

The influence of long-term application of mineral fertilizers on the biological activity of *Cambisols*

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Long-term fertilization trials were established in Lithuania on moraine sandy loamy *Epicalcari-Endohypogleyic Cambisol* in 1971 and limnoglacial silty loamy *Endocalcari-Epihypogleyic Cambisol* in 1990. This paper presents experimental data influence on the of long-term application of mineral NPK fertilizers on soil biological activity in the period 1999–2002.

The results show that in the soils developed on different glacial deposits the enzymatic activity and respiration intensity are different: in limnoglacial silty loamy *Cambisol* the urease activity is higher, while in moraine sandy loam activity of saccharase, dehydrogenases and soil respiration intensity are higher.

In sandy loamy *Cambisol* with permanent annual fertilization of 180 kg ha⁻¹ phosphorus in combination with nitrogen and potassium fertilizers, the content of available phosphorus in soil increases from 43–61 to 499–503 mg kg⁻¹ and therefore lowers the activity of enzymes. Thus, such fertilization is unacceptable from the ecological point of view. However, in silty loamy *Cambisol*, the change of available phosphorus in soil under intensive fertilization is insignificant, and changes of enzymatic activity are slight.

Mineral fertilizers inhibited the activity of oxidation–reduction enzyme dehydrogenases in both soils, while saccharase did it only in silty loamy cambisol. The highest soil respiration intensity expressed as carbon dioxide content was determined in sandy loamy *Cambisol* on fertilizing the agricultural plants with nitrogen and potassium fertilizers.

Key words: *Cambisol*, enzyme activity, soil respiration, mineral fertilizers

INTRODUCTION

Present-day concern regarding the long-term productivity and sustainability of agro-ecosystems is leading to the development and protection of soil resources (Kanchikerimath et al., 2001; Svirskienė, 2003). Biological activity of soil is an important factor of productivity from agricultural and ecological standpoints and a sensitive indicator of anthropogenic impact (Ros et al., 2003; Svirskienė, 2003; Shimek et al., 1999; Михайловская, 1997). Like enzymatic activity, microbial biomass and soil respiration respond more rapidly to changes in crop management practices or environmental conditions than do the agrochemical characteristics of soil (Dick, 1992; Ros et al., 2003). They are one of the most important and most precise indicators of biological activity (Janušienė, 1996; Trasar Cepeda et al., 2003; Крушельницкая, 2001; Grigaliūnienė et al., 2003).

Investigation data of many authors indicate rational fertilization with mineral and organic fertilizers to be one of the best

measures to improve the microbiological characteristics of soil and to stimulate its respiration intensity. It exerts a stronger influence on the activity of these processes and the yield of agricultural plants than does application of mineral fertilizers alone (Svirskienė, 2003; Чундарева, 1973; Zakarauskaitė et al., 2005). In scientific literature, there are different data on the systematic impact and high rates of application of mineral fertilizers, particularly on microbiological processes. Some authors state that at increasing rates of mineral fertilizers microbiological processes become more intensive, while others point out a negative impact of high rates on these processes (Schinner et al., 1995; Svirskienė ir kt., 1997; Чундарева, 1973). According to the data of Lugauskas et al. (1997), the use of high rates (120 kg ha⁻¹) of phosphorus for long time had a negative influence on microorganisms: it changed the ratio, number and species composition of the groups. The amount of residues left by perennial grasses is three times higher than that left by cereals and sugar beet. The ratio of carbon and nitrogen is very important; the lowest value

of this ratio has been estimated in clover residues and the highest one in the residues of winter wheat and oat (Svirskienė et al., 1997; Дудкин и др., 1987).

This research was undertaken to ascertain changes in soil urease, saccharase and dehydrogenases activity and soil respiration intensity under the effect of a long-term (31 and 10 years) application of mineral fertilizers in moraine sandy loamy and limnoglacial silty loamy *Cambisols*.

