ISSN 1392-3196 / e-ISSN 2335-8947
Zemdirbyste-Agriculture, vol. 106, No. 3 (2019), p. 219–226
DOI 10.13080/z-a.2019.106.028

The influence of natural and anthropogenic conditions on the earthworm population in different grassland ecosystems

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Abstract

Grassland ecosystems are influenced by natural and anthropogenic factors, of which soil, predominance of agriculturally valuable perennials, climatic conditions and management practices are the most important ones. The data on earthworms in grasslands are still scarce despite their importance for ecosystem functioning. Experiments were carried out at the Vėžaičiai Branch of Lithuanian Research Centre for Agriculture and Forestry during the period 2014–2016 on plain and hilly relief. The objective of this study was to investigate changes of the earthworm population in different grassland ecosystems under natural and anthropogenic conditions. The soil of the experimental sites was *Bathygleyic Dystric Glossic Retisol*. On the plain, where the soil acidity (pH) of the experimental site was 3.9 and 5.0, four mixtures of legumes and grasses were cultivated. The four mixtures were composed of one legume variety and two grass varieties – timothy (*Phleum pratense* L.) 35% and meadow grass (*Poa pratensis* L.) 15%, 50% of each: 1) red clover (*Trifolium pratense* L.), 2) white clover (*Trifolium repens* L.), 3) hybrid clover (*Trifolium hybridum* L.) and 4) alfalfa (*Medicago sativa* L.). On hilly relief, samples were collected from the soil of the 1) temporary: alfalfa (*Medicago sativa* L.) 50% + timothy (*Phleum pratense* L.) 35% + meadow grass (*Poa pratensis* L.) 15%, and 2) permanent (established in 1983): timothy (*Phleum pratense* L.) 20%, red fescue (*Festuca rubra* L.) 20%, meadow grass (*Poa pratensis* L.) 20%, white clover (*Trifolium repens* L.) 20%, common bird's-foot trefoil (*Lotus corniculatus* L.) 20%, grasslands grown on the slopes of the southern exposition, with a slope of 14–16° and the northern exposition, with a slope of 11–13°. The number and biomass of earthworms depended on the soil acidity, hill exposition and grassland age (permanent grassland or temporary grassland in a crop rotation). In temporary grasslands on the plain, the greatest number and biomass of earthworms were determined in the soil with an acidity of 5.0. On hilly relief with different soil moisture conditions, where the soil acidity was ≥5.7, the greatest number and biomass of earthworms were determined in the soil of the northern exposition, irrespective of the sward age.

Key words: *Lumbricidae*, relief, soil pH, temporary and permanent grasslands.

Introduction

Soil biology is a key to maintaining soil health, and soil health is fundamental to the sustainability of agricultural systems (Crotty et al., 2014). Agricultural grasslands usually support a relatively stable and numerous soil biota that contributes to soil functioning and fertility (Murray et al., 2012).

Earthworms (*Lumbricidae*) as members of macrofauna exert a significant impact on soil formation processes (Butenschoen et al., 2009), especially in perennial swards (van Eekeren et al., 2007; Eisenhauer, Scheu, 2008). Through their interactions with plants, earthworms are involved in the provision of food, wood and fibre. They also directly influence major services, such as climate and flood regulation and water purification, and can play a role in remediation and restoration (Dominati et al., 2010). Earthworms participate in soil mixing, create micropores and bond soil particles to bigger aggregates thus improving soil aeration and water infiltration. Moreover, they improve not only physical but also chemical soil composition by supplementing the soil with humic and fulvic acids. The burrows of earthworms create favourable conditions for drainage, so plant roots can reach deeper layers of the soil together with concentrated and easily-accessible nutrients. Lubbers et al. (2013) indicate that earthworms affect CO_2 emission directly and indirectly by changing soil aggregation and moisture dynamics as well as gas metabolism. Barré et al. (2009) reported that earthworms were shown to bring initially loose or compacted soil to an intermediate mechanical state that is more favourable for structural stability and root growth. In addition, soil aggregate size distribution is significantly affected by earthworms in the 0–2 cm layer of soil (Snyder et al., 2009).

Please use the following format when citing the article:
Skuodienė R., Tomchuk D., Kinderienė I. 2019. The influence of natural and anthropogenic conditions on the earthworm population in different grassland ecosystems. Zemdirbyste-Agriculture, 106 (3): 219–226. DOI 10.13080/z-a.2019.106.028

Earthworms are extremely important in organic residue decomposition, as they transfer a great part of organic matter through the gut. The abundance and incidence of earthworms in arable land strongly depend on the soil type, texture, acidity (pH) and moisture as well as quantity and quality of organic matter (Peigne et al., 2009; Dighton, Krumins, 2014; Orgiazzi et al., 2016; Schon et al., 2017). It is known that earthworms are sensitive to acid conditions; however, they can concentrate even in the acid soil if the place is rich in nutrients, for instance, in plant residues or root rhizosphere (Lavelle et al., 1995). Grassland conversion to arable land can affect the soil organic matter and also decrease earthworm populations (Lemtiri et al., 2014). Indeed, van Eekeren et al. (2008) found a strong decrease in earthworm abundance after grassland conversion to arable land. Ploughing disrupts earthworm soil habitats and exposes earthworms to predation and desiccation (Holland, 2004). Conversely, arable land conversion to grassland stimulated richness and abundance of the earthworm species, even in the second year after conversion (van Eekeren et al., 2008).

