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Enzymatic activity and enchytraeids abundance in agricultural mountain soils

Abstract: The number of soil mesofauna and enzymatic activity of soils are good indicators of changes in soil influenced by cultivation. The aim of this study was to compare density of enchytraeids and the activity of dehydrogenases (ADh), urease (AU), and invertase (AI) in the soils of grassland and arable land. Relationships that exist between those biological parameters and the basic soil properties (the content of total organic carbon (TOC) and nitrogen (TN), pH, texture, and total porosity) were defined. In the research, soil material from humus horizon of 12 soils which were located in the Mały Beskid and Silesian Foothills (S Poland) was used. The main density of enchytraeids in grassland soils (12 982 ind·m^{-2}) was twice higher than in arable land soils (6099 ind·m^{-2}), and the differences were statistically significant. Grassland soils were characterised by higher enzymatic activity than arable land soils. However, only ADh, which were almost three times higher in grassland than in arable soils (2024 and 742 µmol TPFkg^{-1}h^{-1}, respectively), showed significant differences. In grassland soils more favourable edaphic conditions for the development of soil organisms occurred in comparison with arable land.

Keywords: Enchytraeidae, dehydrogenases, urease, invertase, land use

INTRODUCTION

Enchytraeids (Enchytraeidae*,* potworms, microannelids) play an important role in the formation of soil and its properties, especially in acidic soils in which the activity of earthworms is limited. They are important in the circulation of organic matter and elements and the energy flow in ecosystems (e.g. Kasprzak 1986, Römbke et al. 2013). The relation between enchytraeids and soil properties such as pH value, soil organic matter content or texture are generally known (Beylich and Graefe 2009, Severon et al. 2012, Römbke et al. 2013). The influence of land use on microannelids were also investigated (Jänsch et al. 2005, Schlaghamerský et al. 2007).

The measurement of the soil enzymatic activity, is commonly used to evaluate the health and fertility of the soil (Russel 2005, Karaca et al. 2011), anthropogenic effects on soil stress factors (Ciarkowska et al. 2014), and fertilisation and cultivation practices (Bandick and Dick 1999). Bandick and Dick (1999) claim that the measurement of enzyme activity is a good and sensitive indicator of changes in soil influenced by cultivation. The measurement of the soils enzymatic activity using accepted methods indicates the maximum potential soil enzyme (Alef and Nannipieri 1995). For the purpose of this work, three of them were chosen: soil enzymes – urease and invertase, which are responsible for the metabolism of organic matter and the release of nutrients available to plants (Alef and Nannipieri 1995), and dehydrogenases (intracellular enzymes), which are used to determine the total soil microbial activity (Włodarczyk 2000).

Little is known about the relation between enchytraeids and enzymatic activity in soil. The purpose of this work was to provide information on these organisms, their amount in agricultural soils and links with selected soil properties, including enzyme activity and the soil use.

MATERIALS AND METHODS

Sites and soils

The study was carried out on six sites located in two geographic region of the Polish Carpathian Mts. (S Poland): the Silesian Foothills (sites P1–P3) and the Mały Beskids (sites P4–P6). All sites were situated in lower mountain locations at an altitude of 320–545 m above sea level, in a moderately warm climate floor. The mean annual sum of precipitation in this area ranges from 700 to 1400 mm, and the mean annual temperature is 6–8°C. At each site, 2 soil pits were performed: one on the arable land (A) and one

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on the grassland (G). In manuscript was used the abbreviation according to the scheme: P1A – site 1 (P1) arable land (A), P1G – site 1 (P1) grassland (G) etc. The bedrock for soils from Silesian Foothills was the loess-like carbonateless material. According to WRB (IUSS Working Group WRB 2015), these soils were classified as Gleyic and Haplic Retisols. The soils at Mały Beskids was formed from the Carpathian flysch and was classified, in most cases, as Haplic and or Stagnic Cambisols. The study area was dominated by the cultivation of cereals (mainly wheat) and potatoes (Table 1).

