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The content of carbon, nitrogen, phosphorus and sulphur in soil against the activity of selected hydrolases as affected by crop rotation and fertilisation

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Abstract

The aim of the paper was to determine the effect of crop rotation and different fertilisation with manure and nitrogen on the content of phosphorus and sulphur against the activity of phosphatase and arylsulphatase. The soil was sampled from a long-term field experiment carried out in a three-factor design, which covered two crop rotation types (A – enriching and B – depleting the soil from organic matter), manure fertilisation (0, 20 and 60 t ha^{-1}) and nitrogen fertilisation (N_0, N_1 and N_2). The experiment was performed on a *Haplic Luvisol* (*LVha*). The soil was sampled in each year during a 4-year (2009–2012) rotation. There was found a significant effect of the experiment factors on the content of available forms of the elements. According to the criteria provided for in Chemical and Agricultural analysis – determination of the content of available phosphorus in mineral soils (PN-R-04023:1996) the soil represented the second highest class of richness with available phosphorus. The activity of the hydrolytic enzymes depended both on the application of manure and ammonium nitrate. The synthesis of the results demonstrated a significant effect of the type of the crop rotation on the content of total organic carbon, total nitrogen, available phosphorus and sulphur considering a selected hydrolases activity. Growing crops in a crop rotation enriching the soil with organic matter increased the richness of the soil with available forms of phosphorus and sulphur and increased the activity of acid phosphatase and arylsulphatase.

Key words: alkaline and acid phosphatases, arylsulphatase, crop rotation, fertilization, phosphorus, sulphur.

Introduction

Over the last ten years in the countries of the European Union there has been observed a loss of soil organic matter by abandoning the use of natural or organic fertilisation for the benefit of mineral fertilisers (Rutkowska, Pikuła, 2013). The adequate cultivation and plant fertilisation affect the physicochemical properties even at greater depths as well as the yield size (Zhu et al., 2007). The use of organic fertilisation combined with mineral fertilisation increases the content of organic matter as well as macro- and micronutrients in soil (Wei et al., 2013). A long-term application of manure as a source of phosphorus in soil affects its dynamics by increasing the solubility and availability of P as well as a decreases in the risk of P losses by leaching (Geisseler et al., 2011). An adequately planned crop rotation considering the application of undersown crops, catch crops and the share of papilionaceous plants combined with balanced fertilisation counteracts a decrease in humus, nutrients, activity of soil enzymes as well as enhances the use of mineral fertilisers (Dodor, Tabatabai, 2003; Lauringson et al., 2004; Bakšienė et al., 2009; Kizilkaya, Dengiz, 2010).

Enzymes catalyze all biochemical reactions and are an integral part of nutrient cycling in the soil. Soil enzymes are believed to be primarily of microbial origin but also originate from plants and animals. They are usually associated with viable proliferating cells, yet enzymes can be excreted from a living cell or released into soil solution from dead cells. The free enzymes with humic colloids may be stabilized on clay surfaces and organic matter (Piotrowska, Wilczewski, 2012). Investigations on a limited number of enzymes show that agricultural management practices affect their activities (Gianfreda, Ruggiero, 2006).

The aim of the current research was to determine the effect of a varied fertilisation with manure and nitrogen as well as crop rotation on the content of available phosphorus and sulphur during a 4-year rotation. There was also investigated the activity of the enzymes participating in the transformations of phosphorus and sulphur.

Materials and methods

Site description and experimental design. During 2009–2012, the research material was sampled from a many-year field experiment located in the area of the Agricultural Experiment Station at Grabów, the Mazowieckie Province, the Zwoleńsk County, the Przyłęk Commune, Central Poland. The location of the experiment station is determined by the latitude (51°21′8″ N) and longitude (21°40′8″ E); the lowland climate of moderate latitudes. The experiment was established in 1980 by the Department of Plant Nutrition and Fertilization, the Institute of Soil Science and Plant Cultivation in Puławy, on the soil typical of Poland, classified as light loamy and sand texture (*Haplic Luvisol*, *LVha*) according to FAO soil classification (Rutkowska, Pikuła, 2013).