Bathygleyic Distric Glossic Retisols prevailing in Western Lithuania are acid, low in organic matter and contain a high level of toxic mobile Al^{3+} (Repsiene, Karcauskiene, 2016), with an intermediate-humus content (2–3%) and higher retention of nutrients and water (Volungevicius et al., 2018). Therefore, grasslands are often recommended for soil protection and erosion prevention. According to these observations, it was hypothesized that by properly choosing swards and their management practices it is possible to affect not only chemical and physical characteristics but also mesofauna of the soil. Given that management practices, different natural and anthropogenic conditions can have a major impact on grassland ecosystems, we utilised the data from a field experiment to test the effects of environmental factors on the earthworm population. The aim of the study was to investigate changes of the earthworm population in grassland ecosystems under different natural and anthropogenic conditions.

Materials and methods

Site and soil description and experimental design. Experiments were carried out at the Vėžaičiai Branch of Lithuanian Research Centre for Agriculture and Forestry during the period 2014–2016 in two different sites of Western Lithuania, varying in relief (on plain and hilly relief) and soil characteristics.

Before the experiment, in the autumn of 2013, the soil was treated once with dolomitic lime. The content of calcareous material was calculated on the basis of hydrolytic acidity and lime applied at a rate of 1.0 (7 t ha^{-1} $CaCO_3$). Due to the liming, the soils were of different acidity. Agrochemical and physical properties of soils in the 0–10 and 10–20 cm layers at the beginning of the experiment are presented in Table 1.

Table 1. Agrochemical and physical properties of the soil arable layers of experiment I, 2014

Soil properties	Soil pH_{KCl} 3.9		Soil pH_{KCl} 5.0	
	0–10 cm	10–20 cm	0–10 cm	10–20 cm
Acidity (pH_{KCl})	4.01 ± 0.02	3.85 ± 0.02	5.48 ± 0.03	4.67 ± 0.05
Mobile Al^{3+} mg kg^{-1}	88.59 ± 11.73	89.44 ± 12.58	3.88 ± 2.22	43.27 ± 6.95
Mobile P_2O_5 mg kg^{-1}	162.63 ± 10.33	161.88 ± 8.88	135.31 ± 6.49	135.44 ± 6.96
Mobile K_2O mg kg^{-1}	201.38 ± 5.68	200.38 ± 3.80	195.50 ± 6.69	192.00 ± 5.83
Organic carbon (C_{org}) %	1.43 ± 0.05	1.42 ± 0.04	1.46 ± 0.04	1.43 ± 0.04
Total nitrogen (N_{tot}) %	0.13 ± 0.00	0.13 ± 0.00	0.13 ± 0.00	0.14 ± 0.00
Soil bulk density Mg m^{-3}	1.27 ± 0.02	1.33 ± 0.03	1.31 ± 0.02	1.38 ± 0.03
Soil moisture[1] %	15.5 ± 1.32	16.0 ± 1.26	15.4 ± 1.42	15.8 ± 1.27

Note. [1] – soil moisture is average soil moisture during the growing season; mean values ± standard deviation.

Experiment I – temporary grasslands on the plain. The geographical location of the experiment I: latitude 55°70′ N, longitude 21°49′ E, 68.0 m a.s.l. The soil of the experimental site was Bathygleyic Dystric Glossic Retisol (WRB, 2014) with a texture of sandy light loam (clay particles <0.002 mm – 13–15%). Regarding the pH_{KCl} index, it has a smooth, highly acidic profile up to 300 cm with very high mobile aluminium (Al^{3+}) content (up to 300 mg kg^{-1}) in the EB and B horizons (Repsiene, Karcauskiene, 2016).

Factor A. Soil acidity (pH): 1) soil pH_{KCl} 3.9, 2) soil pH_{KCl} 5.0. Factor B. Legume-grass swards: 1) red clover (*Trifolium pratense* L.) 50% + timothy (*Phleum pratense* L.) 35% + meadow grass (*Poa pratensis* L.) 15%, 2) white clover (*Trifolium repens* L.) 50% + timothy (*Phleum pratense* L.) 35% + meadow grass (*Poa pratensis* L.) 15%, 3) hybrid clover (*Trifolium hybridum* L.) 50% + timothy (*Phleum pratense* L.) 35% + meadow grass (*Poa pratensis* L.) 15%, 4) alfalfa (*Medicago sativa* L.) 50% + timothy (*Phleum pratense* L.) 35% + meadow grass (*Poa pratensis* L.) 15%. The experiment was established in four replications. Treatments were arranged randomly. The area of the trial field was 3.0 × 8.0 m = 24 m^2. In the sowing year (2014), cover crop barley was fertilized with $N_{60}P_{60}K_{90}$. In the second and the third years of development (2015–2016), the grassland was fertilized with $P_{60}K_{90}$.