TABLE 1. Fertilisation and management of the investigated soils

Site	The way of use*	Fertilization **	Crop rotation /age of grassland
P1	A	M	potatoes, aftercrop lupins – wheat
	G	0	meadow for more than 20 years, after cutting hay is left in the meadow
P2	A	M+N	potatoes, aftercrop lupins – wheat
	G	0	hay meadow for 8 years
P3	A	0	maize – wheat – wheat
	G	0	meadow for 10 years
P4	A	M+N	potatoes – wheat – wheat
	G	M+N	pasture for a long time, sometimes mowed
P5	A	M+N	potatoes – wheat – mixture of grasses – oat
	G	0	meadow for 3 years, before it was cultivated limb and rye, 1 or 2 swaths or grazing
P6	A	0	wheat – wheat
	G	0	meadow around 15 years old, 1–2 swaths

* A – arable land, G – grassland; ** M – mineral, N – natural, 0 – none.

Soil sampling and analysis

Twelve soil pits were excavated for description and sampling. For the purposes of this study, soil materials were collected only from the humus horizons. The soil samples were divided into two parts. One part was immediately sieved (2 mm mesh size) and stored field-moist at 4°C for enzymatic measurements. The second part of the soil was air-dried, sieved (2 mm sieve soil), and used for analyses. Undisturbed soils were taken using 100 cm^3 cylinders.

In the fresh samples, dehydrogenase activity (ADh) was determined by the method of Casida et al. (1964), urease activity (AU) by that of Tabatabai and Bremner (1972, cit. after: Alef and Nannipieri 1995)), and invertase activity (AI) by Frankenberger and Johanson (1983). The analyses were conducted in 3 replications.

In dry soil samples, properties such as a particle size distribution by the Casagrande-Prószynski areometer method (PN-R-04032) and soil pH value in H_2O by the potentiometer method (1:2.5 soil: water ratio) were measured. For total soil nitrogen (TN) estimation, the Kjeldahl method was used. Total organic carbon (TOC) content in soil samples was determined by dry combustion (Euro Thermoglas TOC-TN 1200).

Bulk density was measured in undisturbed soil samples. Solid phase particle density was determined by the pycnometer method. Total porosity (P) of soil was calculated based on bulk and particle density. To specify macro-, meso- and micro-pores, wilting point (WP) and water field capacity (WFC) were determined based on curves of the soil water retention capacity (pF curves), using a porous plate in pressure chambers (Eijkelkamp's apparatus) (Klute and Driksen 1986).

Enchytraeids sampling and extracting

Samples intended for the extraction of enchytraeids were collected in 10 replicates designated along the transect. For this purpose, the sampler was used where the soil cores were obtained with a height of 10 cm and a surface area of 16.6 cm^3. From the soil cores collected in the field, enchytraeids were extracted using a method modified by O'Connor Baermann's wet funnels (Adl 2008).

Statistical analysis

Data sets were statistically analysed using the Statistica 10 software (StatSoft, Inc. 1984–2011). A nonparametric Mann-Whitney U test was used to test the significance of differences between mean values and a Spearman rank correlation test was used to determine the strength of the association between the studied characteristics of soils. Differences at $p <0.05$ were considered significant.

RESULTS

Based on the granulometric composition (Polskie Towarzystwo Gleboznawcze 2009), soils were assigned to the following textural classes: loam, silty clay, and silt loam. Soils from sites 1–3 were more silty (silt loam and silty clay) than soils from sites 4–6 (mostly loam soil). It was observed that the humus horizons

TABLE 2. Selected soil properties, and results of the Mann-Whitney U test (U and p) showing the significance of differences between grassland and arable land soils

