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THE ROLE OF HABITAT HETEROGENEITY IN THE RELATIONSHIPS BETWEEN SOIL PROPERTIES AND EARTHWORM ASSEMBLAGES: A CASE STUDY IN POMERANIA (NORTHERN POLAND)

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Abstract

The vastness of the research on earthworm assemblages in agricultural lands focus on the comparison of agricultural treatments of different intensity. Consequently, spatial complexity of the landscape is less emphasised. Our study addresses this knowledge gap. The field study taken in West Pomeranian Lake District in Poland during four campaigns (spring and autumn) revealed that the mosaic of fields (MF) supported higher earthworm abundance than the similarly managed but homogeneous field (HF). Number and biomass of earthworm individuals both reflect the relationships with electrical conductivity, pH and in some situations also soil organic carbon and soil moisture effectively. We argue that autumn sampling is more preferable for biomonitoring.

Key words

soil properties • earthworms • landscape structure • biological monitoring

Introduction

Earthworms are well-known for being sensitive and reliable indicators of the quality and health of soils. Certain parameters of earthworm assemblages may provide information on the progress of chemical and physical processes occurring in soils that improve or degrade their overall condition (Paoletti 1999; Keith et al. 2012 as cited in Regulska & Kołaczowska 2015). Since earthworms

are closely connected with the soil substratum and have restricted mobility, they rely on habitat and food resources at a local scale. In recent studies on soil biology and ecology, earthworm assemblage characteristics have been discussed against the background of such soil properties as: acidity, electrical conductivity, soil organic carbon and total nitrogen contents, C/N ratio, soil organic matter content, soil moisture content and soil texture (Regulska 2008; Valckx et al.

2009; lordache & Borza 2010). However, conclusions drawn from the vastness of research are still ambiguous (Chan & Barchia 2007).

Most studies have focused on the comparison of agricultural treatments and cultivation methods of different intensity (Ivask et al. 2007; Ouellet et al. 2008). Consequently, there has been less emphasis on the spatial complexity of the landscape (Regulska & Kołaczowska 2015).

The objective of our study was to clarify the nature of the relationships between earthworm assemblages and soil properties in two areas of arable land of different spatial structure. It was hypothesised that more structurally complex farmlands: (a) support more abundant earthworm assemblages and (b) are characterised by different relationships between selected belowground fauna and soil parameters, compared to homogeneous, large-sized units.

The foregoing hypothesis was tested in another region of Poland – the Suwałki Lake District (54°17'N, 22°30'E), over 300 km to the East of the study area presented below (Regulska & Kołaczowska 2015). Both field studies were conducted in young glacial landscapes as parts of the project entitled “Faunistic and landscape indicators and their usefulness for the estimation of the sustainable development of the rural landscape (one example of a selected area)”.

The areas described in the previous article (Masuria Region) and in this paper (Pomerania Region) were characterised by different arable farming methods up to World War II. The former cultural practices are reflected in the contemporary spatial arrangement of fields. The fields analysed in the previous article are generally smaller and represent less intensive agriculture than the fields from this paper. Since the 1990s (after the cessation of food production by State farms in Poland) the use of agrochemicals in the Suwałki Lake District has shown a distinct decrease. At present, most of the farmers in this area use very little or no mineral fertilisers and pesticides – contrary to the area presented in the manuscript. All the above-mentioned differences

have been reflected in our results and that is why we treat each region as a separate case study.

Materials and methods

Site description

The study area is located in a young glacial landscape within an undulating morainic plateau near Potęgowo in the Damnicka Upland (54°29'N, 17°29'E), which is part of the West Pomeranian Lake District in Northern Poland (Fig. 1).

The soil cover was formed as a result of long-term pedological processes in the Late Pleistocene, within the range of the Pomeranian phase of the last Weichselian/Würm glaciation (Marcinkowska et al. 2013).

Local climatic conditions are influenced by the Baltic Sea. According to meteorological observations from 1991 to 2009 in Lębork, the long-term mean monthly temperature was 14.5°C in the warm season (April-September) and 3.0°C in the cold season (October-March); mean annual temperature was 8.8°C. Mean annual precipitation for this period was 716 mm.