The experiment, established in the split-plot design, was carried out in a 4-year crop rotation. Factor I involved the type of crop rotation: A – recognized as soil exhausting from humus (2009 – potato cultivar 'Alicja', 2010 – winter wheat cv. 'Korweta', 2011 – spring barley cv. 'Stratus', 2012 – grown for silage maize cv. 'Nimba') as well as B – considered to enrich soil with humus (2009 – potato cv. 'Alicja', 2010 – winter wheat cv. 'Korweta' and white mustard, 2011 – spring barley cv. 'Stratus' and undersown grass, 2012 – red clover cv. 'Jubilatka' and grass-meadow fescue cv. 'Cykada'). Factor II was made up by the cattle manure (FYM) fertilisation at the following rates: 0, 20, and 60 t ha^{-1} every four years under potato (autumn 2008). The chemical composition of FYM applied in the experiment (nutrient % fresh weight) was as follows: dry matter 21%, nitrogen (N) 0.5%, phosphorus (P_2O_5) 0.29%, potassium (K_2O) 0.68%, calcium (CaO) 0.45%, magnesium (MgO) 0.16%, sodium (Na_2O) 0.11%. Factor III involved fertilisation with nitrogen in a form of ammonium nitrate (34% N) at the rates of N_0, N_1 and N_2, where rate N_2 was an adequate multiplication of rate N_1. Rates N_1 in kg ha^{-1} N in crop rotation A are as follows: 45 kg under potato and maize as well as 40 kg under winter wheat and spring barley; in crop rotation B: 45 kg under potato, 40 kg under winter wheat and under mustard, 30 kg under spring barley with undersown crop and in total 120 kg for three cuts (3 × 40 kg) under the mixture of clover with grasses. In total during a single rotation in treatments, N_1 was introduced into soil with mineral fertilisers 170 kg N in both crop rotations.

The fertilisation with phosphorus (granulated triple superphosphate – 46% P_2O_5) and potassium (60% potassium salt) in all the experimental treatments was the same and it was as follows: potato – 54 kg ha^{-1} P_2O_5, 160 kg ha^{-1} K_2O, winter wheat – 54 kg ha^{-1} P_2O_5, 100 kg ha^{-1} K_2O, spring barley – 54 kg ha^{-1} P_2O_5, 85 kg ha^{-1} K_2O, maize for silage – 54 kg ha^{-1} P_2O_5, 120 kg ha^{-1} K_2O, clover with grass – 54 kg ha^{-1} P_2O_5, 115 kg ha^{-1} K_2O.

Based on the analysis of active and exchangeable acidity, it was found that the soil represents acid and slightly acid soils. All the tillage and cultivation practices complied with the commonly applied principles of good agricultural practice. Soil was sampled once in each year, during a 4-year rotation. The sampling dates were as follows: 12 09 2009, 14 09 2010, 17 09 2011 and 14 09 2012.

Analysis of soil properties. In an adequately prepared soil material the following were assayed: the content of available phosphorus (P_{E-R}) (PN-R-04023:1996), which involves the spectophotometric measurement of the intensity of the colour of phosphorus-molybdenum blue created by orthophosphoric ions with molybdenum ions in the acid environment in the presence of $SnCl_2$, sulphate sulphur (SO_4^{2-}) following the Bardsley and Lancaster (1960) turbidimetric method modified by Department of Plant Nutrition and Fertilization, the Institute of Soil Science and Plant Cultivation, the activity of alkaline phosphatase (AlP) and acid phosphatase (AcP) with the Tabatabai and Bremner (1969) method, the activity of arylsulphatase according to the Tabatabai and Bremner (1970) method. The activity of hydrolases is based on the colorimetric assaying of released substrate: p-nitrophenol after the incubation of soil with modified universal buffer (MUB) at pH 5.5 for acid phosphatase and pH 8.5 for alkaline phosphatase and pH 5.8 acetate buffer for arylsulphatase samples for 1 h at a temperature of 37°C. Carbon of total organic compounds was assayed with the analyser "TOC Primacs" ("Scalar", Netherlands), total nitrogen – with the Kjeldahl method (PN-ISO 11261:2002).

