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The Influence of Type, Composition and Dosage of Exogenous Organic Matter on Selected Biochemical Soil Properties

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Abstract

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Organic matter in soil is exposed to decomposition and other changes, and excessive loss of such matter is one of the most serious forms of degradation. One of the possible solutions to this problem is the application of various types of organic matter. These include exogenous organic matter (EOM), which originates, to a large extent, outside the agro-ecosystem. The aim of the presented research was to evaluate the influence of type and dosage of applied EOM on soil characteristics, specifically on the activity of selected soil enzymes which can respond quite sensitively to changes in land management as well as changes in environmental conditions. Nitrogen was supplied to the soil in a combination of organic and mineral fertilizers: in variants from 0–100% in various types of EOM, and 0–100% in mineral form respectively, with a resulting dosage of 200 kg N/ha. Enzymes from the N, P and C cycles were chosen for evaluation of the influence of EOM on enzyme activity, focusing on the activity of urease, phosphatase and cellulase. In the research it was proven that application of EOM leads to relatively rapid changes in enzyme activity. Soil micro-organisms, and the processes they control, showed varying sensitivity to EOM application. Enzyme activity (cellulase, acid and alkaline phosphatase) was generally stimulated by the highest dosage of the tested EOM. This was not statistically confirmed in the case of urease activity. The research also confirmed that the decisive factor for cellulase, acid and alkaline phosphatase activity was the amount of carbon, nitrogen and phosphorus supplied via EOM. The ratio of C : N in the applied EOM had an influence on the activity of urease and a slight dependence was proven in cellulase activity and acid phosphatase activity.

Keywords: enzyme activity; exogenous organic matter; phosphorus; soil, carbon

Soil organic matter in agro-ecosystems comprises of all organic compounds present in the soil (Diacono & Montemurro 2010), including plant residue in various stages of decomposition, microorganisms, metabolites of microorganisms produced during their growth and decomposition, and humic compounds. This organic matter breaks down and excessive loss of such matter is one of the most serious degradation factors. This problem is evident in the Czech Republic, where humus loss is at a rate of 5–15% on drained land and in soil on light-grained substrate, however the greatest danger of this form of soil degradation is due to inadequate supply of good-quality organic matter (Ministry of Agriculture 2015). Therefore the recommendation is for types of management which lead to an increase in the carbon content of soil (van Camp *et al.* 2004). One possible solution is the application of various types of exogenous organic matter (EOM), such as sewage plant slurry, bi-products from the food industry, composted waste from industrial production or communal waste, biogas fermentation waste, digestates, bonemeal and farmyard manure (Diacono & Montemurro 2010), which influence physical, chemical and biological properties/characteristics. As with chemical and physical parameters, biological soil parameters are also sensitive to changes in the form of soil management or changes in environmental conditions, but react to these changes much more quickly (Lynch *et al.* 2004). Although the influence of the supply

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of organic matter on soil characteristics has been extensively tested, unlike the influence on physical and chemical characteristics (Gong *et al.* 2009; Guo *et al.* 2012), there has been little research in terms of biological characteristics.

The ability of microorganisms to effectively ensure the transfer of organic compounds (decomposition, mineralisation, immobilization of nutrients) is dependent on their ability to react quickly to changes in external conditions, such as substrate input. Soil microorganisms, including the enzymes they produce, contribute to overall biological activity as they are closely involved in the catalytic reaction essential for stabilization of soil structure and decomposition of organic matter (Allison & Vitousek 2005), mineralization and the nutrient cycle (Dick *et al.* 1994; Tabatabai 1994), transfer of energy and quality of the environment. Therefore, soil microorganisms influence not only the current content of available nutrients, chemical and physical characteristics, but consequently also primary production (Rutigliano *et al.* 2004).