The main site selection criterion was the existence of two spatially different configurations of arable fields in close proximity (< 1 km) to reduce the potential influence of other local factors on soil biota. The selected farmlands are old-structured landscapes and have been under constant management for at least 100 years. We established two study sites of approximately 4.5 ha each and about 800 m apart from each other (Fig. 1). The study site named MF (Mosaic of Fields) comprised seven small fields crossed by balks and tracks with a Mean Shape Index MSI (McGarigal & Marks 1995) of 6.10. The second study site named HF (Homogeneous Field), was located within a single large-sized field with MSI of 1.21. The study plots were distributed near the field margins as well as in-field in the HF. All the study plots were placed in a relatively flat area, with elevation differences not exceeding 1.67%. The actual vegetation comprised arable weed communities

consisting predominantly of annual plants accompanying the cultivated crops (phytologically, the *Aphanenion arvensis* alliance [R. Tx. et J. Tx. 1960]). The dominant soil type at both sites was Epicutanic Luvisol with a texture of loamy sand.

Earthworm assemblage abundances are mostly higher in crop rotation systems, including both commercial and cover crops, than in less complex systems (Hubbard et al. 1999). In our study crop rotation was very simple over the study period and both sites were managed in a conventional way. The main differences between the sites were apparent only in autumn. In spring, cereals were grown at both sites in nearly 100%

of the field areas (Tab. 1). At the HF site, cereals had been grown twice sequentially during the spring before the study was begun. Some of the details of each study site are given in Table 1.

Design of the field survey and statistical analysis of the data

The number of sampling points was adapted to reflect the spatial complexity of the landscape. Accordingly, we established 34 plots in the MF site, and 16 in the HF site, each 25×25 cm in area and 30 cm deep. A total of 200 samples were analysed. The earthworms were collected in May (spring season)

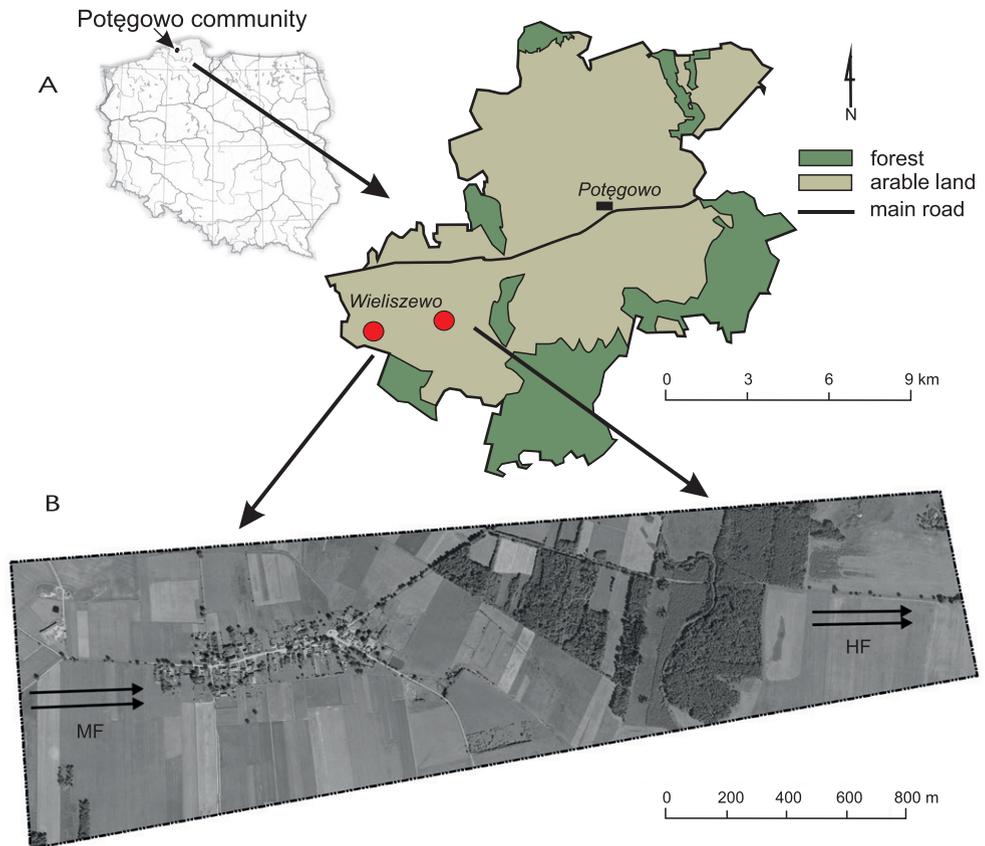


Figure 1. The study area: (A) Location, (B) Orthophotomap with range of study sites: MF – mosaic of fields, HF – homogeneous field

