in OF. The low activities in IF area could be explained by the fact that it had accepted small amounts of C_{org} (mean 1.66 %), very often resulting in low enzyme activities (Kremer & Li, 2003).

Enzyme activity responded differently to manure application and farming management. Therefore soil hydrolytic enzymatic activity was successfully used to

detect short–term changes in soil and can be used as an indicator under different farming management (Lagomarsino et al., 2009).

CONCLUSIONS

Hydrolytic enzyme activity responded to different forms of fertilizers, farming management and growth period. Decreasing activity (after manure apply after the 2nd clover) of hydrolytic enzymes (saccharase and urease) varied between the four OF soils studied. Mineral fertilisation resulted in the lowest enzyme activity presumably explained by the small amounts of organic residues (C_{org}) in the IF area. Urease and saccharase activity were higher at the beginning of vegetation than that in autumn and reached a peak value in mid-summer due to untapped resources of substrates in the soil and increasing temperature and humidity.

The responses of soil hydrolytic enzyme activity to nutrient addition detected short-term changes during the vegetation period and rotation in the soil and qualify them as indicators under different farming management.

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