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Assessment of Relationships between Earthworms and Soil Abiotic and Biotic Factors as a Tool in Sustainable Agricultural

Radoslava Kanianska [1,*], Jana Jaďuďová [1], Jarmila Makovníková [2] and Miriam Kizeková [3]

[1] Faculty of Natural Sciences, Matej Bel University, Tajovského 40, Banská Bystrica 97401, Slovakia; jana.jadudova@umb.sk

[2] National Agricultural and Food Centre—Soil Science and Conservation Research Institute Bratislava, Regional Station Banská Bystrica, Mládežnícka 36, Banská Bystrica 97421, Slovakia; j.makovnikova@vupop.sk

[3] National Agricultural and Food Centre—Grassland and Mountain Agriculture Research Institute, Mládežnícka 36, Banská Bystrica 97421, Slovakia; kizekova@vutphp.sk

* Correspondence: radoslava.kanianska@umb.sk; Tel.: +421-48-446-5807

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Abstract: Earthworms are a major component of soil fauna communities. They influence soil chemical, biological, and physical processes and vice versa, their abundance and diversity are influenced by natural characteristics or land management practices. There is need to establish their characteristics and relations. In this study earthworm density (ED), body biomass (EB), and diversity in relation to land use (arable land—AL, permanent grasslands—PG), management, and selected abiotic (soil chemical, physical, climate related) and biotic (arthropod density and biomass, ground beetle density, carabid density) indicators were analysed at seven different study sites in Slovakia. On average, the density of earthworms was nearly twice as high in PG compared to AL. Among five soil types used as arable land, Fluvisols created the most suitable conditions for earthworm abundance and biomass. We recorded a significant correlation between ED, EB and soil moisture in arable land. In permanent grasslands, the main climate related factor was soil temperature. Relationships between earthworms and some chemical properties (pH, available nutrients) were observed only in arable land. Our findings indicate trophic interaction between earthworms and carabids in organically managed arable land. Comprehensive assessment of observed relationships can help in earthworm management to achieve sustainable agricultural systems.

Keywords: earthworm; agro-ecosystem; sustainability; climate; soil type; soil moisture; soil temperature; arthropod; ground beetle; carabid

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1. Introduction

Soil macro-invertebrates play a key role in soil organic matter transformations and nutrient dynamics at different spatial and temporal scales through perturbation and the production of biogenic structures for the improvement of soil fertility and land productivity [1,2]. As such, they influence soil chemical, biological, and physical processes [3] or contribute to biological control [4], food web dynamics and soil processes [5,6]. Among numerous macro-invertebrates in the soil, earthworms are the most important components of soil biota in terms of soil formation and maintenance of soil structure and fertility [7]. Due to earthworms' activity in soil they are classified as "ecosystem engineers", which means that they affect their environment, either biotic or abiotic. Ecosystem engineers are keystone species that modify their habitat so strongly that it in turn affects other organisms. Christensen and Mather [8] showed that earthworm number and biomass reflect both natural soil parameters, e.g., sand content, and agricultural practices. Kernecker et al. [9] confirmed the effect of soil moisture, vegetation diversity, and soil nutrient concentrations on earthworm species compositions.

There are many studies in different parts of the world which have assessed earthworm abundance and functions in the soils [10–16]. Focus has been given to integrate earthworms into agriculture management in order to increase the crop yield [17]. However, cultural practices are widely recognized to affect earthworms in agricultural fields [18]. Tillage, crop type and pesticide inputs have been demonstrated to affect earthworms in agroecosystems. Earthworms can serve as a tool reflecting the environmental impact of tillage operations, soil pollution, different agricultural input, trampling, industrial plant pollution, etc. [19]. Several studies have shown that earthworm abundance and diversity are reduced in agricultural fields, compared to uncropped soils [4]. Significant loss of soil faunal biodiversity or species richness has been found to accompany conventional agricultural systems based on intensive cultivation, high fertilizer inputs and crop monoculture.

In terrestrial systems the majority of ecosystem services arise from soil functions that are dependent to a greater or lesser extent on interactions between organisms, organic and mineral fractions of soil [20]. Earthworms come into interaction with other soil organisms directly or indirectly. So, to investigate the potential of the earthworms to integrate into agriculture management, knowledge on different physical, chemical, biological and management factors that affect the distribution and abundance of earthworm population is important, which will help to identify the ecological appropriateness of the earthworms in order to supplement their existing population and quantity the impact of earthworms on agricultural land [21]. Incorporating the functional roles of earthworms in agro-ecosystem management is key to sustainable agriculture. Earthworms have a potential to contribute to the management of soil fertility for plant growth, enhanced farming efficiency and agricultural sustainability. For this to be realised, it is important to understand the roles they play in driving soil based processes, their biology and ecology [22].

The linkages between earthworm activity, biotic and abiotic factors, climate change and land management practices are numerous, interrelated, and in many cases shape ecosystem service provision. Here, we present a survey of earthworm species in differently managed arable systems and permanent grasslands of Slovakia with the following objectives; (1) to record different species present in various agroecosystems; (2) to evaluate variations of earthworm density, biomass productivity, and diversity in relation to management, abiotic and biotic factors; and (3) to evaluate trophic interactions between earthworms and arthropods that can be utilised to enhance agricultural sustainability.