MATERIALS AND METHODS

Long-term fertilization trials were established in soils developed on different glacial deposits – moraine sandy loamy *Epicalcari-Endohypogleyic Cambisol* (further in the text sandy loamy *Cambisol*) in 1971 (Skėmiai, Radviliškis district) and limnoglacial silty loamy *Endocalcari-Epihypogleyic Cambisol* (further in the text silty loamy *Cambisol*) in 1990 (Kriūkai, Šakiai district). In the soil texture of the ploughing (Ap) horizon of moraine sandy loamy *Cambisol* there prevails the sand fraction (54.7%), with lower amounts of silt (31.2%), and 14.1% of clay fractions in the Ap horizon of limnoglacial silty loamy *Cambisol* is determined by silt fraction (69.9%), lower amounts (17.2%) of clay and (12.9%) sand fractions (Fig. 1).

The mineralogical composition of this fraction was determined with a Dron-6 X-ray diffractometer. In the ploughing horizon of silty and sandy loamy *Cambisols* there were found almost the same minerals (Table 1); however, the amount of clay particles and minerals was different in these soils. In silty loamy soil, which had a higher content of clay, there was estimated a higher content of the main potassium mineral muscovite as compared with sandy soil. It shows that plant-available potassium resources are relatively more intensive in silty loamy than in sandy loamy soils. Furthermore, in these soils a low content of potassium aluminium sulphate was obtained. In the clay fraction of silty and sandy loamy soils, the expanding-crystal-lattice mineral montmorillonite was found. In silty loamy soil, this mineral was more hydrated than in sandy loamy soil, therefore, it had a higher ability to fixate ions of soil solution in the interlayer of the crystal lattice. A comparatively low content of greenalite from the mineral group of kaolinite was estimated in the test soils. The content of this mineral was lower in sandy loam than in silty loam. However, the content of quartz debris was lower in the clay fraction of silty loamy than sandy loamy soil.

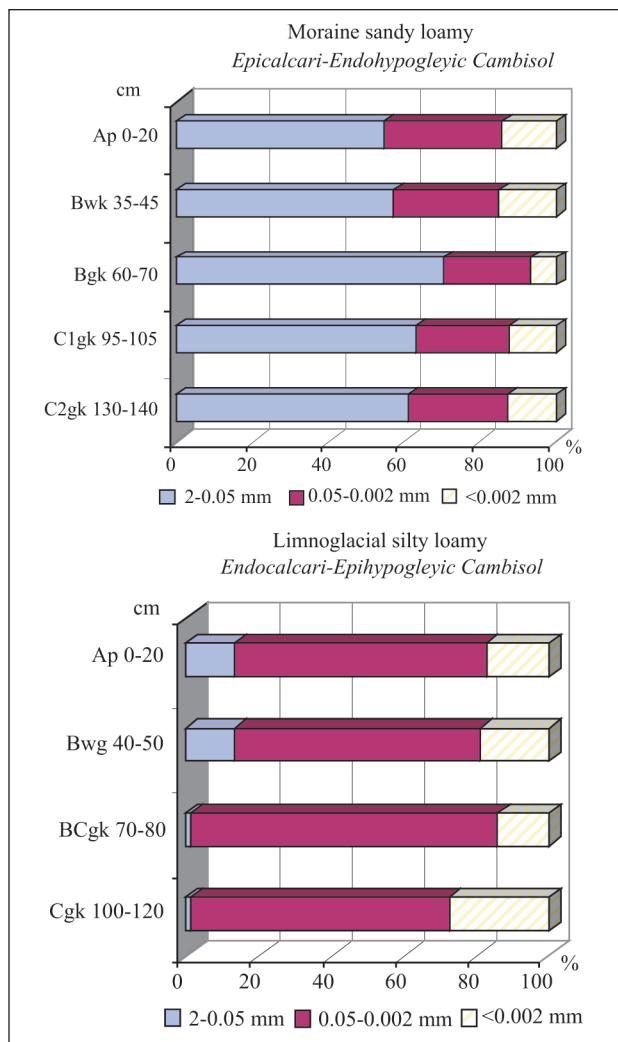


Figure. Soil texture of moraine sandy loamy and limnoglacial silty loamy *Endocalcari-Epihypogleyic Cambisols*