Site		Depth of humus horizon	Fraction of: 2–0.05 mm	0.05–0.002 mm	<0.002 mm	pH H_2O	TOC	TN	P	Pore size < 0.2 µm	0.2–30 µm	< 30 µm
			%				g·kg^{-1}		%			
P1	A	23	18	70	12	5.5	13.15	1.13	44.34	14.69	23.03	6.62
	G	30	18	74	8	4.9	29.44	2.59	47.40	15.17	23.48	8.75
P2	A	23	14	73	13	5.3	14.45	1.54	46.92	14.83	20.91	11.17
	G	21	12	78	10	5.1	23.78	2.23	55.27	11.93	28.50	14.84
P3	A	27	17	59	24	5.5	12.52	1.55	45.72	18.17	18.06	9.49
	G	23	19	61	21	5.6	15.00	1.75	44.63	16.67	22.12	5.84
P4	A	23	49	32	19	7.1	15.15	1.41	39.56	16.94	15.26	7.36
	G	17	47	50	3	5.1	34.39	3.21	55.53	15.79	21.15	18.60
P5	A	23	50	36	14	5.7	14.05	1.00	45.93	10.45	22.17	13.32
	G	19	44	51	5	6.0	16.55	1.66	48.47	12.57	22.51	13.40
P6	A	22	35	49	16	6.4	15.57	1.65	44.74	22.27	13.14	9.33
	G	20	30	62	7	6.1	21.53	2.20	58.31	10.73	35.08	12.51
U		9.0	17.0	10.0	5.0	11.0	2.0	0.0	4.0	12.0	5.0	11.0
p		0.173	0.936	0.230	0.045*	0.298	0.013*	0.005*	0.031*	0.378	0.045*	0.298

Statistical significance shown: * $p<0.05$.

of arable land had a significantly higher content of clay fraction (<0.002 mm) than in the corresponding horizons of grassland (Table 2).

The pH value of tested soils ranged from 4.9 to 6.4 (Table 2). In most of the investigated soils, the arable land soil showed a slightly higher pH than grassland soils, but the differences were not significant.

There was higher content of TOC in soil humus horizon of grassland soils than in the same horizon of arable land soils. TN content was analogous to TOC content. The differences between mentioned soil properties were statistically significant.

Soil from humus horizons of grasslands, except for a P3G, were characterised by higher total porosity and mesopore content than the corresponding soil from arable land. Differences in soil porosity and the quantity of mesopores between grassland and arable land soils were statistically significant.

The density of enchytraeids in the soils varied between the soils studied. In grassland soils, the density of these Oligochaeta ranged from 3414 ind·m^{-2} to 24548 ind·m^{-2}. In the soil of arable land, this feature varied from 2410 to 8434 ind·m^{-2} (Fig. 1). Density of enchytraeids in most of the soils was