Statistical analysis. Multiple-replication numerical data being a result of the analysis of the soil material reported over 2009–2012 were statistically verified. For the results of the contents of C, N, P, S, the activity of acid phosphatase, arylsulphatase in soil, there was performed the analysis of variance of multiple three-factor experiments, in split-plot design. The results were statistically validated using the analysis of three-factor variance (*ANOVA*). The significance of the differences between means was verified with the Tukey test at the level of significance/confidence of $p = 0.05$. Besides the results of the analyses were subjected to the analysis of simple correlation ($p < 0.05$) which determines the relationship between respective characters. The analysis of correlation was made using *Statistica 8.1* for *Windows*.

Results and discussion

The analysis of variance showed a significant effect of the experimental factors applied on the changes in the content of total organic carbon in the soil (Table 1). The content of total organic carbon was greater in the soil where crop rotation B was used (enriching the soil with organic matter) in all the years of the rotation (2009–2012). A positive effect of crop rotation B with the share of legumes and a mixture of grasses was also reported by Rutkowska and Pikuła (2013). However, a long-term intensive use of the soil fertilised with manure cannot ensure a positive total organic carbon balance. For that reason the use of crop rotation with papilionaceous plants and catchcrops allows for maintaining the optimal humus level. The manure application significantly increased the content of total organic carbon in the *Haplic Luvisol* under study. Similar results were reported by Cvetkov and Tajnšek (2009). The highest content of total organic carbon was noted in the soil from the treatments where the rate of 60 t ha^{-1} was applied (on average about 26% higher content as compared with the objects where no manure was applied).

The total nitrogen content in the soil ranged from 0.694 to 1.075 g kg^{-1} (Table 1). The greatest effect on the soil total nitrogen content was attributed to the FYM fertilization (rate of 60 t ha^{-1}) and fertilisation with N (highest rate). Similar results were earlier reported by Lemanowicz et al. (2014). The value of the C:N ratio in soil during crop growing in crop rotation A ranged from 7.82 to 10.45, whereas in the case of the second crop rotation B it was higher and ranged from 9.67 to 16.7 (Table 1). The application of the rotation of "enriching" crops in organic matter widened the C:N in the soil uptaken from the crop rotation, which can affect the biological activity in those soils, mostly the increase in the population of microorganisms which use both nutrients readily. Soil microorganisms, transforming organic and mineral compounds, enrich the soil with nitrogen, growth, antibiotic and biologically active substances (Janvier et al., 2007), the essential role in those transformations is played by enzymatic processes (Piotrowska, Wilczewski, 2012**)**.

One can thus assume that a high activity of the hydrolases, especially in the soil sampled in 2009 from the crop rotation "enriching" with organic matter is of microbiological origin. The content of available phosphorus ranged from 87.58 to 106.9 mg kg^{-1} (Table 2). The content classifies the soil as class I with a very high status of that nutrient (PN-R-04023:1996). Based on the 4-year research, there was found a significant effect of the factors applied on the content of P_{E-R} in soil. The content of that phosphorus form was higher in the soil from the crop rotation "enriching" the soil with organic

Table 1. Content of total organic carbon (g kg^{-1}), total nitrogen (g kg^{-1}) and C:N ratio in soil