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Table 1. Spatial characteristics of the study sites (total area of spatial units applies for the whole connected space managed in the given way, not only within the study site)

Study site	Spatial unit	Total area (ha)	Rotations in the fields during the survey period			
			2007		2008	
			spring	autumn	spring	autumn
MF ^a	field	5.29	cereal	no vegetation	cereal	no vegetation
	field track	0.13				
	field	4.68	cereal	after-crop	cereal	stubble
	balk	0.02				
	field	3.67	cereal	stubble	cereal	no vegetation
	field track	0.09				
	field	4.44	no vegetation	potatoes	cereal	stubble
	field	2.17	cereal	stubble	cereal	stubble
	balk	0.06				
	field	1.46	cereal	after-crop	cereal	no vegetation
	balk	0.06				
	field	3.45	cereal	no vegetation	cereal	after-crop
HF ^b	field	167.00	cereal	no vegetation	cereal	no vegetation

Explanations: ^a mosaic of small fields, balks and field tracks; ^b large, homogeneous field.

and in October (autumn season) over a two-year period (2007-2008). Each spatial unit of the MF site was covered by at least two sampling points every season. Earthworm individuals were extracted *in situ* by a combination of sifting and hand-sorting and fixed immediately in 70% ethanol. All the individuals were identified (following Kasprzak 1986; Plisko 1973), counted and weighted. Most of the juvenile individuals could only be identified to the genus level, thus individuals identified at least to the genus level were also taken into account in the statistical analyses.

Soil samples were collected from the same sampling points as the earthworms. The soil moisture content (MC), pH in H₂O and electrical conductivity (EC) were determined in the soil samples, which were taken separately from the 0-15 cm and 15-30 cm soil depth bands during each season. Total soil nitrogen content (TN) and soil organic carbon content (SOC) were measured in samples (at 0-30 cm depth) obtained in autumn 2008 and were correlated with the earthworm assemblage parameters relating only to this season.

The soil moisture content (MC) was measured on a mass basis following PN-ISO 11465:1999, pH in H₂O – in accordance with ISO 10390:2005 (pH-meter SensoDirect pH 200) and electrical conductivity (EC) – according to ISO 11265:1994 (HACH conductimeter type). The total soil nitrogen content (TN) was measured using the Kjeldahl method (ISO 11261:1995), and the soil organic carbon content (SOC) analysis was performed using the Tyurin method (mineral samples), following which the C/N ratio was calculated.

We implemented a data mining procedure (random forests – Breiman 2001) to identify the most important factors that determine the number of individuals and the biomass of the earthworm assemblages studied. This method is relevant for numerical as well as for nominal data and is insensitive to the distribution and multicollinearity of independent variables. The models computed using this algorithm are also robust against overfitting (Breiman 2001). The random forest models were computed in R with package 'randomForest' (Liaw & Wiener 2002) in version 4.6-12. The environmental variables were

the abovementioned soil parameters, the seasons (spring/autumn), and the spatial type of arable ecosystem (MF/HF). The variables which were chosen by the model as the most important for earthworm abundance were further statistically analysed in detail.

Statistical calculations were made using Past 2.03 software with the significance level set at 0.05. The correlations between earthworm assemblage characteristics and soil properties were calculated using Spearman's rank correlation coefficient, preceded by the Shapiro-Wilk W test. Statistically significant differences between soil and earthworm parameters in two types of arable landscapes (i.e. with differing spatial structures) were consistently identified using the Mann-Whitney U test. Mean earthworm abundance (MF versus HF) was compared using the t-test since the faunistic data met the requirements for parametric testing. Field-scale species richness was measured as the total number of species found during a single sampling campaign. This metric is commonly used as a diversity measure in studies evaluating the effects of agri-environmental management because of its local application (Concepción et al. 2012).

Results

Characteristics of earthworm assemblages

In total, six earthworm species were found at the study sites. The species composition was the same at both sites, excluding *Octolasion*

lacteum [Örley 1885], which was reported occasionally at the HF site. The most common species at both sites was *Aporrectodea caliginosa* [Savigny 1826], which composed over 80% of the community – both in terms of biomass and number of individuals. Other species were: *Aporrectodea rosea* [Savigny 1826], *Lumbricus castaneus* [Savigny 1826], *Lumbricus rubellus* [Hoffmeister 1843] and *Lumbricus terrestris* [Linnaeus 1758].