The agrochemical properties of the soil ploughing horizon (0–20 cm) before establishing the experiments are presented in Table 2. The soil pH (7.1–7.4) was estimated potentiometrically (0.01 M CaCl₂), and the estimation of exchangeable acidity (1 M KCl) of exchangeable cations (Ca²⁺, Mg²⁺, K⁺, Na⁺) was based on extraction with 1 M ammonium acetate. The humus was determined by the Tyurin method and total nitrogen (N_t) by the Kjeldahl method. Available P and K were determined by the

Table 1. Mineralogical composition of clay fraction in the ploughing horizon of *Cambisols*

Mineral type	Sandy loamy	Silty loamy
	Clay content in soil, %	
	9.1	18.5
Clay mineralogical composition		
Quartz SiO ₂	+++++*	++++
Muscovite-2 KAl ₂ (Si ₃ Al)O ₁₀ (OH, F) ₂	++	+++
Greenalite-1 Fe ₃ Si ₂ O ₅ (OH) ₄	++	+++
Montmorillonite-14A Na _{0.3} (Al, Mg) ₂ Si ₄ O ₁₀ (OH) ₂ ·H ₂ O	++	-
Montmorillonite-15A Na _{0.3} (Al, Mg) ₂ Si ₄ O ₁₀ (OH) ₂ ·4H ₂	-	+
Potassium aluminium sulphate K ₃ Al(SO ₄) ₃		+

* Diffraction intensity: +++++ very high; ++++ high; +++ medium; ++ low; + very low.

Table 2. Average agrochemical properties of *Cambisol* ploughing horizon (0–20 cm) before establishing the experiments

Agrochemical properties	Moraine sandy loamy <i>Epicalcari-Endohypogleyic Cambisol</i> (1971)	Limnoglacial silty loamy <i>Endocalcari-Epiphypogleyic Cambisol</i> (1990)
pH _{KCl}	7.1	7.4
Exchangeable acidity (mmol kg ⁻¹)	0.30	0.29
Exchangeable cations (mmol kg ⁻¹)	136.70	221.87
Organic C (%)	1.27	1.34
Total nitrogen N _t (%)	0.17	0.12
Available phosphorus (mg kg ⁻¹)	57	110
Available potassium (mg kg ⁻¹)	109	84

A-L method with extract of ammonium lactate. The enzymatic activity was determined from air-dried soil samples. Saccharase (invertase) (EC 3.2.1.26) and urease (EC 3.5.1.5) were determined by the modified Hofmann method (Чундарева, 1973). Determination of dehydrogenase activity (EC 1.1.1.1) was based on 2, 3, 5-triphenyltetrasol chloride (TTCH) colourless oxidized form reaction with red formasane (Методические..., 1978). Soil respiration intensity was established by the Öhlinger method (Schinner et al., 1995).

Investigation of soil biological activity was carried out in 1999–2002. Soil samples for analyses of biological activity were taken each year two times per plant vegetation period – before fertilization and after harvesting.

In this period, the crop rotation was as follows: on sandy loamy *Cambisol*: sugar beet – spring barley – annual grasses (vetch-oat mixture for green forage) – winter wheat; on silty loamy *Cambisol*: potatoes – spring barley – annual grasses – winter wheat.

The following mineral fertilizers of the same forms were used in both experiments: ammonium saltpetre, granulated super phosphate, potassium chloride.

Phosphorus and potassium fertilizer rates, available P and K contents in soil are presented in the oxide form (P₂O₅, K₂O).