Biochemical transformations in the soil environment are dependent on the presence of relevant enzymes, and many transformations of organic matter may be catalysed by enzymes which occur outside microorganism cells (Burns *et al.* 2013). A plant´s root system and an individual soil may therefore be characterized by a unique composition of specific enzymes (Marinari *et al.* 2014), and enzyme activity is thus a suitable instrument for evaluation of the functional diversity of microbial communities and one of the indicators of soil quality (Kujur & Patel 2014). Soil enzymes are sensitive to changes in conditions caused by agrotechnical interventions and agricultural management, and react to changes much sooner than other soil parameters (Lynch *et al.* 2004; Odlare *et al.* 2008; Sarapatka *et al.* 2014), and enzyme activity obtains greater stimulation from organic material than from mineral fertilizer (Marinari *et al.* 2000).

The main aim of our research was to evaluate the influence of the application of various types and dosage of exogenous organic matter (of both agricultural and industrial origin) on the activity of selected soil enzymes. Emphasis was put on the influence on soil enzyme activity, in relation to the composition of organic matter, expressed as the basic macro-elements supplied via EOM, i.e. C, N and P. The enzymes selected were from the N, P and C cycles, namely urease, phosphatases and cellulase.

MATERIAL AND METHODS

Study site. The research was carried out in 2013 and 2014 in small-parcel field trials in the Pusté Jakartice locality of the Czech Republic (50°34'02.6''N, 16°48'07.1''E), 295 m a.s.l. The soil was classified as luvisol with pseudogleyic properties with the following basic soil characteristics: C_{org} – 1.131%, pH 6.5, soil colloid fraction 28–31%, supply of available nutrients in Mehlich 3 solution: P – 101 mg/kg, K – 209 mg/kg, Mg – 90 mg/kg, Ca – 1820 mg/kg. The average precipitation for the locality is 640mm and the average annual temperature is 8.0°C. In both years maize was grown on the plots of land, N K Terada FAO 26 variety in 2013 and Ułan – FAO 270 in 2014.

Experimental design and soil sampling. The field trial, carried out on randomly assigned blocks of land, comprised of 10 combinations of fertilization, where each combination was repeated 4 times. The area of each individual land parcel was 25 m^2. EOM with varying parameters was applied to the soil (Table 1) in the form of compost made from farmyard manure (Ag), bone meal (Mb) made from by-products of animal origin, and industrial compost (Ra) made from sludge from waste-water treatment plants, sawdust and biologically degraded waste from parks and gardens.

The same level of fertilization with nitrogen was set for all variants (200 kg/ha), but the ratio of nitrogen supplied by organic or mineral fertilizer (ammonium nitrate) varied, as described in Table 2. One-off fertilization with other nutrients (P, K, Ca, Mg) was carried out in autumn 2012 to ensure a good supply of accessible nutrients.

Table 1. Chemical composition of organic fertilizers tested in field trial (Source: Niedźwiecki *et al.* 2015)

Tested fertilizer	Short form	Total carbon (%)	Total nitrogen (%)	C:N	Total phosphorus (%)
Bone meal	Mb	40.1	8.4	4.77	6.42
Industrial compost	Ra	17.9	2.3	7.78	0.75
Compost	Ag	24.1	2.6	9.27	3.35

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Table 2. Test variants and nitrogen supplied via exogenous organic matter (EOM) and mineral fertilizer

Variant No.	Variant, EOM	N supplied (%, kg N/ha)	
		via EOM	via NH_4NO_3
1	Control	0, 0	100, 200
2, 5, 7	Mb, Ra, Ag	50, 100	50, 100
3, 6, 9	Mb, Ra, Ag	75, 150	25, 50
4, 7, 10	Mb, Ra, Ag	100, 200	0, 0

Mb – bone meal; Ra – industrial compost; Ag – compost

Table 3. Interventions on trial plots

Month/year	Intervention	Month/year	Intervention
IV/2013	soil tillage	IV/2014	soil tillage
V/2013	sowing	IV/2014	sowing
VI/2013	soil sampling	V/2014	soil sampling
X/2013	harvest	IX/2014	harvest
XI2013	soil sampling	IX/2014	soil sampling

Organic material was applied in spring 2013, specifically on 30. 4. and 21. 5. (Mb), and on 22. 4. 2014 the following year (Table 3). Soil samples were taken from the 0 – 0.2 m horizon, for analysis, one month after application, on 10. 6. 2013 (24. 6. 2013 – Mb) and on 6. 5. 2014. After the maize was harvested soil samples were again taken in autumn on 6. 11. 2013 and 24. 9. 2014. Exogenous organic matter was worked into the soil to depths of 15–20cm.