		Total organic carbon				Total nitrogen				C:N			
		2009	2010	2011	2012	2009	2010	2011	2012	2009	2010	2011	2012
Factor I crop rotation	A	6.45	8.32	6.60	9.74	0.824	0.886	0.694	0.932	7.82	9.39	9.51	10.45
	B	12.5	9.25	8.44	10.4	0.751	0.942	0.753	1.075	16.7	9.82	11.2	9.67
Factor II manure t ha^{-1}	0	8.75	8.22	6.41	8.55	0.771	0.874	0.647	0.858	11.3	9.41	9.91	9.96
	20	9.38	8.34	7.48	10.18	0.771	0.911	0.713	1.056	12.2	9.15	10.5	9.64
	60	10.4	9.79	8.66	11.47	0.820	0.956	0.810	1.096	12.7	10.2	10.7	10.5
Factor III nitrogen (N) kg kg^{-1}	N_0	9.40	8.82	7.54	10.2	0.766	0.900	0.709	0.957	12.3	9.79	10.6	10.7
	N_1	9.85	8.54	7.43	10.1	0.786	0.920	0.723	1.010	12.5	9.28	10.3	10.0
	N_2	9.28	8.99	7.59	9.81	0.810	0.921	0.739	1.042	11.5	9.76	10.3	9.41
Mean		9.50	8.78	7.52	10.1	0.787	0.914	0.724	1.003				
$LSD_{0.05}$	factor I	0.063	0.432	0.450	0.718	0.025	ns	ns	ns				
	factor II	0.047	0.568	ns	0.470	0.013	0.074	0.019	0.058				
	factor III	0.089	0.611	0.637	1.015	0.017	ns	0.005	0.035				
Interaction	II × I	0.089	0.611	0.637	1.015	0.018	ns	0.028	0.081				
	I × II	0.165	0.557	0.557	2.773	0.024	ns	0.07	0.139				
	III × I	0.067	ns	ns	0.664	ns	ns	0.006	0.050				
	I × III	0.150	ns	ns	2.684	ns	ns	0.075	0.127				
	III × II	0.082	0.983	0.432	0.814	ns	ns	0.008	0.061				
	II × III	0.091	0.906	0.566	0.968	ns	ns	0.020	0.076				

ns – non-significant

Table 2. Content of available phosphorus (P_{E-R}) (mg kg^{-1}), sulphur ($S-SO_4^{2-}$) (mg kg^{-1}) and P:S ratio in soil

		P_{E-R}				$S-SO_4^{2-}$				P:S			
		2009	2010	2011	2012	2009	2010	2011	2012	2009	2010	2011	2012
Factor I crop rotation	A	84.86	100.7	81.07	81.18	19.51	12.83	14.09	9.903	3.59	4.46	5.75	5.47
	B	98.03	113.2	94.62	93.99	23.66	22.56	29.88	14.85	5.02	8.83	3.17	9.49
Factor II manure t ha^{-1}	0	78.33	89.27	74.68	75.17	21.88	11.526	21.83	10.053	3.58	7.74	3.42	7.48
	20	85.53	95.62	80.32	79.62	21.77	18.369	21.67	10.503	3.93	5.21	3.71	7.58
	60	110.4	136.0	108.5	107.9	21.09	23.187	22.45	16.58	5.23	5.87	4.83	6.51
Factor III nitrogen (N) kg kg^{-1}	N_0	85.13	93.60	73.90	76.35	22.02	17.06	21.70	12.44	3.87	5.29	3.41	6.14
	N_1	98.80	123.9	102.2	100.4	22.62	16.87	21.89	11.58	4.37	7.35	4.67	8.57
	N_2	90.40	103.9	87.41	85.94	20.10	19.15	21.37	13.11	4.50	5.43	4.09	6.56
Mean		91.44	106.9	87.84	87.58	21.58	17.69	21.98	12.38				
$LSD_{0.05}$	factor I	2.019	7.913	6.975	2.308	2.728	3.444	1.412	0.445				
	factor II	2.050	4.726	2.214	1.713	ns	0.542	0.788	0.916				
	factor III	1.068	1.036	0.879	2.108	1.058	0.851	0,626	0.490				
Interaction	II × I	ns	ns	ns	ns	ns	0.766	1.115	1.206				
	I × II	ns	ns	ns	ns	ns	3.231	1.398	1.103				
	III × I	ns	ns	ns	ns	1.496	1.203	0.885	0.694				
	I × III	ns	ns	ns	ns	2.264	2.833	1.204	0.631				
	III × II	ns	1.794	ns	3.651	1.862	1.476	1.083	0.849				
	II × III	ns	4.927	ns	3.415	2.392	1.313	1.173	1.137				