2. Materials and Methods

2.1. Study Area

Slovakia is a highly diversified country in respect to its natural environment. It is largely located in the mountainous territory of the western Carpathian arch, which forms the boundary between important physical and bio-geographic zones and several main European watersheds. Only a small part of Eastern Slovakia belongs to the eastern Carpathian region, and the southwestern part to the Pannonian basin. The mountain regions cover more than 55% of the total land territory. The climate is temperate but is influenced locally by elevation and type of relief. Communities vary from thermophilous in the southern parts of the country, to mountainous in the higher altitudes [23].

Seven study sites with two different land uses (arable land—AL, and permanent grasslands—PG) located in different natural conditions were selected. Only the VO study site situated at Danubian Upland with the most productive soil type (Black Chernozem) is used only as arable land (Figure 1, Tables 1 and 2).

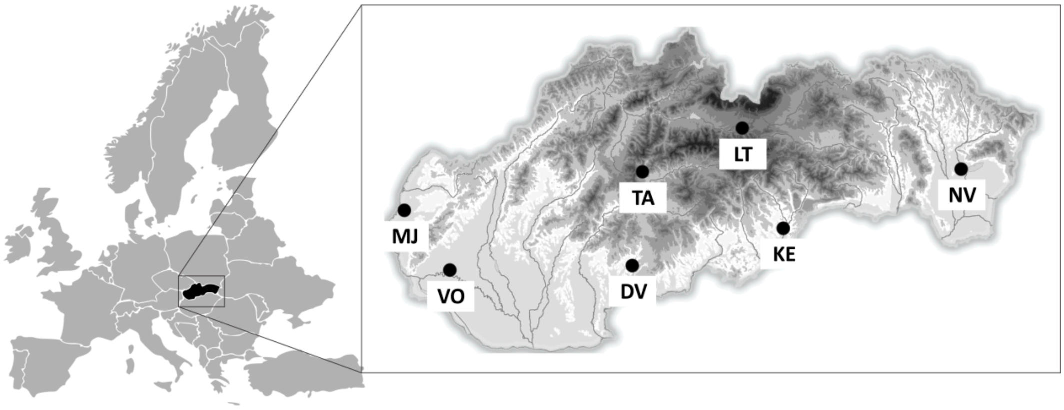

Figure 1. Map of the location of Slovakia in Europe and seven study sites.

Table 1. Geographic characteristics and land management of the seven study sites.

Study Site	Geographical Location	Soil Type	Soil Texture	Altitude (m)	Land Management
NV	Eastern Slovak Upland	Humic Regosol (Fluvisol)	Clayey	AL-121 PG-123	AL-intensive farming PG-cattle pasture
VO	Danubian Upland	Black Chernozem (Chernozem)	Loamy	AL-137	AL-intensive farming
DV	Krupina Plain	Humic Regosol (Fluvisol)	Sandy-loam	AL-157 PG-155	AL-intensive farming PG-alluvial meadow
MJ	Borská Lowland	AL-Regosol (Regosol) PG-Humic Regosol (Fluvisol)	Sandy Sandy	AL-157 PG-160	AL-intensive farming PG-meadow
KE	Slovak Karst	Eutric Cambisol (Cambisol)	Loamy	AL-360 PG-344	AL-extensive farming PG-cattle pasture
TA	Kremnica Mountain	Dystric Cambisol (Cambisol)	Loamy	AL-595 PG-597	AL-extensive farming PG-sheep pasture
LT	Low Tatras	Regosol (Rendzina)	Loamy	AL-950 PG-931	AL-organic farming PG-meadow

Abbreviations: NV, Nacina Ves; VO, Voderady; DV, Dvorníky; MJ, Moravský Ján; KE, Kečovo; TA, Tajov; LT, Liptovská Teplička; AL, arable land; PG, permanent grasslands.

Table 2. Climatic characteristics from the nearest meteorological stations.

Study Site	Meteorological Station	Long-Term Average Air Temperature (°C)	Two Months Average Air Temperature before Sampling (°C)	Long-Term Average Rainfall (mm)	Two Months Rainfall before Sampling (mm)
NV	Michalovce	8.9	4.3	559	28
VO	Kráľová pri Senci	9.5	4.1	560	57
DV	Dudince	8.7	3.5	606	67
MJ	Moravský Ján	9.2	4.1	525	53
KE	Rožňava	8.6	3.4	620	33
TA	Banská Bystrica	8.1	7.2	795	106
LT	Poprad	6.2	4.7	950	41

Abbreviations: NV, Nacina Ves; VO, Voderady; DV, Dvorníky; MJ, Moravský Ján; KE, Kečovo; TA, Tajov; LT, Liptovská Teplička.

2.2. Methods

At each study site the biotic (earthworm density—ED, earthworm biomass—EB, earthworm diversity—EDI, earthworm species, arthropod density—AD, arthropod biomass—AB, ground beetle density—GBD, and carabid density—CD) and abiotic parameters (soil temperature—ST, soil moisture—SM, and penetration resistance—PR) were measured for two land use categories (arable land—AL and permanent grasslands—PG) with the site specific land management.