Experiment II – temporary and permanent grasslands on hilly relief. Geographical location of the experiment II: latitude 55°31′ N, longitude 22°30′ E, 161.5 m a.s.l. The soil of the northern exposition slope was *Bathygleyic Dystric Glossic Retisol* (slightly eroded). The soil of the southern exposition slope due to very severe erosion in the upper part was *Orthieutric Regosol* (severely eroded). The soil in the middle and lower parts of the slope was the same as in the northern slope – *Bathygleyic Dystric Glossic Retisol* (slightly eroded). The soil texture was medium and light loam. Agrochemical and physical properties of the soil in the 0–10 and 10–20 cm layers at the beginning of the experiment are presented in Table 2.

Factor A. Hill exposition: 1) southern exposition, slope of 14–16°, 2) northern exposition, slope of 11–13°. Factor B. Parts of the hill: 1) summit, 2) midslope, 3) footslope. Factor C. Grassland age: 1) temporary grassland, 2) permanent grassland.

Accounting length of the northern slope was 90 m and that of the southern slope – 43 m. Soil samples for analysis were collected from three replications of the summit, midslope and footslope of the hill.

Table 2. Agrochemical and physical properties of the soil arable layers of experiment II, 2014

Soil properties	Southern exposition		Northern exposition	
	0–10 cm	10–20 cm	0–10 cm	10–20 cm
Acidity (pH_{KCl})	5.74 ± 0.07	5.96 ± 0.08	5.88 ± 0.03	6.08 ± 0.05
Mobile P_2O_5 mg kg^{-1}	101.67 ± 12.10	157.78 ± 9.16	120.72 ± 14.69	200.56 ± 16.22
Mobile K_2O mg kg^{-1}	109.00 ± 11.71	112.50 ± 8.67	102.00 ± 10.02	119.33 ± 7.98
Organic carbon (C_{org}) %	1.07 ± 0.02	1.37 ± 0.09	1.29 ± 0.07	1.70 ± 0.08
Total nitrogen (N_{tot}) %	0.12 ± 0.01	0.13 ± 0.01	0.14 ± 0.01	0.15 ± 0.01
Soil bulk density Mg m^{-3}	1.34 ± 0.09	1.42 ± 0.06	1.33 ± 0.08	1.41 ± 0.04
Soil moisture[1] %	14.9 ± 0.56	14.7 ± 0.57	16.8 ± 0.44	16.2 ± 0.55

Note. [1] – soil moisture is average soil moisture during the growing season; mean values ± standard deviation.

Temporary grassland: alfalfa (*Medicago sativa* L.) 50% + timothy (*Phleum pratense* L.) 35% + meadow grass (*Poa pratensis* L.) 15%, was sown with the cover crop spring barley. In the year of sowing (2014), the cover crop was fertilised with $N_{60}P_{60}K_{90}$. Permanent grassland was established in 1983. Mixture of perennials was sown: timothy (*Phleum pratense* L.) 20%, red fescue (*Festuca rubra* L.) 20%, meadow grass (*Poa pratensis* L.) 20%, white clover (*Trifolium repens* L.) 20% and common bird's-foot trefoil (*Lotus corniculatus* L.) 20%. Permanent grassland was fertilised with $N_{90(60+30)}P_{60}K_{90}$.

Soil sampling, chemical, physical and biological measurements. Soil samples for chemical analyses were collected from the 0–10 and 10–20 cm soil depths with a drill from each plot. Soil agrochemical characteristics were determined by the following methods: the soil acidity was determined by potentiometric method in the extraction of 1 M KCl (pH_{KCl}) according to ISO 10390:2005 (Soil quality - Determination of pH); total nitrogen (N_{tot}) content – by the Kjeldahl method; mobile phosphorus (P_2O_5) and potassium (K_2O) in the soil – using the Egner-Riehm-Domingo (A-L) method; organic carbon (C_{org}) – by the dry combustion Dumas method; mobile aluminium (Al^{3+}) content in the soil – by the Sokolov method ISO 11260:1994 (Soil quality - Determination of effective cation exchange capacity and base saturation level using barium chloride solution) and ISO 14254:2018 (Soil quality - Determination of exchangeable acidity using barium chloride solution as extractant). Soil bulk density (as an indirect structure evaluation indicator) in the 0–10 and 10–20 cm layers was determined to estimate the aeration and moisture conditions; the soil bulk density – with a 100 cm^3 cylindrical drill (by the Kachinsky method). The soil moisture content during the plant vegetation period was determined by weighing the samples as fresh field mass and then again after being dried out.