ns – non-significant

matter. The manure application at the rate of 60 t ha^{-1} significantly increased the content of P_{E-R} in all the research years (Table 2), which is mostly due to the fact of introducing on average about 13.5 kg ha^{-1} P of that component into soil with manure. Similar results were earlier reported by Lemanowicz et al. (2014). However, in the soil sampled in 2010 (two years after the FYM application), the content of that macronutrient was highest both in crop rotation A (mean of 101.7 mg kg^{-1}) and in crop rotation B (mean of 114.7 mg kg^{-1}). The use of catch crops and application of crop rotation prevent a potential loss of nutrients remaining from the previous cultivation due to their leaching deep the soil profile or from microbiological and biochemical decomposition.

The content of sulphate sulphur in soil over the four research years fell within a wide range of 9.903 to 19.51 mg kg^{-1} in the soil sampled from crop rotation A and from 14.85 to 29.88 mg kg^{-1} in the samples from crop rotation B (Table 2). In most soils under agricultural use the content of sulphate sulphur in Poland does not exceed 25 mg kg^{-1} of soil. Most soils, namely 70% of the agricultural acreage, show the content of that sulphur fraction from 5.0 to 20.0 mg kg^{-1} (Lipiński et al., 2003). Such a content range was noted in the soil sampled from the experiment from crop rotation A, while the soil samples from the crop rotation "enriching" with organic matter in the first three research years (2009–2011) can be classified as the soil class with a very high richness of that nutrient. Such an amount of sulphates (SO_4^{2-}) guarantees a good supply of the plants with that nutrient (Lipiński et al., 2003). The lowest content of sulphur available to plants was found in the soil sampled in the last research year. The concentration of sulphates in soil below 10 mg kg^{-1}, which was also reported in the soil from the experiment in Grabów, is considered low and it does not ensure the plants with an adequate supply with sulphur. Sulphur deficit in plant production has become a problem of contemporary agriculture, especially in the North of Europe (Eriksen et al., 2004; Scherer, 2009). Besides mineral fertilisers, some amounts of sulphur are provided by natural fertilisers. FYM contains from 0.9 to 1.2 kg ha^{-1} of sulphur and it is estimated that on average 20% of total sulphur occurs in a form of sulphates available to plants, 40% accounts for the organic bonds and another 40% – organic and inorganic sulphates (Kaczor, Zuzańska, 2009). The present research showed that only a year after the application of FYM fertilisation (2009) there was found no effect of that fertiliser on the content of sulphur available to plants (Table 2). However, in successive research years it was found that the rate of 60 t ha^{-1} was the best natural fertiliser rate for which the highest contents of sulphates in soil were assayed (Table 2).

Values of the P:S ratio ranged from 3.17 to 9.49 depending on the FYM and N rate applied (Table 2). A similar tendency was earlier noted by Lemanowicz et al. (2014). Enzymes may respond to changes in soil management more quickly than other soil variables and might be useful as early indicators of biological changes (Gianfreda, Ruggiero, 2006). Activity of phosphatase, both alkaline and acid, got significantly transformed due to crop rotation and fertilisation with FYM and N. The analysis of variance demonstrated a significantly higher activity of both phosphatases in the soil sampled from the treatment with crop rotation B (considered to enrich soil with humus) (Table 3), which is due to the increasing content of total organic carbon which is one of the substrates needed for the development of soil microorganisms being the basic source of enzymes in soil (Dodor, Tabatabai, 2003). There was noted a higher activity of acid phosphatase, as compared with the activity of alkaline phosphatase (on average by 55% for the entire experiment). The increasing N rates inhibited the activity of alkaline phosphatase and stimulated the activity of acid phosphatase in the soil. Ammonium nitrate is an acid fertiliser; hence such an effect on both phosphatases. Such conditions enhance activity of acid phosphatase which dominates in acid soils (Dick et al., 2000; Dodor, Tabatabai, 2003; Lemanowicz, 2013).