Before the earthworm collection and traps installation, measurement of the soil physical parameters was done in the same places where earthworms were collected. Soil temperature, soil moisture and penetration resistance were measured at seven points on each site at the arable land and permanent grasslands. Soil temperature (ST) was measured in °C at 0.05 (ST05) and 0.20 (ST20) m depth by insertion of a thermometer in °C. Soil moisture level (SM) was measured in soil moisture volume percentage at 0.05 (SM05) and 0.20 (SM20) m depth using a soil moisture sensor (ThetaProbe, type ML2x: Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands, 2013) in the soil moisture volume percentage. Penetration resistance (PR) was measured with an electronic penetrometer (Penetrologger, 6987 EM: Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands, 2013) with a cone diameter of 1 cm^2 and a 60° top angle cone. Cone resistance was recorded in MPa.

On each site 1 kg of soil samples for chemical analysis were collected from four points placed at the apices of a 10-m side square. Soil samples were air dried, homogenized, sieved thoungh 2 mm sieves and analysed for soil pH in KCl, total oxidizable organic carbon (TOC) according to Tjurin's method (a modification of Nikitin) [24], total nitrogen (N_t) according to a modified Kjeldahl method in accordance with the Slovak standardized method (STN ISO 11261), and available nutrients (P, K, Mg) according to Mehlich III [25]. The mean values were used as soil chemical status characteristics.

Earthworms were sampled in seven study sites, in seven arable land and six grasslands (at VO study site the land is not used as permanent grasslands). Sampling was done in the spring 2015 (March–April) when earthworm populations are the most active. At each site, earthworms were sampled by digging of seven pits of 35×35 cm^2 to the depth of 20 cm placed in a line with 3 m distance between the pits. Soil from pit was replaced on plastic bag and hand sorted. The earthworms were counted and collected. Deeper-dwelling earthworms were expelled by 1.5 L of 0.2% formalin. Formalin was applied directly to the holes. After a few minutes earthworms were collected. The collected samples of earthworms were transported in glass cups with sufficient amount of soil in portable fridge to the laboratory. The next day, the collected earthworms were washed, weighed, and the sexual maturity and body colour were noted. The earthworm density and biomass from soil monoliths were recalculated per square meter. Earthworms were sacrificed, fixed in 4% formalin and mature individuals identified by the Zajonc Earthworm key [26] using a binocular microscope. Shannon-Wiener index (H') analysis was performed to determine a measure of estimated diversity within each study site.

Soil arthropods including ground beetles and carabids were sampled in the same places where earthworms were collected. Seven plastic pitfall traps of 1.5 L volume were placed flush with the surface. The traps were filled with 200 mL 4% formalin solution. After one month the traps were collected. The captured individuals were counted, weighed, identified and preserved in formalin solution.

Correlation analysis was conducted using the Spearman's Rank correlation coefficients to identify relationship among earthworms and selected soil abiotic and biotic factors. Differences between two different land use systems (AL, PG) were tested by a paired samples test. The statistical analysis was conducted using the PASW Statistics software version 18.0 (SPSS Inc., Chicago, IL, USA, 2016).

3. Results and Discussion

3.1. Earthworm Biomass, Density and Diversity in Differently Managed Agroecosystems

In total, 1091 earthworms were sampled representing an overall biomass of 609.8 g. Average fresh body biomass of the earthworms per 1 m^2 ranged from 0 to 66.4 $g \cdot m^{-2}$ and 28.2 to 134.5 $g \cdot m^{-2}$ in arable land and permanent grasslands, respectively (Table 3).

Table 3. Basic statistical characteristics of earthworm fresh biomass at seven study sites under different land use in 2015 (g·m^{-2}).

Study Site	AL				PG			
	Min	**Max**	**Mean ± SD**	**Median**	**Min**	**Max**	**Mean ± SD**	**Median**
NV	0.0	101.6	40.8 ± 31.10	32.6	64.8	235.6	134.5 ± 57.49	132.7
VO	0.0	93.3	28.2 ± 30.26	23.2	-	-	-	-
DV	13.3	143.5	66.4 ± 49.76	46.8	84.9	194.0	122.0 ± 39.11	129.4
MJ	0.0	0.0	0.0	0.0	15.2	171.0	84.4 ± 64.54	78.0
KE	42.7	90.4	63.3 ± 17.82	58.3	7.3	188.5	67.8 ± 63.42	51.4
TA	21.6	39.5	27.4 ± 6.25	25.1	19.2	69.1	40.8 ± 19.00	37.5
LT	0.0	21.39	7.23 ± 8.35	2.12	8.7	49.8	28.2 ± 16.35	28.8

Abbreviations: NV, Nacina Ves; VO, Voderady; DV, Dvorníky; MJ, Moravský Ján; KE, Kečovo; TA, Tajov; LT, Liptovská Teplička; AL, arable land; PG, permanent grasslands.

The measured mass values indicate the fact that earthworms belong to the largest soil macrofauna species and they are a major component of soil fauna communities in most ecosystems [27,28]. In temperate zones earthworm biomass often exceeds the biomass of all other soil biota having major impacts on other soil biota and soil conditions [29].

The density of earthworms ranged from 0 to 226.2 ind·m^{-2} and from 74.6 to 277.6 ind·m^{-2} in AL and PG, respectively (Table 4).