The number of earthworms (m^{-2}) and their biomass (g m^{-2}) were determined by manual excavation from 0.25 m^2 up to 20 cm depth (Anderson, Ingram, 1994) at the end of the growing season in late autumn.

Samples of belowground phythomass (roots) were collected and mass was determined by the quantitative measuring method: the roots were dug in two places of each plot with a cylinder having an area of 78.5 cm^2 from the soil layers of 0–10 and 10–20 cm, washed with running water through a mesh of 0.25 mm, air-dried and weighed (Rosário et al., 2000). Plant chemical analyses were carried out using the following methods: total N – according to the Kjeldahl method, organic C according to the Dumas method of dry combustion (Kimble et al., 2001).

Environmental and weather characteristics. Lithuania has a humid continental climate; it is in middle latitude with snowy winters and warm summers (big contrast between winter and summer). Western regions of Lithuania are strongly affected by the maritime climate (in winter it is warmer and in summer it is cooler than in eastern regions). The soil is more podzolized and acid compared to the middle and east regions of Lithuania. It receives the highest rate of precipitation, which has amounted to an average of 865 mm annually (from 18% to 107% more compared to the middle of Lithuania) during the last 10 years. Meteorological conditions during the 2014–2016 period were diverse (Table 3).

Table 3. The average daily air temperature and precipitation during the study period

	2014	2015	2016	SCN
Meteorological conditions of experiment I				
Annual mean temperature °C	8.0	8.1	7.8	7.0
Growing season's mean air temperature °C	13.2	11.9	11.6	12.4
Total annual precipitation mm	720	914	1003	914
Growing season's total precipitation mm	444	462	617	601
Meteorological conditions of experiment II				
Annual mean temperature °C	7.3	7.6	7.1	6.3
Growing season's mean air temperature °C	12.7	11.7	12.5	11.9
Total annual precipitation mm	691	749	967	816
Growing season's total precipitation mm	441	351	551	495

SCN – the standard climate norm

Climatic conditions of the first experimental site were evaluated according to the data of Vėžaičiai Meteorological Station, which is 3.5 km away from the experimental site (Table 3). In 2014, the weather was dry and warmer than usual. The amount of precipitation during the year reached 78.8% and during the plant vegetation period – 73.9% of the standard climate norm (SCN). The vegetation period in 2015 was dry, and the mean air temperature during the growing season was close to the norm. The year of 2016, in regard to humidity, was favourable for the growth of perennials.

Climatic conditions of the second experimental site were evaluated according to the data of Laukuva Meteorological Station, which is 16.5 km away from the experimental location (Table 3). The years of 2014 and 2015 were dry – the amount of precipitation during the year and plant vegetation period was 89.0, 70.9 and 84.7, 94.8 % of the SCN, respectively. In 2016, the weather was more humid compared to the SCN.

Statistical analysis. The research data were statistically processed using the multi-factor analysis of variance (*ANOVA*). The significance of the differences between the treatments of experiment I was evaluated by the two-factor *ANOVA*, and for experiment II – by the three-factor *ANOVA*. The significance of the differences between the means was determined by the least significant difference (LSD_{05}) at 95% and 99% probability level ($p < 0.05$ and $p < 0.01$). The interdependences of individual characteristics were estimated by linear correlation coefficient (r) and regression equations. Data of the soil chemical and physical parameters are presented by arithmetic mean and average square error of the mean; the relations of characteristics were evaluated using the correlation-regression analysis with the software *STAT* (Raudonius, 2017).

Results and discussion

Temporary grassland on the plain. The first year of perennials' development can be characterised as the adaptation period of ecosystem, because perennials are sensitive after sowing and the growth is slow. Therefore, soil acidity (pH) is a very important edaphic factor affecting plant nutrition, fertility and abundance (Skuodienė, Repšienė, 2009; Daugėlienė, 2010). On the other hand, earthworms are an important component of the invertebrate community in soils, in terms of their contribution to overall underground biomass and their effects on the biogeochemical cycles of soil (Ivask et al., 2012).

The growth and development of temporary grassland swards on the plain were essentially dependent on the soil pH and the properties of plant species, which were forming agrocenosis (Skuodienė et al., 2017). The root mass of the ecosystem plants, their distribution and the quality of roots, expressed in carbon (C) and nitrogen (N) ratio, are the key factors affecting the interactions and functions of other ecosystem elements (De Deyn et al., 2009).

Under the climatic conditions of Western Lithuania, grassland ecosystems were influenced by natural and anthropogenic factors. On the plain, where the soil pH was 3.9 and 5.0, development changes of the grassland root mass significantly depended on the soil pH (Table 4). During the year of grassland ecosystem adaptation in the soil with pH 3.9, the total root mass at a depth of 0–20 cm was 2.4 times greater compared to the soil with pH 5.0. In grassland of the second year, root system development was the most intense. In the naturally acid soil, the root mass increased 3.2 times, and in the soil with pH 5.0 – 8.8 times. In the grassland of the third year, after changes in specific composition, the root mass increase was similar (1.5 and 2.1 times) in the soils of different acidity (Tomchuk, 2018). The root C:N ratio on the plain was influenced by the grassland species composition, which depended on the soil pH and grassland age. This was especially evident in the grassland of the second year, when legumes significantly depleted in naturally acid soil (Tomchuk, 2018).