Arylsulphatase catalyses the hydrolysis of an anion by fission of the O-S bond and releases plant-available inorganic S as SO_4^{2-}. The activity of arylsulphatase, similarly as other hydrolases, underwent essential changes due to crop rotation and FYM and N fertilisation (Table 3). In the first year after the application of FYM fertilisation (2009) in crop rotation A there was noted the lowest activity of arylsulphatase in the *Haplic Luvisol*. Such a low activity of arylsulphatase can come from the fact that in those soil samples there was also noted the lowest value of the C:N ratio and the highest content of sulphates (Tables 1–2). According to the concept, the balance between microbial synthesis of ester sulphates and their degradation, and thus the size of the ester sulphate pool in soils, is strongly affected by the activity of sulphohydrolases. Similar results were reported by Vong et al. (2004), who, investigating during plant growth, rhizosphere from fallow, barley and rape, found that low SO_4^{2-} concentration stimulated the biotic production of arylsulphatase. Organic fertilisation by supplying substrates for biochemical reactions and constituting the sources of carbon for soil microorganisms stimulates the enzymatic activity of soil. For that reason there was observed an effect of FYM on the activity of arylsulphatase (Table 3). Increasing the FYM rate from 0 to 60 t ha^{-1} resulted in an increase in the activity of the hydrolase by 1.9 (2010 and 2011) to 3 fold higher (2009) than in the control plots. Arylsulphatase activity is increased after a long-term application of organic manure (Kotkova et al., 2008).

However, there was found no clear effect of ammonium nitrate on the activity of arylsulphatase, as compared with the phosphatases (Table 3). Only in the soil sampled in 2010 and in 2012 there was noted an inhibiting effect of the highest rate of nitrogen fertiliser on the activity of arylsulphatase, which agrees with the results by Vong et al. (2004), who claim that available inorganic nutrients inhibit enzyme synthesis in soil. There was demonstrated, however, a clear effect of crop rotation on the activity of arylsulphatase (Table 2). In agreement with Knauff et al. (2003), with arylsulphatase, alkaline and acid phosphatase activity depended on the crop species. Similarly Acosta-Martinez et al. (2007) showed an effect of the land use: agriculture, forest and pasture on the activity of arylsulphatase in soil.

With the values of the activity of alkaline and acid phosphatase reported, there was calculated the enzymatic index of the pH soil level (Dick et al., 2000). The value of the AlP:AcP ratio during the research was 0.33–0.62 (Fig.). The value optimal for plant growth and development can be considered such a value of soil pH under the conditions of which there is recorded the adequate ratio of the AlP:AcP activity, namely 0.50 (Dick et al., 2000). The AlP:AcP ratio lower than 0.50 indicated an acid soil for which liming is recommended. The lowest values of the enzymatic index of pH were recorded in the soil from treatments where the plants were fertilised with nitrogen at the rate of N_2 as well as in the soil sampled in 2012 – four years after the FYM application (Fig.) The enzymatic indicator of the pH level can be used as an alternative method to determine soil pH as well as the changes in it (Lemanowicz, 2013).

Table 3. Activity of alkaline and acid phosphatases and arylsulphatase in soils (mM pNP kg^{-1} h^{-1})