Table 4. Basic statistical characteristics of earthworm density at 7 study sites under different land use in 2015 (ind·m^{-2}).

Study Site	AL				PG			
	Min	**Max**	**Mean ± SD**	**Median**	**Min**	**Max**	**Mean ± SD**	**Median**
NV	0.0	57.1	29.2 ± 17.54	24.5	114.3	261.2	197.1 ± 53.20	220.4
VO	0.0	122.4	45.5 ± 38.25	40.8	-	-	-	-
DV	32.7	114.3	53.6 ± 27.43	49.0	228.6	383.7	277.6 ± 54.15	253.1
MJ	0.0	0.0	0.0	0.0	16.3	179.6	100.3 ± 59.37	98.0
KE	146.9	351.0	226.2 ± 69.54	195.9	32.7	146.9	84.0 ± 48.23	65.3
TA	32.7	81.6	56.0 ± 21.82	40.8	40.8	179.6	108.5 ± 49.37	89.8
LT	0.0	57.14	20.99 ± 18.77	24.49	16.3	163.3	74.6 ± 50.86	65.3

Abbreviations: NV, Nacina Ves; VO, Voderady; DV, Dvorníky; MJ, Moravský Ján; KE, Kečovo; TA, Tajov; LT, Liptovská Teplička; AL, arable land; PG, permanent grasslands.

The maximum value of 277.6 individuals per m^2 was measured in permanent grasslands at the DV site in Fluvisol. The majority of individuals were juveniles (228.57 ind·m^{-2} of juveniles; 47.72 ind·m^{-2} of matures). On average, the density of earthworms per m^2 was nearly twice as high in permanent grasslands (120.3 ind·m^{-2}) compared to arable land (61.6 ind·m^{-2}).

At the KE site we measured the second highest density of individuals per m^2 (226.2 ind·m^{-2}) in extensively managed arable land. As at the DV site juveniles dominated the samples (205.25 ind·m^{-2} of juveniles; 21.00 ind·m^{-2} of matures). The high earthworm density at the KE site is probably a consequence of ongoing erosion processes that are more intense in arable land than in permanent grasslands with permanent vegetation cover. The sampling was done on the bottom of the slope with accumulated fine materials with suitable content of organic matter, nitrogen and other nutrients that generate good living conditions for earthworms. The findings followed similar patterns as Kanianska et al. found in 2014 [30].

Mean biomass of earthworms was also more than twice as high in permanent grasslands (68.3 g·m^{-2}) compared to arable land (33.3 g·m^{-2}). The differences between arable land and permanent grasslands analysed by a paired samples test confirmed significant differences between the earthworm density and biomass in these two land uses. Results confirmed the opinion that the distribution and biomass of earthworms in the soil depends on land use and management practices. Several authors [31–33] found earthworms to be more abundant and populations to have greater biomass under long-term pasture than under long-term cropping. In addition, in permanent grasslands with managed grazing, positive effects of cattle and sheep slurry manure on earthworm populations were recorded in permanent grasslands at the NV and the TA sites similar to studies of de Goede et al. [34], Murchie et al. [35], and Pommeresche and Loes [36]. Significant loss of soil faunal abundance in arable lands is connected with intensive cultivation [37], high fertilizer inputs, crop monoculture, and machine traffic in arable land [38]. In our study the number of anecic earthworms were also reduced in intensively managed study sites.

A total of 12 species were identified of which 9 were present in arable land and 11 in permanent grasslands (Tables 5 and 6). The percentage of juveniles within community was only slightly higher in arable land (80%) than in permanent grasslands (72.4%).

Table 5. Summary of the numbers of earthworms of each species and Shannon-Wiener index at seven study sites in arable land (ind·m^{-2}).

	NV	VO	DV	MJ	KE	TA	LT	Total
Aporrectodea caliginosa	7.00	5.83	1.17	0.00	11.70	0.00	2.30	27.99
Aporrectodea rosea	3.50	1.17	1.17	0.00	0.00	0.00	2.33	8.16
Eiseniella tetraedra	0.00	1.17	0.00	0.00	1.17	0.00	0.00	2.33
Octolasion cyaneum	0.00	0.00	0.00	0.00	0.00	16.32	0.00	16.32
Octolasion lacteum	0.00	0.00	0.00	0.00	1.17	0.00	1.17	2.34
Sum of mature endogeic species	10.50	8.17	2.34	0.00	14.04	16.32	5.80	57.17
% of mature endogeic species	64.30	22.26	70.01	0.00	66.86	77.79	99.49	66.23
Dendrobaena octaedra	0.00	1.17	0.00	0.00	7.00	0.00	0.00	8.17
Dendrodrilus rubidus	0.00	0.00	0.00	0.00	00.00	4.66	0.00	4.66
Lumbricus rubellus	0.00	2.33	1.17	0.00	0.00	0.00	0.00	3.50
Sum of mature epigeic species	0.00	3.50	1.17	0.00	7.00	4.66	0.00	16.33
% of mature epigeic species	0.00	29.99	11.13	0.00	33.33	22.21	0.00	18.92
Lumbricus terrestris	5.83	0.00	7.00	0.00	0.00	0.00	0.00	12.83
Sum of mature anecic species	5.83	0.00	7.00	0.00	0.00	0.00	0.00	12.83
% of mature anecic species	35.70	0.00	66.60	0.00	0.00	0.00	0.00	14.86
Sum of mature species	16.33	11.67	10.51	0.00	21.00	20.98	5.83	86.30
Sum of juveniles	12.83	33.82	43.15	0.00	205.25	34.99	15.16	345.20
Total sum	29.16	45.49	53.65	0.00	226.25	55.97	20.99	431.50
H′	1.50	1.90	1.36	0.00	1.41	0.79	1.52	2,72

Abbreviations: H′, Shannon-Wiener index; NV, Nacina Ves; VO, Voderady; DV, Dvorníky; MJ, Moravský Ján; KE, Kečovo; TA, Tajov; LT, Liptovská Teplička.