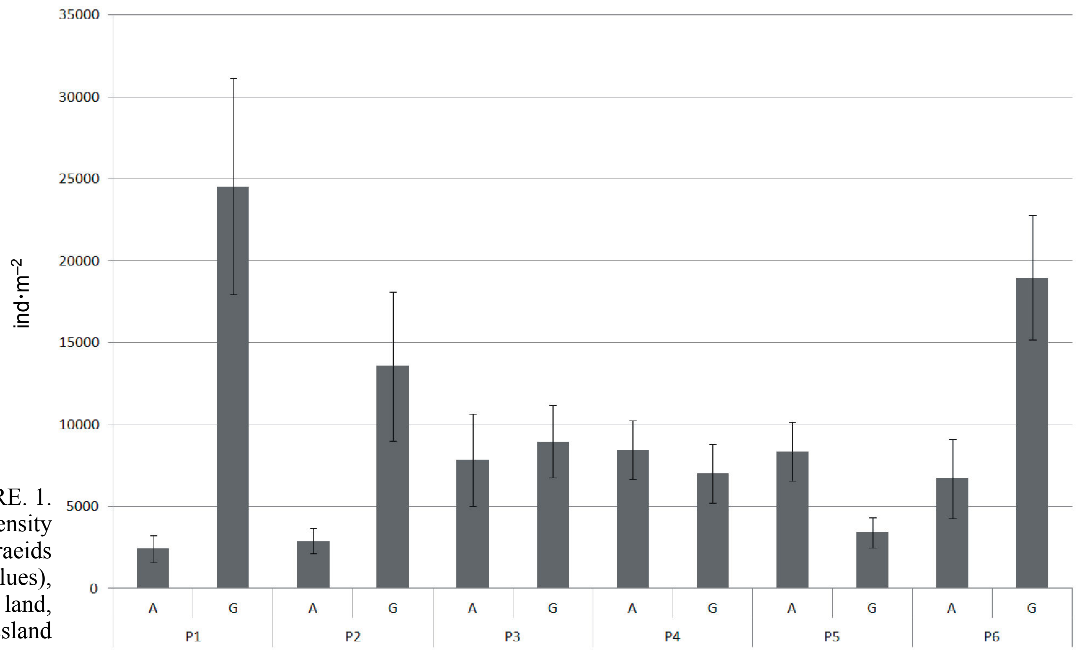

FIGURE. 1. Density of enchytraeids (mean and SD values), A – arable land, G – grassland

higher in grassland than in arable land soils. Differences between density of enchytraeids in soils of arable land and grassland were significant (U=4.0, p=0.031).

Dehydrogenase activity in grassland soils was higher than in the soils of arable land (Fig. 2). This relationship was confirmed by the statistical interpretation (U=4.0, p=0.031). For most of the sites, grassland soils were characterised by a higher invertase activity than arable land (Fig. 2), but these differences were not confirmed in the statistical interpretation (U=8.0, p=0.128). Urease activity had similar character to AI (Fig. 2), however, like in the case of AI, the difference in AU between soils used in different ways was not statistically significant (U=12.0, p=0.378).

The results of the statistical analysis, presented in Table 3, showed that with the increasing content of

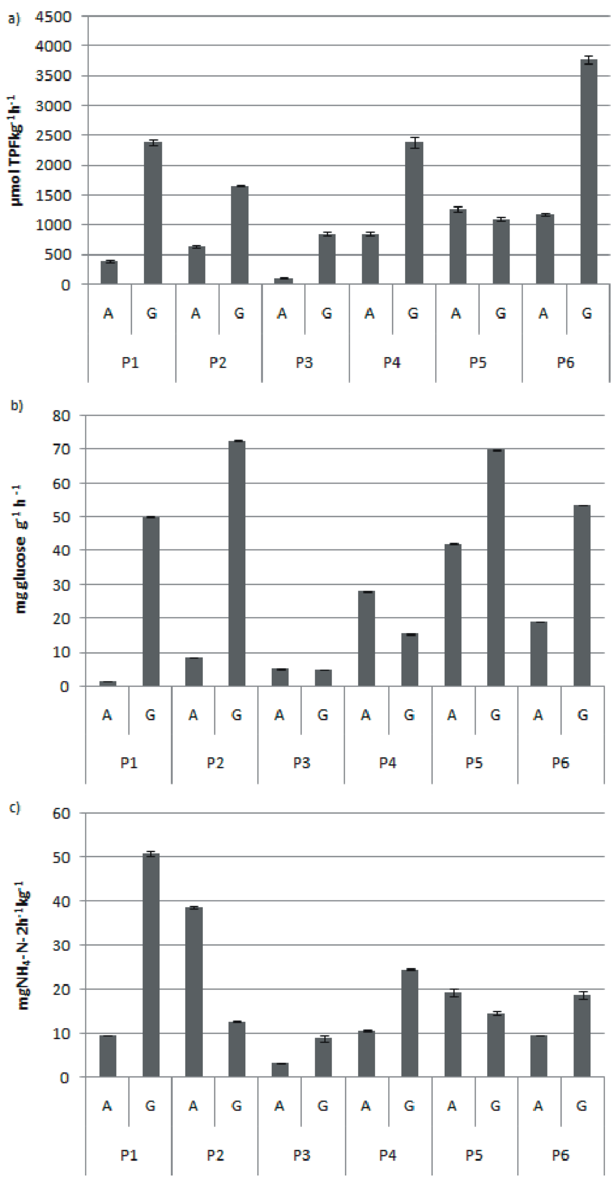

FIGURE 2. Enzymes activity (mean and SD values), a) urease, b) invertase, c) dehydrogenase; A – arable land, G – grassland