		Alkaline phosphatase (AlP)				Acid phosphatise (AcP)				Arylsulphatase			
		2009	2010	2011	2012	2009	2010	2011	2012	2009	2010	2011	2012
Factor I crop rotation	A	0.583	0.553	0.462	0.617	1.261	1.047	1.035	1.852	0.030	0.366	0.265	0.350
	B	0.878	0.573	0.779	0.868	1.976	1.359	1.280	1.847	0.156	0.309	0.085	0.225
Factor II manure t ha^{-1}	0	0.723	0.476	0.555	0.694	1.382	0.957	1.051	1.696	0.043	0.247	0.129	0.180
	20	0.708	0.511	0.623	0.697	1.505	1.081	1.139	1.814	0.109	0.294	0.147	0.295
	60	0.761	0.701	0.684	0.836	1.968	1.571	1.281	2.038	0.129	0.471	0.247	0.388
Factor III nitrogen (N) kg kg^{-1}	N_0	0.743	0.563	0.680	0.847	1.504	1.067	1.097	1.708	0.094	0.320	0.164	0.236
	N_1	0.760	0.600	0.628	0.725	1.620	1.208	1.160	1.589	0.095	0.355	0.185	0.337
	N_2	0.690	0.525	0.554	0.655	1.731	1.225	1.215	1.982	0.090	0.337	0.175	0.290
Mean		0.731	0.563	0.620	0.742	1.157	1.850	1.618	1.203	0.093	0.337	0.175	0.288
$LSD_{0.05}$	factor I	0.070	ns	0.017	0.019	0.023	0.140	0.021	ns	0.072	0.014	0.093	0.035
	factor II	0.007	0.039	0.013	0.026	0.069	0.073	0.017	0.018	0.039	0.051	0.041	0.025
	factor III	0.022	0.024	0.011	0.017	0.051	0.049	0.022	0.016	ns	0.030	ns	0.033
Interaction	II × I	0.009	0.055	0.019	0.037	0.025	0.025	0.097	0.104	0.055	0.073	0.057	0.035
	I × II	0.068	0.073	0.019	0.033	0.025	0.123	0.081	0.136	0.071	0.060	0.086	0.204
	III × I	0.032	ns	0.015	0.023	ns	0.023	0.072	0.069	ns	0.042	0.033	0.047
	I × III	0.057	ns	0.016	0.023	ns	0.118	0.061	0.115	ns	0.035	0.077	0.187
	III × II	0.039	0.041	0.018	0.029	ns	0.028	ns	0.085	ns	0.051	0.040	0.057
	II × III	0.032	0.051	0.020	0.035	ns	0.028	ns	0.100	ns	0.066	0.052	0.053

ns – non-significant

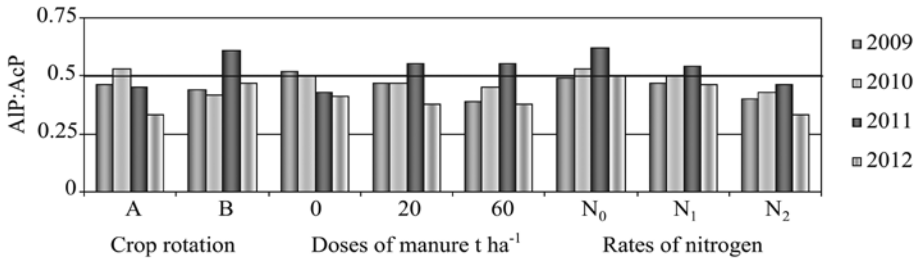

Figure. Ratio of alkaline to acid phosphatase (AlP:AcP) (the enzymatic index of pH)

In the soil from the experiment in Grabów there was shown a positive correlation between the content of the nutrients of organic matter of soil and the activity of the hydrolases. The content of total organic carbon positively correlated with the activity of alkaline phosphatase (r = 0.72, $p < 0.05$) and acid phosphatase (r = 0.82, $p < 0.05$) (Table 4). Ma et al. (2011), investigating polar soils of the Tundra, found a significant value of the coefficient of correlation between the activity of alkaline phosphatase and the content of organic carbon (r = 0.70, $p < 0.001$) and total nitrogen (r = 0.43, $p < 0.002$) as well as with mineral phosphorus (r = 0.40, $p < 0.037$). The content of nitrogen, on the other hand, showed an effect on the activity of arylsulphatase (r = 0.66, $p < 0.05$). There was also recorded a correlation between the activity of arylsulphatase and the phosphorus content in soil (r = 0.52, $p < 0.05$) and sulphates content ($r = -0.47$, $p < 0.05$). Negative correlation confirms the results reported by others (Vong et al., 2004; Kotkova et al., 2008) that a high content of sulphates in soil decreases the activity of arylsulphatase. A lack of the correlation between the content of available phosphorus and the activity of phosphatases is confirmed by earlier reports by Orczewska et al. (2012).