The significance of differences between treatment means was determined using Fisher's LSD₀₅. Dependence of mineral fertilizers on enzymatic activity and soil respiration was determined by correlative-regressive analysis using the STAT_ENG for EXCEL version 1.55 Program (Tarakanovas, 2002). The symbols used in the work: * – significant at the 95% probability level; LSD₀₅ – least significant difference at 95% probability level; η – correlation ratio.

RESULTS AND DISCUSSION

Relationship between soil properties and enzymatic activity

A number of enzymes secreted by microorganisms, mezofauna and plant roots participate in the processes of mineralization and humification that take place in soil. Urease catalyses the hydrolysis of amides to secretion of ammonia and characterizes nitrogen regime in soil (Dick, 1992; Trasar Cepeda et al., 2003; Чундарева, 1973).

Dependence of soil biological activity on agrochemical properties has been analysed by using non-linear second-level equation. This analysis showed a strong correlation (η = 0.65–0.68) between urease activity and the amount of available P accumulated in both soils (Table 3). In the Ap horizon of sandy loamy *Cambisol* where plants had been fertilized for 30 years with 180 kg ha⁻¹ phosphorus in combinations with nitrogen and potassium, available phosphorus increased from 43–61 to 499–503 mg kg⁻¹. Thus, at such a high amount of available phosphorus in soil, the activity of urease significantly decreased. This observation is very important ecologically. It has been estimated also by the other authors. The data indicate that activity of enzymes decreased when the amount of available phosphorus and potassium compounds accumulated in soil exceeded 200–250 mg kg⁻¹ (Svirskienė, 2003; Чундарева, 1973).

A strong correlation (η = 0.68–0.7) was calculated between soil respiration and the content of available P in soil. However, the correlation between available phosphorus and activity of saccharase and dehydrogenases was weak (η = 0.38–0.45 and η = 0.29–0.48) in both soils. However, the results presented by C. Lai et al. (2002) show a significant positive correlation between the activity of urease and dehydrogenases and available P, total N and organic C.

Table 3. Dependence of biological activity on *Cambisol* agrochemical properties

Agrochemical properties	Urease activity	Sacharase activity	Dehydrogenases activity	Soil respiration intensity
	η (correlation ratio)			
Moraine sandy loamy <i>Cambisol</i>				
N _{mineral}	0.34	0.17	0.49	0.33
Available P	0.68*	0.45	0.48	0.70*
Available K	0.08	0.57*	0.44	0.43
pH _{KCl}	0.00	0.36	0.21	0.11
Limnoglacial silty loamy <i>Cambisol</i>				
N _{mineral}	0.66*	0.64*	0.32	0.03
Available P	0.65*	0.38	0.29	0.68*
Available K	0.60	0.47	0.28	0.56
pH _{KCl}	0.10	0.04	0.16	0.06

The content of available K in soil was somewhat higher in sandy loamy *Cambisol*. Urease activity correlated ($\eta = 0.28-0.60$) stronger with available K in limnoglacial silty loamy *Cambisol*. However, only in moraine sandy loamy *Cambisol* saccharase activity correlated with available K significantly at the 95% probability level. The amount of available K accumulated in both soils was quite low (84–118 and 85–194 mg kg⁻¹), and K had no such a great influence on soil enzymatic activity than available P. Soil respiration intensity correlation ($\eta = 0.43-0.56$) with available K in both soils was not significant at the 95% probability level.

Dependence of N_{mineral} accumulated in soil on enzymatic activity was significantly stronger in silty loamy than in sandy loamy *Cambisol*. Notably, the amount of accumulated N_{mineral} was higher in silty loamy *Cambisol*. The correlation between N_{mineral} and urease and saccharase activity was significant at the 95% probability level in silty loamy *Cambisol*. Soil respiration intensity weakly correlated with N_{mineral} in both soils. Dependence of N_{mineral}, available P and K on dehydrogenase activity was stronger ($\eta = 0.44-0.49$) in moraine sandy loamy ($\eta = 0.28-0.32$) than in limnoglacial silty loamy *Cambisol*. Thus, soils had a narrow range of soil pH_{KCl}, and it correlated very weakly with their biological activity.