Table 6. Summary of the numbers of earthworms of each species and Shannon-Wiener index at six study sites in permanent grasslands (ind·m^{-2}).

	NV	DV	MJ	KE	TA	LT	Total
Allolobophora chlorotica	3.50	1.17	0.00	0.00	0.00	0.00	4.66
Aporrectodea caliginosa	39.65	30.32	48.98	13.99	23.32	3.50	159.77
Aporectodea rosea	5.83	7.00	0.00	0.00	3.50	8.16	24.49
Eiseniella tetraedra	1.17	0.00	1.17	0.00	0.00	0.00	2.34
Octolasion cyaneum	0.00	0.00	0.00	0.00	1.17	4.66	5.83
Octolasion lacteum	0.00	0.00	0.00	0.00	0.00	2.33	2.33
Sum of mature endogeic species	50.15	38.49	50.15	13.99	27.99	18.65	199.42
% of mature endogeic species	82.69	80.49	87.74	70.59	100.00	99.95	85.92
Dendrobaena octaedra	1.17	1.17	1.17	1.17	0.00	0.00	4.67
Lumbricus castaneus	0.00	0.00	2.33	0.00	0.00	0.00	2.33
Lumbricus rubellus	0.00	2.33	1.17	0.00	0.00	0.00	3.50
Sum of mature epigeic species	1.17	3.50	4.67	1.17	0.00	0.00	10.51
% of mature epigeic species	1.93	7.32	8.17	5.90	0.00	0.00	4.53
Aporrectodea longa	0.00	0.00	1.17	0.00	0.00	0.00	1.17
Lumbricus terrestris	9.33	5.83	1.17	4.66	0.00	0.00	20.99
Sum of mature anecic species	9.33	5.83	1.17	4.66	0.00	0.00	20.99
% of mature anecic species	15.38	12.19	4.09	23.51	0.00	0.00	9.55
Sum of mature species	60.65	47.82	57.16	19.82	27.99	18.66	232.08
Sum of juveniles	136.44	228.57	43.15	64.14	80.47	55.98	608.75
Total sum	197.09	276.39	100.31	83.96	108.45	74.64	840.83
H′	1.04	1.24	0.85	1.08	0.80	1.85	1.42

Abbreviations: H′, Shannon-Wiener index; NV, Nacina Ves; DV, Dvorníky; MJ, Moravský Ján; KE, Kečovo; TA, Tajov; LT, Liptovská Teplička.

Only one species, *Aporrectodea caliginosa* occur in abundance at all seven study sites and was the most numerous species in both different land use sites. In permanent grasslands, *A. caliginosa* represented 68.8% of identified specimens in the dataset, and 32.4% in arable land. *Aporrectodea rosea* and *Lumbricus terrestris* were found at four of seven study sites. *Octolasion cyaneum* and *Octolasion lacteum* were found abundant in arable land. Other species were found irregularly. At the species level, our results are in line with the observations done of Zajonc [26,39] in agro-climatic zones of Slovakia in the 1970s and 1980s. The most abundant species in our research, *Aporrectodea caliginosa*, belongs to the quite common earthworm species in Europe. The assessment of earthworm field studies from six different European countries revealed that endogeic species *Aporrectodea caliginosa*, *Aporrectodea rosea* and *Allolobophora chlorotica* are the dominant ecological group [40]. *Aporrectodea caliginosa* was confirmed as the second most numerous species in a British earthworm dataset [41].

The overall effect of earthworms on soil processes varies with their ecological category and species. We identified six endogeic (*Allolobophora chlorotica*, *Aporrectodea caliginosa*, *Aporrectodea rosea*, *Eiseniella tetraedra*, *Octolasion cyaneum*, and *Octolasion lacteum*), four epigeic (*Dendrobaena octaedra*, *Dendrodrilus rubidus*, and *Lumbricus castaneus*, and *Lumbricus rubellus*), and two anecic (*Aporrectodea longa*, *Lumbricus terrestris*) species. Endogeic species dominated in both land use categories. Higher endogeic species abundance was observed in PG (85.9%) than in AL (66.2%). Epigeic species represented 4.5% and 18.9% in permanent grasslands and arable land, respectively. The anecic species represented 9.5% and 14.9% in permanent grasslands and arable land, respectively. The distribution of the different ecological categories of earthworms in arable land and permanent grasslands showed a preference of endogeic species, as soil dwellers with horizontal burrows. These worms rarely come to the surface in contrast to the epigeic species that live in the litter and humus layers or can sometimes penetrate a little deeper into loose mineral soil (particularly *Lumbricus rubellus*). Epigeic species are susceptible to soil cultivation, since it destroys the superficial soil layer. Epigeic species are associated with surface organic matter and may be present in agricultural settings where farming practices provide concentrations of organic materials e.g., animal faeces or crop residues. True epigeic species are often rare in natural settings. In general, they are much more abundant in non-arable habitats than in ploughed ones [42,43]. Such effect were observed in intensively managed arable land at the NV, the DV, the MJ sites where the epigeic group of earthworms was absent. In addition, the numbers of endogeic species were higher in permanent grasslands comparing to arable land. Similarly, the deep-burrowing earthworms (*Lumbricus terrestris* and *Aporrectodea longa)*, which collect food from the soil surface, is adversely affected by heavy soil cultivation that destroys its burrow system and reduces food sources [44]. Overall, *Aporrectodea longa* was not found in arable land