We collected 1154 earthworm individuals in the four times repeated field survey: 337 from the HF site and 817 from the MF site. The earthworm biomass was also greater at the MF site than at the HF site (Tab. 2).

The overall mean number of individuals was significantly lower in the homogeneous site than in the complex arable landscape ($t = -2.419$, $p = 0.020$). Moreover, the earthworm response to environmental conditions varied seasonally between sites. There was little difference between the sites in spring, whereas in autumn the MF site was characterised by over 1.5 times higher values of these parameters than the HF site (which was significant in the t-test at $p < 0.05$).

Soil characteristics

The measured values of soil characteristics varied greatly at both study sites. Nonetheless, the sites were significantly different from each other in pH at 0-15 cm depth, MC at 15-30 cm depth, SOC, TN, and C/N ratio (Tab. 3).

Table 2. General earthworm abundance across the study sites and seasons

Site	No. of individuals				Biomass			
	min	max	mean	SD	min	max	mean	SD
MF ^a (N = 136)	0	42	5.90	0.53	0	9.17	1.83	0.16
Spring (N = 68)	0	18	3.47	4.27	0	4.46	0.92	1.11
Autumn (N = 68)	0	42	8.34	6.94	0	9.17	2.73	2.04
HF ^b (N = 64)	0	13	3.91	0.40	0	4.72	1.28	0.15
Spring (N = 32)	0	9	3.00	2.38	0	2.98	0.82	0.76
Autumn (N = 32)	0	13	4.81	3.67	0	4.72	1.74	1.37

Explanations: ^a mosaic of small fields, balks and field tracks; ^b large, homogeneous field.

Table 3. Physical and chemical soil characteristics and the differences between their distributions in the mosaic of small fields (MF) and the homogeneous field (HF)

	Depth (cm)	Site	Min	Max	Mean	SD	Mann-Whitney U test	P-value (the distributions are identical)
pH H ₂ O	0-15	HF	5.27	6.96	5.78	0.66	114.5	0.001
		MF	4.53	7.56	5.32	0.44		
MC (%)	15-30	HF	4.47	6.46	5.38	0.64	213.0	0.224
		MF	4.67	7.72	5.31	0.45		
	0-15	HF	4.65	22.51	11.41	4.28	247.0	0.610
		MF	3.72	16.88	10.91	2.92		
EC ($\mu\text{S cm}^{-1}$)	15-30	HF	3.76	24.10	9.86	3.77	127.0	0.003
		MF	3.15	15.99	11.19	2.35		
	0-15	HF	15.70	114.10	52.35	24.00	183.5	0.067
SOC (mg g^{-1})	15-30	MF	12.00	168.30	47.62	26.00		0.942
		HF	13.50	94.40	40.89	21.13	268.0	
	0-30	MF	12.30	101.70	41.28	19.42		
TN (mg g^{-1})	0-30	HF	9.30	19.30	14.61	2.44	46.0	< 0.001
		MF	11.50	27.30	20.49	3.52		
C/N ratio	0-30	HF	0.90	1.56	1.24	0.17	100.5	< 0.001
		MF	0.84	1.83	1.47	0.23		
	0-30	HF	10.00	13.00	11.75	0.78	9.0	< 0.001
		MF	13.00	15.00	13.94	0.69		

For explanations of abbreviations see chapter Materials and Methods.

The MF site was characterised by a wider range of pH values at both depths than the HF one. The mean values were relatively constant along the soil profile analysed at the MF site. The soil at the HF site was more acidic at 15-30 cm depth than at 0-15 cm. The difference between the sites was considerable, but only at 0-15 cm depth.

The soil moisture content at the study sites varied between 3.2% and 24.1%. The mean moisture value was similar at both sites at 0-15 cm depth. However at 15-30 cm depth it was significantly higher at the MF site. On the other hand, the highest values of this parameter occurred in the soil samples from the HF site.

Electrical conductivity reached mean values of about $50 \mu\text{S cm}^{-1}$ at 0-15 cm and $40 \mu\text{S cm}^{-1}$ at 15-30 cm regardless of the site and consistently there were no significant differences between the sites. The highest values (over $115 \mu\text{S cm}^{-1}$) occurred only occasionally at the MF site.

Soil organic carbon and total nitrogen content were significantly higher at the MF site than at the HF one. The C/N ratio followed a similar pattern.