TABLE 3. Spearman rank correlation between the investigated properties of the soil

		Enchytraeids	AU	AI	ADh
Enchytraeids					
AU		0.273			
AI		0.448	0.455		
ADh		0.727*	0.594*	0.650*	
Fraction of:	2–0.05 mm	-0.014	0.154	0.161	0.301
	0.05–0.002 mm	0.273	0.196	0.091	0.077
	<0.002 mm	-0.203	-0.657*	-0.517	-0.671*
pH H_2O		-0.280	-0.413	0.098	-0.154
TOC		0.587*	0.552	0.622*	0.839*
TN		0.692*	0.315	0.371	0.685*
P		0.503	0.608*	0.622*	0.748*
Pore structure	< 0.2 µm	-0.182	-0.448	-0.517	-0.406
	0.2–30 µm	0.497	0.308	0.490	0.510
	< 30 µm	0.168	0.476	0.587*	0.524

Statistical significance shown: * $p<0.05$.

TOC and TN, the density of enchytraeids increased. This relationship confirms the positive correlation coefficient between the content of TN and TOC and number of enchytraeids. The content of TOC and TN had a positive effect on the ADh. Also, a correlation between TOC and AI was found. The content of clay fraction negatively affected biological parameters in the studied soils. In particular, the AU and the ADh were reported to have a statistically significant negative relationship. The positive effect on the enzyme activity and density of enchytraeids had the total porosity of the soil (Table 3).

DISCUSSION

Schlaghamerský et al. (2007) suggested that a crucial problem of field research is finding a good location to compare different land uses. Very often, it was almost impossible to perform research in the assumed places. Our study showed that, in the small farms that dominate in the Polish Carpathians, it was difficult to obtain accurate data on soil fertilisation. However, the sites in our research were selected in optimal locations, where each grassland neighboured with arable land, so that the impact of soil forming factors unrelated to the method of use was excluded. Each grassland was 10 or more years old and arable lands were constantly cultivated, with the only one exception for P5G. The grassland from P5G was only three years old, and the neighbouring arable land (P5A) had been cultivated for three years (it was a grassland earlier). For this reason, at this site, the differences between grassland and arable land were not as visible. However, the results presented should allow conclusions to be drawn about the effect of different methods of use on soil biological properties.

The manner of land use was one of the factors affecting soil biological activity, as shown in the present study and the extensive data (e.g. Bandick and Dick 1999; Wallenius et al. 2011). Grasslands soils were characterised by higher biological activity than arable land soils. The adverse effect of cultivation on dehydrogenase activity and enchytraeid stood out clearly and was statistically confirmed. In contrast, no clear differences were found for invertase and urease activity between both types of land use. Bandick and Dick (1999) suggested that higher enzymatic activities in the pasture and rescue treatments may be caused by the absence of tillage and the rhizosphere effect. Differences in the manner of land use resulted in variations in the soil properties such as texture (especial <0.002 mm fraction content), TOC and TN content and total porosity. Those soil properties also influenced enzymatic activity. Similar results were reported by many authors (e.g. Gianfreda et al. 2005, Włodarczyk 2000, Kucharski 1997). Some physical properties of the soil, such as clay fraction and total porosity, are known to increase the activity of enzymes in the soil (Włodarczyk 2000,+ Kucharski 1997). In the investigated soils, a positive correlation between ADh, AU and AI, and porosity was found (Table 2). It is noteworthy that clay fraction had a negative effect on the enzyme activity. The positive role of the clay fraction was previously noted in sandy soils, however in clay ones the correlation between the enzymatic activity and fraction below 0.002 mm is negative (Kucharski 1997, Taylor et al. 2002).