Soil enzyme activity assay. Soil enzyme activity was measured in naturally moist samples by means of the spectrophotometric method. In order to evaluate urease activity the soil samples were incubated with a urea solution. Released ammonia ions were extracted in a potassium chloride solution and measured colorimetrically at 690 nm (Tabatabai & Bremner 1972). Cellulase activity was evaluated using CM-cellulose as a substrate. Oligosaccharides cause a reduction in potassium ferricyanide, which reacts with iron sulphate to produce a compound of potassium ferrocyanide, and this was measured colorimetrically (Schinner & von Mersi 1990). Phosphate activity was measured using p-nitrophenyl phosphate as a substrate. The p-nitrophenol produced was extracted and measured photometrically at a wavelength of 400 nm (Tabatabai & Bremner 1969).

Statistical evaluation of results. The results obtained were evaluated by means of the STATISTICA programme (StatSoft Ver. 12) with analysis of correlation dependence (Pearson coefficient), testing the difference between groups (parametric comparison, ANOVA, Tukey test). Results were processed twice a year (both in spring and autumn). Greater statistical dependence was confirmed in the spring period in both years of the study, after application of organic matter, compared with the autumn period. With regard to the size of the file, only an evaluation of the whole extensive file of results from both years is presented in this paper.

RESULTS

Statistical evaluation showed relationships to the activity of individual soil enzymes, including a relationship to the amount of organic matter applied, as is shown in Table 4.

In two-way factor analysis of variance for dosage and type of EOM, no significant interaction was found. The results of single-factor statistical evaluation of soil enzyme activity proved significant differences in activity of cellulase, acid and alkaline phosphatase depending on the dosage and type of EOM. The control variant where only mineral N fertilizer was applied, i.e. no application of organic matter, formed a statistically different group than the variants with organic fertilization. The organic fertilizer variants were divided into other statistically different groups. No statistically conclusive differences were found between variants in terms of urease activity.

Table 4. Correlation between the activity of individual studied enzymes and the amount of EOM applied

	CM-cellulase	Acid phosphatase	Alkaline phosphatase	Urease	Amount of EOM applied
CM-cellulase		**	**	*	**
Acid phosphatase	**		–	**	–
Alkaline phosphatase	**	–		–	–
Urease	*	**	–		–
Amount of EOM applied	**	–	–	–	

EOM – exogenous organic matter; *P < 0.05; **P < 0.01; – no correlation proven

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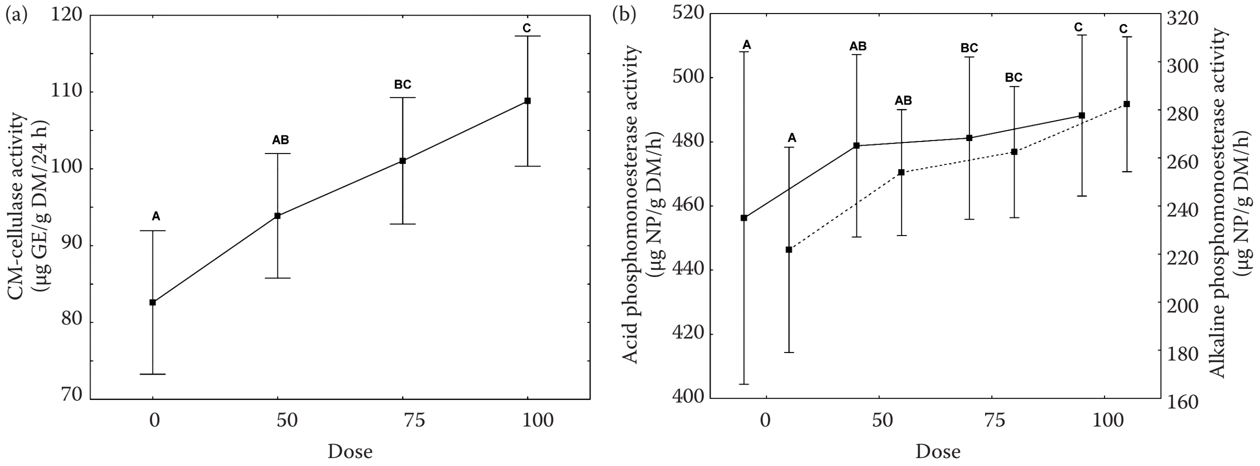