The influence of long-term application of mineral fertilizers on soil biological activity

Organic or inorganic fertilizers are primarily used to increase nutrient availability to plants, but they can also affect soil microorganisms (Marschner et al., 2003). In the present study, the activity of enzymes was significantly affected by the long-term application of mineral fertilizers (Table 4).

In sandy loamy *Cambisol*, a significant trend of urease activity suppression under the effect of fertilizers was determined, especially at high nitrogen and phosphorus fertilizer rates, in the plots planted with sugar beets, barley and winter wheat. Only in 2001, in plots with annual grasses mineral fertilizers promoted urease activity in the soil. The average of experimental data suggests that there were no significant difference in urease activity under the effect of mineral fertilizers. In soil of the trial plots fertilized with moderate mineral fertilizer rates, urease activity was slightly higher than in the unfertilized plot. However, high rates of fertilizers (N₁₃₅, P₁₈₀, K₁₈₀) tended to suppress urease activity.

The average data of the four years of our investigations in silty loamy *Cambisol* show that mineral NPK fertilizers did not have any significant effect on urease activity. Only trends of changes in urease were identified under the effect of fertilizers. A decrease in urease activity is supposed to be due to a

Table 4. Effects of mineral fertilizers on the biological activity of different *Cambisol*

Treatments	Urease mg NH ₃ per 24 h*	Sacharase mg glucose per 48 h*	Dehydrogenases mg formosane per 24 h*	Soil respiration intensity mg CO ₂ per 24 h*	P ₂ O ₅	K ₂ O
					mg kg ⁻¹	
Moraine sandy loamy <i>Cambisol</i>						
Unfertilized	0.34	28.99	6.70	0.29	49	90
P ₉₀ K ₉₀	0.35	29.81	5.69	0.28	263	121
N _{67.5} P ₉₀	0.33	30.39	5.45	0.29	245	85
N _{67.5} K ₉₀	0.38	29.74	5.80	0.23	43	110
N _{67.5} P ₉₀ K ₉₀	0.35	29.33	5.77	0.25	198	115
N _{67.5} P ₁₈₀ K ₉₀	0.34	30.14	5.62	0.27	499	126
N _{67.5} P ₉₀ K ₁₈₀	0.37	31.93	7.14	0.30	249	176
N ₁₃₅ K ₉₀	0.37	29.95	5.27	0.38	53	108
N ₁₃₅ P ₉₀	0.37	29.83	5.61	0.34	286	85
N ₁₃₅ P ₁₈₀ K ₉₀	0.36	30.46	6.14	0.32	360	109
N ₁₃₅ P ₉₀ K ₁₈₀	0.36	31.40	5.64	0.26	234	132
P ₁₈₀ K ₁₈₀	0.33	30.08	5.17	0.28	503	194
N ₁₃₅ P ₁₈₀	0.29	29.08	6.28	0.27	493	82
N ₁₃₅ K ₁₈₀	0.32	29.34	6.41	0.29	61	179
N ₁₃₅ P ₁₈₀ K ₁₈₀	0.31	31.33	5.53	0.31	458	138
LSD ₀₅ / R ₀₅	0.06	4.15	1.04	0.05	37.5	17.3
Limnoglacial silty loamy <i>Cambisol</i>						
Unfertilized	0.45	20.07	6.51	0.28	108	84
P ₉₀ K ₉₀	0.43	19.01	5.63	0.24	128	91
N _{67.5} K ₉₀	0.45	17.95	6.21	0.21	104	98
N _{67.5} P ₉₀	0.43	19.20	5.36	0.26	124	84
N _{67.5} P ₉₀ K ₉₀	0.44	18.09	5.42	0.25	126	91
N _{67.5} P ₁₈₀ K ₉₀	0.42	19.28	5.05	0.28	168	98
N _{67.5} P ₉₀ K ₁₈₀	0.44	17.66	5.51	0.25	123	113
N ₁₃₅ P ₁₈₀ K ₉₀	0.45	19.72	5.50	0.24	158	105
N ₁₃₅ P ₉₀ K ₁₈₀	0.45	18.86	5.65	0.22	124	118
N ₁₃₅ P ₁₈₀ K ₁₈₀	0.47	18.78	5.23	0.22	142	114
LSD ₀₅	0.05	2.37	0.77	0.04	16.3	12.5

* Per 1 g of air-dried soil.