Table 4. The effect of soil acidity (pH) on the average root mass of all perennials and root C:N ratio on the plain

Treatment	2014		2015		2016	
	0–10 cm	10–20 cm	0–10 cm	10–20 cm	0–10 cm	10–20 cm
Average root mass of all mixtures of perennials g m^{-2}						
1. pH 5.0	51.96	5.89	468.52	40.23	708.81	67.04
2. pH 3.9	128.87**	10.72**	424.63	29.51	914.07	38.11
LSD_{05}	*32.101*	*3.510*	*147.347*	*24.125*	*321.135*	*41.113*
Root C:N ratio						
1. pH 5.0	22	–	18	21	21	24
2. pH 3.9	27	–	24**	23	24	25
LSD_{05}	*5.8*	–	*3.1*	*4.6*	*4.6*	*5.9*

* and ** – significant at 0.05 and 0.01 level, LSD_{05} – the least significant difference

In the grassland of the first year of development (2014), the number and biomass of earthworms were significantly greater in the soil with pH 5.0 compared to the soil with pH 3.9 (Table 5). The biomass of earthworms directly depended on the soil acidity: pH increase resulted in an increase in the biomass of earthworms ($r = 0.557$, $p \leq 0.01$).

Irrespective of the soil pH, the greatest amount of the earthworms was determined in the hybrid clover + grass sward and alfalfa + grass sward, 16.0 and 20.0 earthworms m^{-2} in the soil with pH 5.0, and 13.3 and 17.3 earthworms m^{-2} in the soil with pH 3.9, respectively. Cole et al. (2006) reported that nutrient additions and site management had direct and indirect effects on the abundance and structure of soil faunal communities. For example, lime addition affected earthworm community structure.

In the grassland of the second year of development (2015), soil pH had a significant impact on the number of earthworms. The number of earthworms was significantly or 1.9 times smaller in the naturally acid soil compared to the soil with pH 5.0 (Table 5). Besides, the number of earthworms did not depend on the species composition of the grassland. It was observed that the biomass of earthworms was smaller in the soil with pH 3.9 compared to the soil with pH 5.0, but the differences were not significant. The correlation analysis proved that the number of earthworms correlated with the soil pH ($r = 0.423$, $p \leq 0.01$).

However, comparing the grasslands between one other it can be seen that irrespective of the soil pH, the greatest amount of earthworms was determined in the swards of white and hybrid clover + grass. According to Salamon et al. (2004), the abundance of legumes in grasslands increases the abundance of soil biota. Muray et al. (2012) have indicated that white clovers promote a healthier soil environment in comparison to ryegrasses. It is thought that the introduction of legumes has a key influence over how soil biota functions, promoting soil structure, water retention, biodiversity and C and N storage. In the grassland of the third year of development

Table 5. The effect of soil acidity (pH) and legume-grass swards on the earthworm parameters (total number and total biomass) on the plain

Treatment	2014		2015		2016	
	number m^{-2}	biomass g m^{-2}	number m^{-2}	biomass g m^{-2}	number m^{-2}	biomass g m^{-2}
Soil pH (factor A)						
1. pH 5.0	15.7	10.35	28.7	10.93	112.0	45.29
2. pH 3.9	12.7	4.55**	15.3**	6.50	86.0	33.27
LSD_{05}	*6.75*	*3.456*	*6.73*	*5.017*	*46.28*	*20.653*
Legume-grass swards (factor B)						
1. Red clover + grass sward	12.0	5.19	16.7	5.35	118.7	43.59
2. White clover + grass sward	11.3	7.46	25.3	11.34	100.7	50.74
3. Hybrid clover + grass sward	14.7	7.73	27.3*	11.35	117.3	41.51
4. Alfalfa + grass sward	18.7	9.42	18.7	6.83	59.3	21.29
LSD_{05}	*9.54*	*4.888*	*9.5*	*7.095*	*65.46*	*29.207*
Interaction of factors A × B						
A1 × B1	12.0	6.09	22.7	7.61	146.7	55.88
A1 × B2	14.7	13.11*	32.0	12.40	78.7	49.44
A1 × B3	16.0	10.55	33.3	12.52	148.0	51.21
A1 × B4	20.0	11.67	26.7	11.20	74.7	24.63
A2 × B1	12.0	4.28	10.7	3.08	90.7	31.29
A2 × B2	8.0	1.81	18.7	10.28	122.7	52.04
A2 × B3	13.3	4.92	21.3	10.17	86.7	31.80
A2 × B4	17.3	7.17	10.7	2.45	44.0*	17.96
LSD_{05}	*13.49*	*6.913*	*13.46*	*10.034*	*92.57*	*41.305*

* and ** – significant at 0.05 and 0.01 level, LSD_{05} – the least significant difference

(2016), after changes in botanical composition, the number and biomass of earthworms were statistically similar – soil pH and plant biological characteristics had no significant influence. The smallest number of earthworms was estimated in the alfalfa + grass sward in the soil with pH 3.9. However, the number of earthworms increased 3.9–5.6 times and biomass – 4.1–5.1 times compared to the grassland of the second year.