Figure 1. Cellulase activity (a) and acid and alkaline phosphatase activity (b) depending on exogenous organic matter (EOM) dosage and statistically conclusive similarity of groups (A–C) in terms of soil enzyme activity depending on EOM and N dosage supplied by EOM, irrespective of the influence of the type of EOM (Tukey test, $P < 0.05$); DM – dry matter

Activity of cellulase, acid and alkaline phosphatase was greatest in variants with highest amounts of EOM and with 100% N supplied via this organic matter. With reduced EOM dosage the soil enzyme activity decreased. This trend is shown in the activity of cellulase, acid and alkaline phosphatase as illustrated in Figure 1. In urease activity, no dependence was statistically confirmed, therefore no trends for urease are shown in the following graphs.

Evaluation did not focus only on EOM dosage, but also considered the dependence on individual type of exogenous organic matter applied. As with dosage, study of EOM type also proved that there were statistically different groups, where the control variants differed from the variants where various types of EOM was applied. Statistically different groups in the control variants, as well as in individual variants, are for cellulase and both acid and alkaline phosphatase activity after application of tested EOMs shown in Figure 2.

Cellulase activity was most-profoundly influenced by the application of both compost

made from farmyard manure and industrial compost, i.e. organic fertilizer, which was applied in greater amounts and had a wider ratio of C : N compared with applied bone meal. Acid phosphatase activity showed the highest level after application of bone meal, where this organic matter had the high-

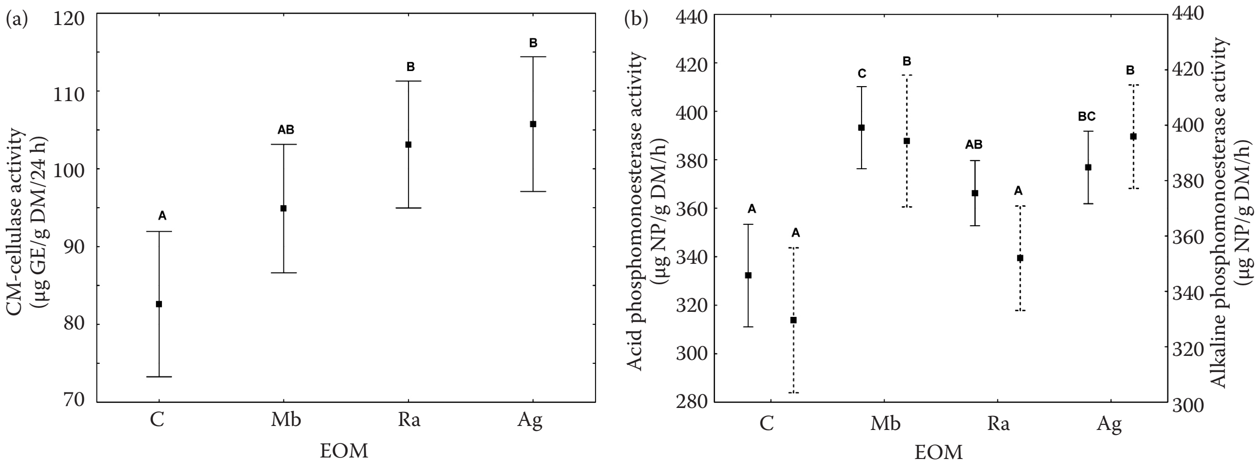

Figure 2. Cellulase activity (a) and acid and alkaline phosphatase activity (b) depending on the type of organic matter applied and statistically conclusive similarity of groups (A–B) according to soil enzyme activity depending on the type of EOM applied, irrespective of the influence of dosage (Tukey test, $P < 0.05$); DM – dry matter