The highest earthworm diversity was found at the VO site ($H' = 1.90$) among arable land study sites, and at the LT ($H' = 1.85$) among permanent grasslands study sites (Tables 5 and 6). Although many authors argue that permanent grasslands have the greatest species diversity of earthworms [45,46] we confirmed the statement in only three study sites (MJ, TA, LT).

3.2. Earthworms in Relation to Soil Type and Soil Chemical Properties

Earthworm abundance and diversity is affected not only by management practices but is also influenced by abiotic and biotic factors. Soil types have an impact on soil biota. We found no earthworms at the MJ study site in Regosol used as arable land situated in a region with low precipitation and high temperature. Regosol used as arable land had unfavourable soil chemical and physical properties. Its texture is sandy, and coarse sand can be a negative factor for earthworms either because of the abrasive action of sand grains that damages earthworm skin or because this soil retains less water [47].

Generally, the abundance of species reflects their preference to site specific conditions. *O. lacteum* prefers soils which are rich in calcium [39]. Therefore *O. lacteum* was found in Rendzina, shallow soil developed on limestone rock at the LT site. *E. tetraedra* as semi-aquatic species [48] with affinity for moist conditions [49], was found more abundant in Fluvisol. In our research we observed the higher earthworm abundance and biomass in Fluvisols, soil types developed in alluvial zones.

Seven study sites differ in soil chemical properties (Tables 7 and 8). In arable land, soil reaction at the VO and LT is neutral, at the DV weakly acidic, at the NV and KE acidic, and at the TA strongly acidic. In permanent grasslands, soil reaction at the NV, MJ and LT is neutral, at the KE weakly acidic, at the DV strong acidic, and at the TA extremely acidic. Content of total organic carbon is medium, high, very high, and only at the MJ is it very low. Content of available phosphorous is very low and low at all study sites. In contrast, content of available magnesium is medium, high and very high at all study sites with the exception of the MJ. Content of available potassium varies, and low content was recorded at the NV in AL, MJ, and TA in PG.

Table 7. Soil chemical parameters at 7 study sites in arable land.

	pH KCl	**TOC ($g \cdot kg^{-1}$)**	**N_t ($g \cdot kg^{-1}$)**	**P ($mg \cdot kg^{-1}$)**	**K ($mg \cdot kg^{-1}$)**	**Mg ($mg \cdot kg^{-1}$)**
NV	5.51	16.67	2.51	10.16	245.59	672.27
VO	7.18	15.89	1.99	18.74	256.53	762.78
DV	6.04	13.54	1.61	48.35	310.84	318.49
MJ	4.58	7.43	0.96	65.77	132.16	39.32
KE	5.46	13.09	1.58	4.84	221.93	184.84
TA	4.84	15.45	1.62	33.56	221.90	127.36
LT	6.70	34.00	3.05	38.23	199.36	949.12

Abbreviations: NV, Nacina Ves; VO, Voderady; DV, Dvorníky; MJ, Moravský Ján; KE, Kečovo; TA, Tajov; LT, Liptovská Teplička; TOC, total organic carbon; Nt, total nitrogen; P, available phosphorus; K, available potassium; Mg, available magnesium.

Table 8. Soil chemical parameters at 6 study sites in permanent grasslands.

	pH KCl	**TOC ($g \cdot kg^{-1}$)**	**N_t ($g \cdot kg^{-1}$)**	**P ($mg \cdot kg^{-1}$)**	**K ($mg \cdot kg^{-1}$)**	**Mg ($mg \cdot kg^{-1}$)**
NV	7.18	23.18	2.78	14.52	293.56	721.78
DV	5.08	13.88	1.91	3.02	140.20	466.68
MJ	6.75	4.80	1.02	31.42	82.33	113.11
KE	5.89	28.20	3.13	22.83	349.30	260.85
TA	4.19	42.30	4.14	0.52	136.22	631.80
LT	6.94	51.30	5.16	3.82	300.33	1233.15

Abbreviations: NV, Nacina Ves; DV, Dvorníky; MJ, Moravský Ján; KE, Kečovo; TA, Tajov; LT, Liptovská Teplička; TOC, total organic carbon; Nt, total nitrogen; P, available phosphorus; K, available potassium; Mg, available magnesium.