Random forest analysis

The analysed environmental predictors explained only 14.66 and 11.67% of variance for earthworm biomass and number of individuals respectively. This means that the environmental conditions of earthworm abundance in agricultural land are much more complex and cover more factors than presented in our study. Nevertheless, the models enable the identification of some trends. The most important variables are 'season' and 'EC 15-30 cm', both for earthworm biomass and number (Fig. 2). Interestingly, the spatial structure of the arable landscape (variable 'field size'), proved to be a weaker predictor than some soil parameters (e.g. conductivity, acidity or SOC).

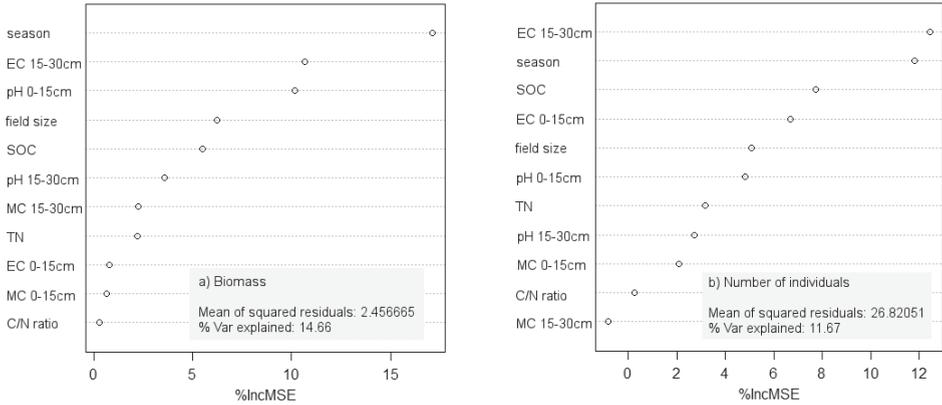


Figure 2. Importance of environmental factors for: A – biomass and B – number of earthworms; type of random forest: regression, number of trees: 500, number of variables tried at each split: 3. For explanations of abbreviations see chapter Materials and Methods

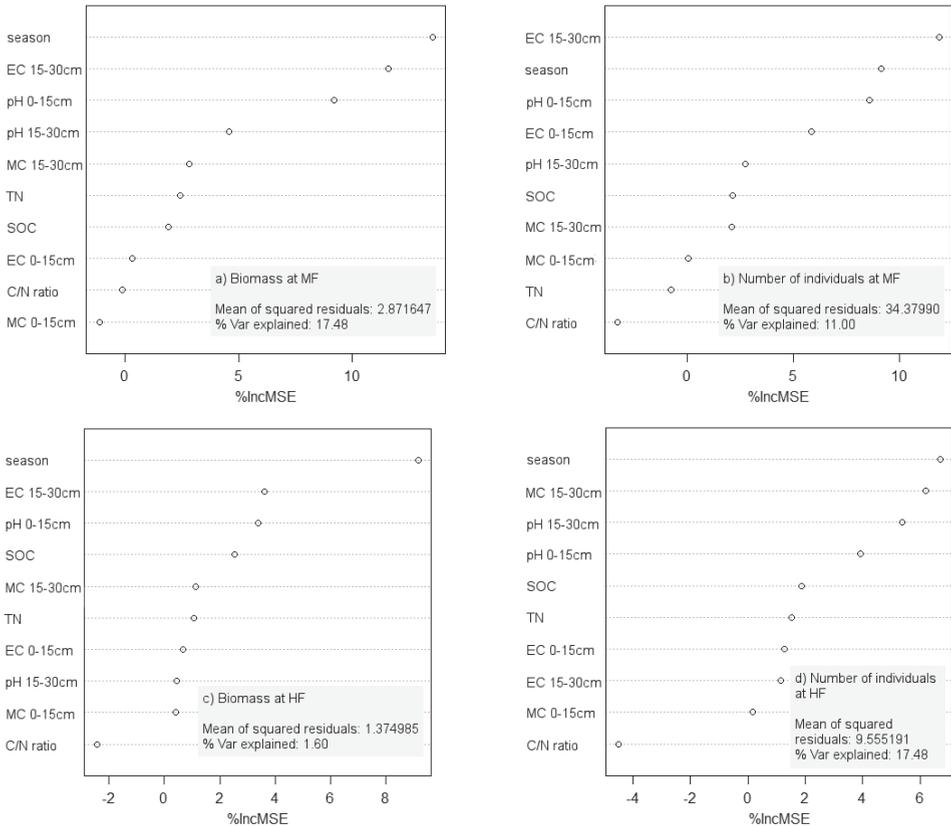


Figure 3. Importance of environmental factors for: A – biomass at MF (mosaic of small fields), B – number of earthworms at MF, C – biomass at HF (homogeneous field) and D – number of earthworms at HF; type of random forest: regression, number of trees: 500, number of variables tried at each split: 3. For explanations of abbreviations see chapter Materials and Methods