Table 5. Dependence of *Cambisol* biological activity (y) on mineral fertilizers (x)

Fertilizers	Urease activity	Sacharase activity	Dehydrogenases activity	Soil respiration intensity
	η (correlation ratio)			
Moraine sandy loamy <i>Cambisol</i>				
N	0.26	0.30	0.12	0.56*
P	0.66*	0.33	0.23	0.66*
K	0.2	0.67*	0.56*	0.28
Limnoglacial silty loamy <i>Cambisol</i>				
N	0.67*	0.62*	0.54*	0.53*
P	0.57*	0.42	0.93*	0.25
K	0.50	0.56*	0.42	0.45

high amount of available P accumulated in soil. On the other hand, urease activity significantly correlated with the application of phosphorus fertilizers in both soils, while with nitrogen fertilizer application it correlated significantly only in silty loam *Cambisol* (Table 5).

Saccharase catalyses the hydrolysis of saccharase into glucose and fructose and characterizes the processes of change of organic C combinations (Крушельницкая, 2001). According to some data (Чундарева, 1973), in this case saccharase activity might be more influenced by the residues of sugar beet roots (usually rich in hydrocarbons). The influence of any rates of fertilizers on saccharase activity in sandy loamy *Cambisol* was significantly higher than in silty loamy soil. In sandy loamy soil, the dependence of saccharase activity on mineral fertilizers was weak ($\eta = 0.30-0.66$). In addition, in silty loamy *Cambisol*, with the application of mineral fertilizers, saccharase activity tended to be lower. In comparison with the unfertilised variant, a very slight decrease of activity of this enzyme was established only under application of $N_{67.5}P_{180}K_{90}$ and $N_{135}P_{180}K_{90}$ when the yield of plants and plant residues was higher. Another research showed a weaker humification process in this soil because in humus synthesis, saccharase is directly related with its content in soil (Чундарева, 1973).

Dehydrogenase activity is thought to reflect the total scope of activity of soil microflora and consequently may be a good indicator of the microbiological activity of P (Nanipieri et al., 1990). In our treatments, mineral fertilizers inhibited the activity of dehydrogenases in both soils. In sandy loamy soil, a correlation between the activity of dehydrogenases and potassium fertilizers was $\eta = 0.56$, while in silty loamy soil a correlation between the activity of dehydrogenases and phosphorus fertilizers was $\eta = 0.93$ (Table 5).

In literature, there are also data indicating that mineral fertilizers, especially at high rates, decreased the activity of dehydrogenases (Schinner et al., 1995), implying that dehydrogenases are highly sensitive to the inhibitory effects associated with high rates of fertilizers. The effects of fertilization on dehydrogenase activity may be directly related to changes in the availability of nutrients or to heavy metals present in the fertilizers as contaminants.

Researches confirm that activity of biological processes in soil is reflected by the amount of carbon dioxide exuded from the soil, which is called soil respiration (Arlauskienė, 1998). Carbon dioxide (CO_2) was exuded because of disintegration of lifeless organic matter of plant and animal origin in the soil,

plant roots breathing and by physical-chemical processes. The average data of four years of our investigations show the impact of mineral fertilizers on respiration intensity to be stronger in sandy loamy than in silty loamy *Cambisol* (Table 5). In addition, in silty loamy *Cambisol*, under the application of mineral fertilizers, a tendency of soil respiration intensity lowering was noted (except for $N_{67.5}P_{180}K_{90}$ fertilizer). The application of mineral fertilizers in sandy loamy *Cambisol* increased soil respiration intensity only in the following treatments: $N_{135}K_{90}$, $N_{135}P_{90}$, $N_{135}P_{180}K_{180}$. Furthermore, the calculated correlation confirms its significant dependence on the application of phosphorus fertilizer.