During all experimental years, the number and biomass of earthworms were smaller in the naturally acid (pH 3.9) soil by 19.0–46.7% and 26.5–56.0%, respectively, compared to the soil with pH 5.0. Regardless of the soil pH, in the third year of development, the number and biomass of earthworms in the soil of different clover + grass swards were almost twice greater compared to the alfalfa + grass sward. Growing grass over several years favoured the growth of earthworm populations. These data suggest that when cultivation is stopped and a grass fallow or grass ley is established, changes in the soil biota composition take place as well (van Eekeren et al., 2007).

Temporary and permanent grasslands on hilly relief. Depending on the hydro-meteorological conditions, hills with high slopes are prone to water erosion, where sediments and nutrients are washed away and ground water becomes polluted. The process is irreversible (Kinderiene, Karcauskiene, 2016). Therefore, it is very important to form agrophytocenosis, which would be productive on hilly relief and could protect the soil from erosion. Perennials effectively stabilise erosion-sensitive soil destruction, directly protect the soil from unfavourable environmental factors, such as intensive rain and wind, and stimulate soil carbon accumulation (Jarašiūnas, Kinderienė, 2016; Volungevičius et al., 2019).

Under the hilly relief conditions, soil moisture has a significant impact not only on soil biota but also on the development of perennials (Tomchuk, 2018). During the plant vegetation period, soil moisture varied in different expositions of the hill (Table 2).

On hilly relief with different soil moisture regime conditions, when the soil pH was ≥5.4, the root mass was significantly influenced by the age of the grasslands (Table 6). The major difference (13.4 times) of the root mass was determined among the permanent and temporary grasslands in the first year of investigation. Together with temporary grassland development, the root mass differences gradually decreased. The total root length significantly depended on the slope exposition and the part of the hill (Tomchuk, 2018). On hilly relief, the root C:N ratio significantly depended on grassland age. The temporary grassland root C:N ratio ranged from 14 to 19, and the permanent grassland root C:N ratio ranged from 27 to 34 (Tomchuk, 2018).

In the first year of investigation (2014), the number and biomass of earthworms were estimated to be significantly greater in plots of permanent grassland (Table 7).

Table 6. The effect of grassland age on the average root mass and root C:N ratio on hilly relief

Treatment	2014		2015		2016	
	0–10 cm	10–20 cm	0–10 cm	10–20 cm	0–10 cm	10–20 cm
Average root mass g m^{-2}						
1. Temporary grassland	93.52	11.46	538.85	55.17	736.20	148.55
2. Permanent grassland	1256.51**	94.55**	1102.55**	62.70	885.07	69.71**
LSD_{05}	*267.723*	*18.597*	*189.236*	*26.632*	*195.766*	*55.211*
Root C:N ratio						
1. Temporary grassland	14	–	19	19	18	16
2. Permanent grassland	35**	–	28**	34**	27**	30**
LSD_{05}	*2.8*	–	*1.5*	*3.5*	*2.3*	*3.1*

* and ** – significant at 0.05 and 0.01 level, LSD_{05} – the least significant difference

Table 7. The effect of hill exposition, parts of the hill and grassland age on the earthworm parameters (total number and total biomass) on hilly relief