The set of variables presented (excluding 'field size') explained earthworm abundance better for the MF than the HF site due to the % of explained variance (Fig. 3). The hierarchy of variables in relation to the spatial type of the site varies very little - the most noticeable dissimilarity is the exceptionally high importance of soil moisture at 15-30 cm for the number of earthworm individuals at the HF site.

Correlations between earthworm abundance and soil characteristics

The soil parameters that were the most important in the random forest analysis were subsequently chosen to the detailed correlations with the earthworm biomass and with the number of individuals across the sites and seasons. Earthworms were generally relatively tolerant of soil acidity as they were captured from acidic to neutral soils at the MF site and from acidic to slightly acidic soil at the HF site (Tab. 4). However, the greatest number of earthworms was noted in soils of pH exceeding 5.6 at both sites. Both earth-

worm number and biomass increased with decreasing soil acidity.

The electrical conductivity at the upper soil level correlated with the number of earthworms at the MF site in spring ($\rho = 0.392$, $p = 0.022$). The number of individuals and SOC correlated relatively strongly and significantly at the HF site ($\rho = -0.550$, $p = 0.027$). Earthworm biomass and number of individuals decreased with increasing soil moisture at 0-15 cm in the spring seasons, and cumulatively at the HF site, unlike at the MF site.

Discussion

Our observations of five-six species in agricultural landscapes are consistent with other Central European studies (Metzke et al. 2007; Decaëns et al. 2008). The number of earthworm species in a farmland usually does not exceed 10, and decreases with increasing human pressure as the susceptible species are eliminated (Valckx et al. 2006; Kovács-Hostyánszki et al. 2013). In our study, the species composition varied in a very small range between sites of different spatial

Table 4. Correlations (Spearman's rho) between earthworm and soil parameters calculated for spring and autumn seasons and the entire year (in total)

Earthworm parameter	Soil parameter	Depth (cm)	MF ^a			HF ^b		
			spring	autumn	in total	spring	autumn	in total
Biomass (g)	pH H ₂ O	0-15	0.291	0.427*	0.473**	0.153	0.402	0.465*
		15-30	0.087	-0.005	0.173	-0.046	0.295	0.259
	EC ($\mu\text{S cm}^{-1}$)	0-15	0.231	0.271	0.325	0.141	0.225	0.209
		15-30	0.302	-0.062	0.220	-0.041	-0.134	-0.332
	SOC (mg g^{-1}) ^c	0-30		0.156			-0.259	
No. of individuals	pH H ₂ O	0-15	0.451**	0.391*	0.408*	0.572*	0.444	0.633**
		15-30	0.167	-0.065	0.107	0.273	0.247	0.299
	EC ($\mu\text{S cm}^{-1}$)	0-15	0.019	0.146	0.166	0.149	0.080	0.164
		15-30	0.392*	-0.125	0.142	-0.069	-0.064	-0.147
	MC (%)	15-30	0.136	0.245	0.203	0.130	-0.353	-0.320
	SOC (mg g^{-1}) ^c	0-30		0.239			-0.550*	

Explanations: * $p < 0.05$, ** $p < 0.01$; ^a mosaic of small fields, balks and field tracks; ^b large, homogeneous field; ^c determined in soil samples collected in autumn 2008. For explanations of other abbreviations see chapter Materials and Methods.