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Table 5. Enzyme activity dependence on C : N ratio and content of C, N and P supplied via EOM

	C : N in EOM	C supplied via EOM	N supplied via EOM	P supplied via EOM
Cellulase activity	0.1595 (0.056)	**0.3236 (0.000)**	**0.2816 (0.000)**	**0.2207 (0.005)**
Acid phosphatase activity	0.1562 (0.062)	**0.1826 (0.021)**	**0.3333 (0.000)**	**0.2753 (0.000)**
Alkaline phosphatase activity	0.0448 (0.594)	**0.1875 (0.018)**	**0.2661 (0.001)**	**0.3343 (0.000)**
Urease activity	**0.2026 (0.015)**	0.1289 (0.104)	0.0339 (0.670)	0.0510 (0.522)

P-values are given in parenthesis; statistically significant dependence is marked in bold

est content of phosphorus and nitrogen of all forms of applied EOM. Alkaline phosphatase activity was similarly significantly influenced by the application of bone meal. A high level of activity was also apparent after application of farmyard manure compost, in which the overall content of phosphorus was also relatively high.

Further statistical evaluation, which is shown in Table 5, confirmed the influence of the amount of carbon supplied via EOM on the activity of cellulase, acid phosphatase and alkaline phosphatase. The supply of phosphorus and nitrogen was also the decisive factor for activity of cellulase, acid phosphatase and alkaline phosphatase. A certain dependence was apparent in the relationship between urease activity and C supplied via EOM ($P = 0.104$), although this was not statistically confirmed. The tested ratio of C : N in the applied fertilizer confirmed the statistical dependence of urease activity and slight statistical dependence of cellulase activity ($P = 0.056$) and acid phosphatase activity ($P = 0.062$).

DISCUSSION

The results show the evident influence of the application of various types of EOM in varying dosage on microorganism communities and the production of extracellular enzymes. Mineral and organic fertilizers stimulate the development of microorganisms as the main source of soil enzymes (Krämer & Green 2000). Activity of hydrolytic soil enzymes can increase after regular application of organic fertilizer (García-Ruiz *et al.* 2008). This is true for enzyme activity, including phosphatase, after application of organic fertilizer, compared with mineral fertilizers or the control variants (Li *et al.* 2015), or after application of compost (Hernández *et al.* 2015). The stimulation effect of applied organic material on microbial and enzyme activity is described by Franco-Otero *et al.* (2012). The presented research obtained similar results. Soil microorganisms and the processes they control showed varying degrees of sensitivity to the application of EOM, and enzyme activity (cellulase, acid and alkaline phosphatase) was generally stimulated by the highest doses of EOM tested, specifically C supplied via this matter.

Not only organic matter itself, but also individual elements bound in organic matter can be important for the development of certain microflora and for the production of enzymes. In the case of phosphatases, it is the transformation of organic P which is bound in phosphate esters and is mineralized independently into C through catalysis by phosphatase, which is influenced by the source of phosphorus (Kandeler *et al.* 1999; Ross *et al.* 1999; Marinari *et al.* 2000). Earlier studies (e. g. Nannipieri *et al.* 1978) show that phosphomonoesterase activity in soil is influenced by content of available phosphorus. If the level of this phosphorus is low, microorganisms and plant roots are active producers of phosphatase enzymes (Chabot *et al.* 1996). On the other hand, higher levels of phosphatase activity are described by Saha *et al.* (2008a) as dependent on high dosage of organic fertilizer and the consequently higher content of C in the soil. Organic phosphorus may be important for short-term biological consumption, while mineral fertilization may reduce enzyme activity relating to the C, N and P cycles (DeForest *et al.* 2012; Fan *et al.* 2012; Wang *et al.* 2012). The importance of added amounts of P, type and dosage of EOM are shown in our study. According to published research, however, there may be other dependencies. For example, Zou *et al.* (1995) describe the positive influence of increased N on phosphatase activity. Similarly, Olander and Vitousek (2000) found that N addition increased phosphatase activity in an N-limited site.