In permanent grasslands, there was no correlation between earthworm characteristics (earthworm density, biomass, and diversity) and soil chemical parameters (Table 9). In arable land, we found a positive correlation between earthworm biomass and available potassium. The available nutrients are supportive not only for plants but also for soil edaphon biomass. In contrast, nutrients and their availability are influenced by soil fauna. Zhu et al. [50] found that earthworms can activate and transform K into effective K that is more readily taken up by plants through feeding, digestion, absorption, and excretion. Earthworm diversity was positively correlated with Mg and pH in arable land (Table 9).

Table 9. Results of a Spearman's correlation analysis for earthworm density, biomass, diversity and selected soil, climatic and geographic parameters (*n* = 7 for AL, *n* = 6 for PG).

	AL			**PG**		
	ED	**EB**	**H′**	**ED**	**EB**	**EH′**
pH	−0.036	0.286	0.893 **	−0.257	0.257	−0.200
TOC	−0.214	−0.071	0.750	−0.543	−0.771	−0.029
Nt	−0.214	−0.071	0.750	−0.543	−0.771	−0.029
P	−0.571	−0.464	−0.500	−0.371	0.257	0.371
K	0.464	0.857 *	0.393	−0.429	−0.200	−0.429
Mg	−0.179	0.143	0.929 **	−0.086	−0.257	−0.486

** Correlation is significant at the 0.01 level; * Correlation is significant at the 0.05 level; Abbreviations: ED, earthworm density; EB, earthworm biomass; H′, Shannon-Wiener index; TOC, total organic carbon; Nt, total nitrogen; P, available phosphorus; K, available potassium; Mg, available magnesium; AL, arable land; PG, permanent grasslands.

3.3. Earthworms in Relation to Climate Related and Physical Soil Parameters

Observation of relationships between biotic and climate related soil parameters are very important because rapid climate changes make it unlikely that earthworm communities and their ecosystem services will survive in the current form, because the environmental filters may change faster than worms can respond [51].

The development of the present ecosystems in the postglacial period (*Holocene*) depended on significant changes in climate, on the distance of refuges in which organisms could survive unfavourable conditions of the glacial period, and on the nature and development of soils. Glacial periods in the Carpathians caused the local extinction of many living forms. Warming in the post-glacial period, about 10,000 years ago, created conditions that facilitated migration of individual species from their refuges (dominantly in southern Europe), where they were protected during glacial periods. After the neolitic revolution, human society began to influence more noticeably the development of natural ecosystems in the lower warmer altitudes of Central Europe. During the formation period of neolitic agriculture, the Carpathians were almost completely covered by forests. Only high-montane and alpine rocky localities were without forest cover—i.e., rocky walls, avalanche slopes, and narrow belts of some river banks [52].

Anthropogenic changes relevant to earthworm communities include habitat alteration, invasive species, and climate change. Disturbance intensity and frequency are important variables for both extinction of endemics and colonization by invasive species [53]. Climate change effects on earthworm communities have not been closely explored with experimental procedures. Distributional data in temperate zones indicate a strong influence of glaciation history on modern distributions, and a very slow rebound from refugia to deglaciated land, where invasive species now dominate [54]. Under the conservative estimates of climate change, the future of earthworm ecosystem services may depend on invasive species, and topographically simple regions. Otherwise, medium-term evolutionary change may be the only response mechanism available to earthworms [55].

In relation to climate, climatic factors reflected in soil moisture and temperature are considered to be the primary factors limiting survival of earthworms [56,57]. Soil temperature and moisture varied among the study sites in different soil depths in arable land and permanent grasslands (Table 10).

In arable land, earthworm density and biomass were positively correlated with soil moisture. In permanent grasslands with significantly higher moisture content values (Table 10), temperature became a limiting factors affecting earthworm density and biomass, which is reflected in significant positive correlation (Table 11).

Table 10. Mean soil temperature and soil moisture at seven study sites under different land use in 2015 (°C, %).

Study Site	**Soil Temperature (°C)**				**Soil Moisture (%)**			
	AL		**PG**		**AL**		**PG**	
	5 cm	**20 cm**	**5 cm**	**20 cm**	**5 cm**	**20 cm**	**5 cm**	**20 cm**
NV	10.4	5.2	7.7	5.2	10.4	27.0	23.0	22.9
VO	9.1	6.2	-	-	21.1	20.6	-	-
DV	8.9	7.9	7.9	7.7	39.0	31.9	47.6	29.9
MJ	7.4	5.6	7.9	5.2	12.9	13.3	34.9	35.3
KE	6.0	4.1	7.2	5.1	22.0	19.7	35.3	18.6
TA	5.0	6.9	6.9	6.6	12.4	30.7	34.7	33.0
LT	4.6	4.3	4.3	4.4	22.7	-	40.4	16.4

Abbreviations: NV, Nacina Ves; VO, Voderady; DV, Dvorníky; MJ, Moravský Ján; KE, Kečovo; TA, Tajov; LT, Liptovská Teplička; AL, arable land; PG, permanent grasslands.

Table 11. Spearman's Rank correlation coefficients (n = 49 for AL, n = 42 for PG) for earthworm density, biomass and selected abiotic and biotic parameters.