structure, thus the environmental heterogeneity of the MF site appeared to have little effect on the number of earthworm species. The study taken in the Masuria region revealed a higher disparity in the number of species (five at the HF site versus eight at the MF one), however, those fields were cultivated less intensively in general and the occurrences of some species were rare (Regulska & Kołaczowska 2015). According to Wolters (2001), additional habitats contribute either nothing or very little to the species richness of earthworms as they do not act as a source of species replacing others that are functionally equivalent. We argue that the earthworm biomass and number of individuals provide better measures of the ecological status of arable soils than the number of species. The MF site, which was more structurally complex and characterised by higher connectivity to other non-productive habitats (e.g. margins, balks or meadows) than the HF site, maintained a larger earthworm community in terms of number of earthworms than the latter. A similar trend, albeit slightly exceeding the significance threshold, was observed for biomass. These findings are in line with other studies that have similarly reported higher earthworm density and biomass in small-sized fields (Roarty & Schmidt 2013; Regulska & Kołaczowska 2015) or in less intensively farmed fields (Schmidt et al. 2001, 2003; Johnson-Maynard et al. 2007; Riley et al. 2008). As number of individuals and biomass showed the same trends in each type of arable unit, both appeared to be equally effective in assessing earthworm abundance. The study conducted in the Masuria region, North-Eastern Poland (Regulska & Kołaczowska 2015) also supports the results. We argue that number of individuals is more appropriate for monitoring purposes for practical reasons.

Presumably, our results are applicable to farmlands on a cross-regional scale in Central-Eastern Europe, however the differences between earthworm responses to soil physico-chemical properties in arable fields having diverse spatial structure are

more pronounced in agricultural areas where human pressure is relatively low – e.g. in the Masuria region. The higher values of pH at the HF site than at the MF site are probably a consequence of intensive fertilising and liming over the decades (Braun 2010). These differences were only slight, but were closely connected with earthworm abundance. Numerous authors consider pH and nitrogen content as the major influencing factors in determining the earthworm community in a variety of soils (Edwards & Bohlen 1996; Hernández et al. 2003; Joschko et al. 2006). Our study confirms these findings regarding the upper soil level (0-15 cm). Also in the analogous study (Regulska & Kołaczowska 2015) none of the earthworm parameters was significantly related to acidity at a soil depth of 15-30 cm. We agree with Jänsch et al. (2013) who concluded that a lack of relationship between these traits was a consequence of the acidity level being within the tolerance range of the earthworm species. Soil moisture content within the observed range of values was not a decisive factor for earthworms excluding the number of individuals at the HF site. It contradicts our previous result where soil moisture was the most significantly and frequently correlated soil physical parameter with the characteristics of earthworm communities (Regulska & Kołaczowska 2015).

The C/N ratio indicates soil biological activity and is therefore one of the most important parameters of habitat quality. The significantly higher values of SOC, TN and C/N ratio at the MF site than at the HF site presumably result from the effect of sowing after-crops and leaving stubble after harvesting, which contribute to enrichment of the soil with nutrients and protect its surface from unfavourable weather conditions. Moreover, it is likely that water conditions at 15-30 cm depth were better at the MF site than the HF one because of these treatments. However, the different values of TN and C/N between the sites had little importance for earthworm abundance. The weak connections between earthworm assemblage parameters and soil chemical

properties are in agreement with the study by Huerta et al. (2007), which revealed a significant relationship only for clay content. Blackshaw et al. (2007) found no significant correlations for soil chemical properties except for the number of individuals and C/N ratio at 0-10 cm. Mele and Carter (1999) related a series of soil parameters (SOC, TN, soil potassium and phosphorus contents, pH, MC) to earthworm density and distribution, and found a relatively strong positive association only for pH and MC.

The flow of nutrients in large crop monocultures is more open than in semi-natural ecosystems and the content of chemical elements in soil is strictly controlled by farmers. Comparative research in less impacted agroecosystems revealed a significant impact of organic soil carbon and total soil nitrogen on the earthworm community, but only in the heterogeneous site (Regulska & Kołaczowska 2015). Presumably, the overall human impact is so strong here that it disguises the influence of other factors.

Conclusions

The relationships between earthworm abundance and soil properties are more pronounced in the mosaic than in the homogeneous agricultural system of fields.

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- Among the soil parameters, soil conductivity was the most important for the characteristics of earthworm assemblages regardless of the site.
- Number and biomass of earthworm individuals both effectively reflect the relationships with EC, pH, and in some situations also SOC and MC.
- Assessing the number of individuals is preferable for monitoring purposes because it is less time-consuming than assessing the biomass.
- The number and biomass of the earthworms, as well as the correlations between them and soil properties reached higher values in autumn than in spring, thus we conclude that autumn sampling is preferable for biomonitoring.

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Unless otherwise stated, the sources of tables and figures are the authors', on the basis of their own research.

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