Different types of amendments may also have varying composition of organic matter (e.g. C : N), affecting the rate of decomposition and influencing the structure of microbial communities. For example, digging in straw favours the competitive ability of cellulolytic microorganisms compared to those that

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cannot degrade cellulase (Jensen & Nybroe 1999). An important factor in these processes is cellulase, which is involved in the decomposition of cellulose as an element of organic matter (Dilly & Nannipieri 2001). Schimel and Weintraub (2003) describe a model according to which the addition of C to a N-limited system increases respiration, while adding N actually decreases respiration. Supply of a source of energy – carbon influenced enzyme activity (also in our research), specifically the activity of cellulase, as is evident e.g. in Figures 1a, 2a and in Table 4. The ratio C : N in the applied EOM was at the limit of statistical confirmation in terms of cellulase activity.

In our research we did not register a statistically significant influence of increasing dosage of EOM on urease activity. Neither was there any confirmation of the influence of various types of EOM on the activity of urease enzymes. Statistically significant dependence was confirmed between the activity of urease and the ratio of C : N in the applied organic matter, while an indication of a relationship was apparent (P = 0.104) in regard to the amount of C supplied via EOM. Relationships between urease activity and applied organic matter are described in research reports, for example, in relation to the dosage of organic matter (Reynolds *et al.* 1985; Pascual *et al.* 1999; Chakrabarti *et al.* 2000; Geisseler *et al.* 2010). Increased activity of the majority of other enzymes (e.g. phosphatase, cellulase) after manure application was described by Saha *et al.* (2008b). On the other hand, in research by Saha *et al.* (2008b) urease activity was uninfluenced by manure treatment. Urease activity may reduce with increased application of NH_3 base N fertilizers (Dick *et al.* 1988; McCarty *et al*. 1992). This was not confirmed in our research in evaluation of urease activity in the case of only mineral fertilization, in some cases with the lowest amount of EOM compared with variants with the lowest dosage of mineral nitrogen and the highest dosage of EOM. Neither did we find a conclusive difference in urease activity in variants with application of EOM made by composting manure in comparison with other types of EOM.

Soil enzymes are important not only in terms of their role in the nutrient cycle, their suitability as indicators of specific biochemical reactions in the soil and managed analytical methods (Dick *et al.* 1994; Kizilkaya & Bayrakli 2005), but they also react relatively quickly to changes in soil environment (Kandeler *et al.* 1999; Zhang *et al.* 2010). In the research we now present, we have proved that application of EOM to soil leads to a relatively rapid reaction in enzyme activity in particular. The biochemical characteristics which we studied reacted much more sensitively during the research than other characteristics identified in the described small-plot trials. In the course of two seasons no statistically significant influence of any individual type of EOM was found on characteristics relating to ground water, infiltration, water-resistance, or on aggregate stability (Lipiec *et al.* 2015). In the case of chemical characteristics, it can also be stated that, with the application of the tested EOM, there was no proof of any significant influence on unstable fractions of organic carbon (Kaczyński & Siebielec 2015).

CONCLUSION

The organic matter content of soil is a basic characteristic which influences the physical, chemical and biological qualities of the soil. In our research we have proven the influence of applied carbon on the activity of cellulase. In our observations, decomposition occurred in material where there was an adequate level of nitrogen, as the C : N ratio in all the studied types of EOM was lower than 10 : 1. The composition of material also figured similarly in phosphate activity, where the supply of overall phosphorus had an influence on this activity. The presented study proves/shows that the composition of various types of organic waste must be taken into consideration when it is applied to agricultural use. This organic matter can then be a benefit to agricultural land as a fertilizer while also solving the problem of waste management.

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