Enchytraeids are small soil organisms which act in the soil in similar way as earthworms do (Pączka et al. 2015). However, the role of Enchytraeids in soils is not yet fully understood and appreciated (Ivask et al. 2008). The comparison of the density of these organisms showed that there were fewer organisms in cultivated soils than in those covered by grass. However, at two sites, density of enchytraeids was higher in soils of arable land than grassland. This may be associated with a resistance of these organisms in the soil disturbance related to the cultivation. This is in accordance with the opinion of Topoliantz et al. (2000), who stated that the representatives of small invertebrates, e.g. enchytraeids, have a higher resistance to the stress associated with the cropping. If the cultivation had not yet disturbed the soil, as takes place in a P5A site, or if the supply of organic matter, e.g. manure is significant, as takes in a P4A place, the benefits associated with the introduction of organic residues may outweigh the negative mechanical damage caused by the effects of cultivation (Lagerlof et al. 1989 cit. after: Parmelee et al. 1990). Also, Schlaghamersky et al. (2007) emphasised the great ecological adaptability of enchytraeids, but mentioned the low ability to compete with other soil-dwelling groups. The density of enchytraeids corresponded with the manner of use, ADh and the content of TOC and TN.

CONCLUSIONS

1. Grassland has a positive impact on the soil biological activity. The dehydrogenase activity was three times higher and the density of enchytraeids was two times higher in a grassland than in an arable land soil.
2. The content of the total organic carbon and the total nitrogen are the most important soil properties correlated positively with the enzymatic activity and density of enchytraeides.
3. In silty loamy and silty clay soils the enzymatic activity is negatively correlated with the clay fraction and positively with the total soil porosity.
4. Density of enchytraeids is correlated with the activity of dehydrogenase. Therefore, it can be assumed that a high density of enchytraeids will be reflected by a high microbial activity in the soil.

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Aktywność enzymatyczna i liczebność wazonkowców w glebach górskich użytkowanych rolniczo

Streszczenie: Liczebność mezofauny glebowej i aktywność enzymatyczna gleb są dobrymi wskaźnikami zmian zachodzących w glebie pod wpływem uprawy. Celem pracy było porównanie zagęszczenia wazonkowców i aktywności dehydrogenaz (ADh), ureazy (AU) i inwertazy (AI) w glebach użytków zielonych i gruntów ornych. Określono także zależności jakie występują pomiędzy wspomnianymi parametrami biologicznymi a podstawowymi właściwościami gleby, takimi jak: zawartość węgla organicznego (TOC) i azotu ogółem (TN), pH, uziarnienie gleby i porowatość. Do badań wykorzystano materiał z poziomów próchnicznych 12 profili glebowych zlokalizowanych w Beskidzie Małym oraz na Pogórzu Śląskim. Średnie zagęszczenie wazonkowców w glebach użytków zielonych (12 982 os. m^{-2}) było ponad 2-krotnie wyższe niż w glebach gruntów ornych (6099 os. m^{-2}), a różnice te były istotne statystycznie. Gleby użytków zielonych charakteryzowały się także wyższą aktywnością enzymatyczną, niż gleby gruntów ornych. Jedynie w przypadku ADh, która w glebach użytków zielonych była prawie 3-krotnie wyższa niż w glebach gruntów ornych (odpowiednio 2024 i 742 μmol $TPF kg^{-1} \cdot h^{-1}$), różnice te były istotne. Wyższa aktywność biologiczna gleb użytków zielonych, w porównaniu do gleb gruntów ornych wynikała m. in. z występowania w nich korzystniejszych dla rozwoju organizmów warunków edaficznych. Poziomy próchnicze gleb użytków zielonych charakteryzowały się wyższą zawartością TOC i TN oraz porowatością, a także niższą zawartością frakcji iłu niż gleby gruntów ornych. Wysokie zagęszczenie wazonkowców szło w parze z wysoką aktywnością enzymatyczną, zwłaszcza z ADh (istotny współczynnik korelacji). Zagęszczenie wazonkowców oraz ADh istotnie zależały od zawartości TOC i TN. Ponadto, ADh istotnie dodatnio korelowała z porowatością gleby oraz ujemnie z zawartością frakcji iłu.

Słowa kluczowe: dehydrogenazy, inwertazy, sposób użytkowania, ureazy, wazonkowce