	AL		**PG**	
	ED	**EB**	**ED**	**EB**
ST05	−0.118	0.185	0.371 *	0.457 **
ST20	−0.055	0.034	0.481 **	0.309 *
SM05	0.309 *	0.242	0.107	−0.113
SM20	0.387 **	0.528 **	0.250	0.225
PR80	−0.137	−0.307 *	0.133	0.072

** Correlation is significant at the 0.01 level; * Correlation is significant at the 0.05 level; Abbreviations: ED, earthworm density; EB, earthworm biomass; ST05, soil temperature in 5 cm depth; ST20, soil temperature in 20 cm depth; SM05, soil moisture in 5 cm depth; SM20, soil moisture in 20 cm depth; PR80, penetration resistance in 80 cm depth; AL, arable land; PG, permanent grasslands.

A negative correlation rate was measured between penetration resistance and earthworm biomass in arable land confirming the negative effect of soil compaction on earthworms. The results are in line with other studies that found a reduction in earthworm populations as a result of soil compaction in arable land [58,59].

3.4. Earthworms in Relation to Arthropods

We observed relationships between earthworms, arthropods, ground beetles and carabids (phylum *Arthropoda*, order *Coleoptera*, family *Carabidae*). A total of 3849 arthropod individuals were trapped representing an overall biomass of 302.3 g. The numbers of arthropods, ground beetles and carabids varied between study sites with different land management practices (Table 12).

On average, the number of arthropods was roughly twice as high in permanent grasslands compared to intensively managed arable land (NV, DV, and MJ) including extensively managed arable land at the KE. Order *Coleoptera* belonged to the dominant orders at all study sites.

The highest number of carabids was counted at the LT study site in organically managed arable land. Carabid recruitment is enhanced by proper organic fertilization and green manuring [60] applied at the LT study site. The second highest number was recorded again in arable land at the TA, study site with extensive land use management, located in mountain area surrounded by pastures and forests. The results suggest that land use and management determine the arthropods' assemblages. Regarding to the effect of management on soil arthropods there are different research results. Many studies reported that extensive or organic management has positive effects on biodiversity [61], but these effects differed between species groups and spatial scales [62]. Attwood et al. [63] observed significantly higher arthropod richness in areas of less intensive land use. There are also authors who observed no effect of management on selected arthropod orders [64] or they found higher mesofauna abundance under conventional management than in under organic [65].

Table 12. Summary of the mean numbers of arthropods, ground beetles and carabids (ind·trap^{-1}) at seven study sites in arable land and permanent grasslands.

		NV	VO	DV	MJ	KE	TA	LT
AL	*Arthropods*	15.86	29.43	20.29	40.00	45.00	39.43	32.71
	of which *Ground beetles*	12.43	17.00	12.00	7.14	13.29	23.14	28.43
	of which *Carabids*	11.6	3.1	0.4	1.1	3.1	21.3	24.4
PG	*Arthropods*	36.57	-	43.86	102.57	78.14	31.43	23.29
	of which *Ground beetles*	19.57	-	9.71	34.57	20.00	8.00	10.14
	of which *Carabids*	13.9	-	0.1	17.7	8.0	2.0	4.9

Abbreviations: NV, Nacina Ves; VO, Voderady; DV, Dvorníky; MJ, Moravský Ján; KE, Kečovo; TA, Tajov; LT, Liptovská Teplička; AL, arable land; PG, permanent grasslands.

Our findings indicate trophic interactions between earthworms and arthropods, particularly because carabid beetles can act as predators of earthworms. Moreover, they are considered predators of crop pests, including slugs and aphids in agricultural systems. Symondson et al. [66] found out that at times when target prey (pests) is limited in ecosystem, earthworms are the most frequently detected prey in the diet of these predators. King et al. [67] found out in the field that, with little evidence of prey choice that earthworms were being predated in proportion to their densities. During the spring when our research was done target prey (pests) was limited in the agroecosystem, and earthworms could become the main prey in the diet of these predators. In the LT the lowest number of earthworms was recorded (20.99 ind·m^{-2}, Table 3) which could be affected by the highest number of carabid beetles among all study sites. However, much is still unknown regarding the biotic interactions, and much more interdisciplinary research is needed to assess the potential role of earthworms in soil systems and the potentially beneficial or harmful effects this regulation may have on ecosystem functions in different ecosystems [68].

4. Conclusions

In spite of the fact that this paper has explored seven study sites in Slovakia, the findings have a wider relevance because of high variability in natural conditions and management practices of selected study sites. The assessment showed high relevance of dynamic but also more stable factors influencing earthworms in soil. Earthworm density and biomass were higher in permanent grasslands than in arable land. Among different abiotic soil parameters, climate related and water availability characteristics affected the distribution of earthworms. They will play an important role in the future connected with ongoing climate changes. Specifically, Fluvisols developed in alluvial zones created the most suitable conditions for earthworm abundance and biomass. These soils are influenced by sufficient water content. Relationships between earthworms and some chemical (pH, available nutrients) and physical (penetration resistance) factors were observed only in arable land. Observed biotic relations indicate trophic interactions between earthworms and arthropods, indicating that land use management may have a top-down effect on trophic interactions that shape earthworm communities. Further research is needed to link the biotic and abiotic factors, processes including land management to more complex ecosystem services.

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