CONCLUSIONS

1. In soils developed on different glacial deposits, the enzymatic activity and respiration intensity are different: in limnoglacial silty loamy *Cambisol* urease activity is higher, while in moraine sandy loam saccharase, dehydrogenases activity and soil respiration intensity are higher.
2. In sandy loamy *Cambisol* with a permanent annual fertilization of 180 kg ha^{-1} phosphorus in combination with nitrogen and potassium fertilizers the content of available phosphorus in soil increased from 43–61 to 499–503 mg kg^{-1} , and the activity of enzymes was lowered. From the point of view of soil ecology, a long-term application of mineral fertilizers at very high rates is unacceptable.
3. In silty loamy *Cambisol*, the change of available phosphorus under intensive fertilization is not significant; therefore, changes of enzymatic activity are slight.
4. Mineral fertilizers inhibited activity of oxidation–reduction enzyme dehydrogenases in both soils and of saccharase only in silty loamy *Cambisol*.
5. The highest soil respiration intensity, expressed by carbon dioxide content, was determined in sandy loamy *Cambisol* under agricultural plants fertilized with nitrogen and potassium fertilizers.

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ILGALAIKIO TRĘŠIMO POVEIKIS RUDŽEMIŲ BIOLOGINIAM AKTYVUMUI

Santrauka

Ilgalaikiai sėjomaininiai tręšimo bandymai moreninio smėlingo priemolio sekliai karbonatingame giliau glėžiškame rudžemyje (*Epicalcari-Endohypogleyic Cambisols*) įrengti 1971 m., o limnoglacialinio dulkiško priemolio giliau karbonatingame sekliai glėžiškame rudžemyje (*Endocalcari-Epihypogleyic Cambisols*) – 1990 m. Straipsnyje pateikiami 1999–2002 m. tyrimų duomenys apie ilgalaikio mineralinių NPK trąšų naudojimo poveikį dirvožemio biologiniam aktyvumui.

Nustatyta, kad nevienodos kilmės dirvožemiuose fermentų aktyvumas ir kvėpavimo intensyvumas yra skirtingas. Dulkiško limnoglacialinio priemolio rudžemyje buvo didesnis ureazės, o smėlingo moreninio priemolio – sacharazės ir dehidrogenazių aktyvumas bei dirvožemio kvėpavimo intensyvumas.

Dėl ilgalaikio gausaus žemės ūkio augalų smėlingo priemolio rudžemyje tręšimo fosforo (180 kg ha^{-1}), azoto ir kalio trąšomis jame didėjo judriojo fosforo kiekis nuo 43–61 iki 499–503 mg kg^{-1} , todėl mažėjo fermentų aktyvumas. Toks tręšimas ekologiniu požiūriu yra netaikytinas. Tačiau dulkiško priemolio rudžemyje judriojo fosforo kiekio pokyčiai dėl intensyvaus tręšimo įtakos nėra tokie dideli, todėl ir fermentų aktyvumas kito neesmingai. Mineralinės trąšos oksidacinių-reducinių fermentų dehidrogenazių aktyvumą slopino abiejuose dirvožemiuose, o sacharazės – tik dulkiško priemolio rudžemyje. Didžiausias dirvožemio kvėpavimo intensyvumas, išreikštas anglies dvideginio kiekiu, nustatytas smėlingo priemolio rudžemyje žemės ūkio augalus tręšiant azoto ir kalio trąšomis.

Raktažodžiai: rudžemis, fermentų aktyvumas, dirvožemio kvėpavimas, mineralinės trąšos