Table 4. Pearson's correlation coefficients (n = 32)

	Total organic carbon	Total nitrogen	P_{E-R}	$S\text{-}SO_4^{-2}$
Alkaline phosphatase (AlP)	0.72			
Acid phosphatase (AcP)	0.82	0.50		
Arylsulphatase		0.66	0.52	−0.47

Significant at $p < 0.05$; P_{E-R} – available phosphorus, $S\text{-}SO_4^{-2}$ – sulphur

Conclusions

1. A 4-year cultivation of crops in crop rotation enriching soil with organic matter increased the richness of that soil with carbon of organic compounds as well as the forms of phosphorus and sulphur available to plants. Over three years after the manure application the soil sampled from that crop rotation had a very high content of sulphur, well-supplying the plants with that nutrient.

2. The crops in the crop rotation affected the activity of the hydrolases participating in phosphorus and sulphur transformations. The activity of phosphatases was usually higher in the crop rotation enriching the soil with organic matter. The activity of arylsulphatase, except for the first year after the manure, was highest in the crop rotation without undersown crops.

3. The best natural fertiliser rate for which there was noted the highest activity of the hydrolases was 60 t ha^{-1}.

4. The ammonium nitrate affected the content of available forms of phosphorus. Most frequently the highest rate of that fertiliser inhibited the activity of the hydrolases investigated in soil.

5. The values of the coefficients of correlation between the hydrolases activity and content of carbon and nitrogen point to the relationship between the parameters.

6. Long-term mineral nitrogen fertilisation and organic fertilisation significantly influence soil properties, which may lead to the change of enzymatic activity of soil as well as nutrient contents in soil. The results of the study show that it is necessary to continue research on soil chemical and enzyme properties relative to different fertilizers rates for the selection of the proper management system.

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Anglies, azoto, fosforo ir sieros kiekio dirvožemyje sąsajos su hidrolazių aktyvumu, priklausomai nuo sėjomainos ir tręšimo

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Santrauka

Tyrimo metu siekta nustatyti sėjomainos ir tręšimo mėšlu bei azotu įtaką fosforo ir sieros kiekiui dirvožemyje, atsižvelgiant į jų sąsają su fosfatazės ir arilsulfatazės veikla. Dirvožemio ėminiai buvo atrenkami 2009–2012 m. iš ilgalaikio lauko bandymo, vykdyto pagal trijų veiksnių schemą, apimančią du sėjomainos tipus (A – gausinanti, B – eikvojanti dirvožemio organinę medžiagą), tręšimą mėšlu (0, 20 bei 60 t ha^{-1}) ir azotu (N_0, N_1 bei N_2). Bandymo lauko dirvožemis – paprastasis išplautžemis (IDp). Nustatyta esminė bandymo veiksnių įtaka augalams pasiekiamų formų mitybos elementų kiekiui. Taikant standartą „Cheminės ir žemės ūkio analizės – augalams pasiekiamo fosforo kiekio nustatymas mineraliniuose dirvožemiuose“ (PN-R-04023:1996) tirtas dirvožemis pagal augalams pasiekiamo fosforo kiekį priskirtinas antrai aukščiausiajai klasei. Hidrolitinių fermentų veikla priklausė ir nuo tręšimo mėšlu, ir nuo tręšimo amonio salietra. Tyrimo rezultatai parodė reikšmingą sėjomainos tipo įtaką suminės organinės anglies, suminio azoto, augalams pasiekiamų fosforo ir sieros kiekiams, priklausomai nuo tam tikros hidrolazės veiklos. Augalus auginant sėjomainoje, gausinančioje dirvožemio organinę medžiagą, jame pagausėjo augalams pasiekiamų fosforo bei sieros ir padidėjo rūgščių fosfatazės bei arilsulfatazės aktyvumas.

Reikšminiai žodžiai: augalų sėjomaina, fosforas, siera, šarminės ir rūgščios fosfatazės bei arilsulfatazės, tręšimas.