Treatment		2014		2015		2016	
		number m^{-2}	biomass $g\ m^{-2}$	number m^{-2}	biomass $g\ m^{-2}$	number m^{-2}	biomass $g\ m^{-2}$
Hill exposition (factor A)							
1. Southern exposition		78.4	29.67	7.3	1.18	60.0	25.89
2. Northern exposition		89.8	32.73	45.3**	6.99**	91.8**	40.66**
	LSD_{05}	*18.21*	*9.705*	*10.42*	*2.684*	*18.64*	*8.700*
Parts of the hill (factor B)							
1. Summit		80.7	27.62	20.7	3.44	69.7	29.21
2. Midslope		78.0	29.23	25.7	3.73	62.3	31.71
3. Footslope		93.7	36.74	32.7	5.09	95.7*	28.91
	LSD_{05}	*22.30*	*11.886*	*12.757*	*3.287*	*22.83*	*10.655*
Grassland age (factor C)							
1. Temporary grassland		51.1	14.153	39.1	5.28	68.9	28.50
2. Permanent grassland		117.1	48.242**	13.6	2.89	82.9	38.06*
	LSD_{05}	*18.21*	*9.705*	*10.42*	*2.684*	*18.64*	*8.700*
Interaction of factors A × B × C							
A1 × B1 × C1		46.7	24.52	40	0.16	45.3	16.36
A1 × B1 × C2		141.3**	48.41*	40	0.44	76.0	35.16
A1 × B2 × C1		61.3	18.51	107	3.65	52.0	17.97
A1 × B2 × C2		105.3*	41.64	80	0.52	77.3	40.80*
A1 × B3 × C1		34.7	6.53	120	0.84	50.7	14.99
A1 × B3 × C2		81.3	3840	53	1.49	58.7	30.08
A2 × B1 × C1		66.7	1188	347*	2.71	86.7	42.35*
A2 × B1 × C2		68.0	2565	400**	10.45**	70.7	22.96
A2 × B2 × C1		45.3	1171	667**	6.96*	48.0	29.56
A2 × B2 × C2		100.0	4508	173	3.80	72.0	38.52*
A2 × B3 × C1		52.0	1177	1067**	17.39**	130.7**	49.75**
A2 × B3 × C2		206.7**	9027**	67	0.64	142.7**	60.81**
	LSD_{05}	*44.60*	*23772*	*2551*	*6.575*	*45.66*	*21.310*

* and ** – significant at 0.05 and 0.01 level, LSD_{05} – the least significant difference

During the year of grassland adaptation (the first year of temporary grassland), when perennials are developing slowly and the root system is forming, the number of earthworms was estimated 2.3 times and biomass 3.4 times smaller compared to the permanent grassland. The greatest number and biomass of earthworms were determined to be on the northern exposition footslope and southern exposition summit in the permanent grassland. After correlation analysis, it was estimated that the biomass of earthworms in temporary grassland did not depend on the root mass and the correlation was negative (r = – 0.576, $p \le 0.05$). However, it was strongly influenced by plant residues' (roots) C:N ratio – a positive, moderately strong correlation was determined ($r = 0.530$, $p \le 0.05$). The obtained data confirm that the major factor for the incidence of earthworms is not the quantity of plant residues but their chemical composition (van Eekeren et al., 2009). The number and biomass of earthworms in the permanent grassland also correlated with soil moisture ($r = 0.625$, $p \le 0.01$ and $r = 0.699$, $p \le 0.01$, respectively).

The second year of investigation (2015) was especially dry and warmer than SCN, the number of earthworms in the soil was smaller by 56.0% and their biomass was smaller by 78.4% compared to the first year of investigation (Table 5). The number of earthworms was influenced by hill exposition and grassland age, while the biomass was significantly influenced only by hill exposition. The greatest number and biomass of earthworms were determined in the temporary grassland of the northern exposition.

The correlation analysis showed that the number and biomass of earthworms were dependent on the edaphic conditions of the temporary grassland. A negative correlation with soil bulk density was determined (number of earthworms $r = -0.773$, $p \le 0.01$, biomass $r = -0.704$, $p \le 0.01$). Soil moisture positively affected the number of earthworms ($r = 0.876$, $p \le 0.01$) and their biomass ($r = 0.751$, $p \le 0.01$). Positive moderately strong and strong correlations were determined between the number and biomass of earthworms and soil organic carbon as well as total nitrogen content in the soil ($r = 0.510$, $p \le 0.05$ and $r = 0.801$, $p \le 0.01$, respectively). Temporary grassland's root mass was not significant for the incidence of earthworms. Likewise, the lack of rainfall during the plant vegetation period had a significant influence as well. Literature indicates that the lack of soil moisture and aeration in strongly eroded parts of slope as well as dry periods reduce the productivity of the grassland; microbiological processes slow down or cease (Nunes, Nearing, 2011; Kinderiene, Karcauskiene, 2012).

In the third year of investigation (2016), the hill exposition and its parts had an essential influence on the number of earthworms, and the hill exposition as well as the grassland age was significant for the biomass. Independent of the grassland age, the greatest number and biomass of earthworms were estimated on the northern exposition footslope in the temporary and permanent grasslands.

The correlation analysis results of the third year of investigation were similar to those obtained in the second year; the number and biomass of earthworms were dependent on the edaphic conditions: soil organic carbon ($r = 0.544$, $p \le 0.05$ and $r = 0.562$, $p \le 0.05$, respectively), total nitrogen quantity ($r = 0.516$, $p \le 0.05$ and $r = 0.680$, $p \le 0.01$, respectively) and soil bulk density ($r = -0.641$, $p \le 0.01$ and $r = -0.643$, $p \le 0.01$, respectively) as well as soil moisture ($r = 0.601$, $p \le 0.01$ and $r = 0.629$, $p \le 0.01$, respectively).

Permanent grassland and continuous arable cropping represent two types of land use that have distinct effects on biological soil quality (van Eekeren et al., 2008). Giving up intensive soil tillage improves development and increases earthworm population (Bogužas et al., 2010). Development of the temporary grasslands stimulated the abundance of earthworms. Regardless of relief, after two growing seasons, the total number of earthworms increased by 1.4 and 6.8–7.1 times, respectively, on hilly relief and on the plain. Therefore, it seems highly likely that it was not only direct effects of the nutrient manipulations that altered the abundance of earthworms but also site management in general. Grassland botanical composition and age were also important drivers.

Conclusions

1. Soil acidity (pH) increment affected the earthworm community population. During the experimental years, the number and biomass of earthworms were smaller in the naturally acid (pH 3.9) soil, by 19.0–46.7% and 26.5–56.0%, respectively, compared to the soil with pH 5.0. Regardless of the soil pH, in the third year of development, the number and biomass of earthworms in the soil of different clover + grass swards were almost twice greater compared to alfalfa + grass swards.

2. Under the hilly relief conditions, the greatest number and biomass of earthworms were determined in the soil of the northern exposition, irrespective of the grassland age. Regardless of the hill exposition, footslope of the hill was more favourable for the earthworm population due to higher soil moisture and organic carbon content.

3. Development of the temporary grasslands stimulated the abundance of earthworms. Regardless of the relief, after two growing seasons, the total number of earthworms increased by 1.4 and 6.8–7.1 times, respectively, on hilly relief and on the plain.

Acknowledgements

The paper presents research findings, obtained through the long-term research programme "Productivity and sustainability of agricultural and forest soils" implemented by Lithuanian Research Centre for Agriculture and Forestry.

Received 23 01 2019
Accepted 10 06 2019

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ISSN 1392-3196 / e-ISSN 2335-8947
Zemdirbyste-Agriculture, vol. 106, No. 3 (2019), p. 219–226
DOI 10.13080/z-a.2019.106.028

Gamtinių ir antropogeninių sąlygų įtaka sliekų populiacijai skirtingose žolynų ekosistemose

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Santrauka

Žolynų ekosistemos yra veikiamos gamtinių ir antropogeninių veiksnių, iš kurių didžiausią įtaką turi dirvožemis, ūkiškai vertingų daugiamečių žolių rūšių dominavimas, meteorologinės sąlygos ir priežiūra. Nepaisant sliekų svarbos ekosistemų funkcionavimui, vis dar nepakanka duomenų apie jų populiaciją žolynuose. Tyrimas atliktas 2014–2016 m. LAMMC Vėžaičių filiale lygiame ir kalvotame reljefe. Jo tikslas – nustatyti gamtinių ir antropogeninių veiksnių įtaką sliekų populiacijai skirtingose žolynų ekosistemose. Tyrimo dirvožemis – natūraliai rūgštus nepasotintasis balkšvažemio moreninis priemolis (JIj6-b). Lygiame reljefe eksperimentas vykdytas skirtingo rūgštumo (pH 3,9 ir 5,0) dirvožemyje, kuriame auginti keturi pupinių ir miglinių – pašarinio motiejuko (*Phleum pratense* L.) 35 % bei pievinės miglės (*Poa pratensis* L.) 15 % – žolių mišiniai po 50 %: 1) raudonasis dobilas (*Trifolium pratense* L.), 2) baltasis dobilas (*Trifolium repens* L.), 3) rausvasis dobilas (*Trifolium hybridum* L.) ir 4) mėlynžiedė liucerna (*Medicago sativa* L.). Kalvotame reljefe ėminiai imti iš šlaituose, kurių pietinės ekspozicijos nuolydis 14–16°, šiaurinės – 11–13°, augintų 1) trumpalaikio: mėlynžiedė liucerna (*Medicago sativa* L.) 50 % + pašarinis motiejukas (*Phleum pratense* L.) 35 % + pievinė miglė (*Poa pratensis* L.) 15 %, ir 2) ilgalaikio (įrengto 1983 m.): pašarinis motiejukas (*Phleum pratense* L.) 20 %, raudonasis eraičinas (*Festuca rubra* L.) 20 %, pievinė miglė (*Poa pratensis* L.) 20 %, baltasis dobilas 20 % (*Trifolium repens* L.), paprastasis gargždenis (*Lotus corniculatus* L.) 20 %, žolynų dirvožemio. Sliekų kiekis ir masė priklausė nuo dirvožemio rūgštumo (pH) rodiklio, kalvos ekspozicijos ir žolynų amžiaus. Lygaus reljefo trumpalaikiuose žolynuose sliekų didžiausias kiekis ir biomasė nustatyti dirvožemyje, kurio pH 5,0. Esant skirtingam dirvožemio drėgniui kalvoto reljefo sąlygomis, kai dirvožemio pH ≥ 5,4, sliekų didžiausias kiekis ir biomasė, nepriklausomai nuo žolyno amžiaus, nustatyti šiaurinės ekspozicijos šlaito dirvožemyje.

Reikšminiai žodžiai: dirvožemio pH, *Lumbricidae*, reljefas, trumpalaikiai ir ilgalaikiai žolynai.