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The impact of earthworms on collembolan (Collembola) communities in grass mono- and polycultures*

Wpływ dżdżownic na zespoły skoczogonek (Collembola) w uprawach trawiastych jedno- i wielogatunkowych

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Abstract: In 2004-2005, a lysimetric experiment was designed to determine whether and how plant diversity and earthworms could influence diversity and densities of collembolan communities. We compared two types of crops: monoculture of *Festuca rubra* L. and a polyculture, the mixture of grasses (8 species) commonly used in agriculture. In addition to two plant communities of different diversity, we used in our experiments an engineering species of earthworm – *Aporrectodea caliginosa* (Sav.). The experiment was initiated in May 2004, when the lysimeters were sown with *F. rubra* or with a mixture of grasses. The following year, in May 2005, we introduced 6 individuals (that correspond with average densities of 200 ind. m-2 in the natural environment) of *A. caliginosa* to half of the lysimeters. Soil samples were taken at the depths of 0-5 cm, 5-10 cm, 10-15 cm and 15-20 cm, before introducing earthworms and after 60, 120 and 180 days from introducing earthworms. We found that soil layer (Kruskal-Wallis test: N=432, H=18.25, P=0.0004), biodiversity of grass culture (N=432, H=14.59, P=0.0001) and the period of the season (N=432, H=24.24, P=0.0000) had an important effect on densities of collembolan communities independently of earthworms presence (N=432, H=1.99, P=0.16). We found 18 species of springtails and the number of species decreased with soil layer. The most abundant were *Proisotomodes bipunctatus* and *Isotomodes productus*..

Keywords: earthworms, springtails, species diversity, grass monoculture, grass polyculture

Streszczenie: W latach 2004-2005 przeprowadzono eksperyment wazonowy, w którym badano wpływ zróznicowania roslinnosci oraz obecności dżdżownic glebowych na zespoły skoczogonek. Porównano dwa typy upraw trawiastych: monokultura kostrzewy czerwonej *Festuca rubra* L. oraz trawiasta uprawa wielogatunkowa (mieszanka 8 gatunków traw). Ponadto w każdej z upraw zbadano wpływ obecności dżdżownic należących do gatunku *Aporrectodea caliginosa* (Sav.). W maju 2004 roku doświadczalne wazony obsiano po połowie kostrzewą czerwoną oraz mieszanką traw. W lipcu 2005 roku do połowy wazonów z każdej uprawy wprowadzono dzdzownice, po 6 osobników, co odpowiada średniemu zagęszczeniu 200 osobników na metr kwadratowy w środowisku naturalnym. Próby glebowe pobrano na głębokości: 0-5 cm, 5-10 cm, 10-15 cm i 15-20 cm, przed wprowadzeniem dżdżownic oraz po 60, 120 and 180 dniach od wprowadzenia dżdżownic. Wykazano, że warstwa gleby (test Kruskala-Wallisa: N=432, H=18.25, P=0.0004), róznorodność traw (N=432, H=14.59, P=0.0001) oraz sezon (N=432, H=24.24, P=0.0000) miały istotny wpływ na zagęszczenie zespołów skoczogonek niezależnie od obecności dżdżownic (N=432, H=1.99, P=0.16). Ogółem stwierdzono 18 gatunków skoczogonek, a ich liczebności w wierzchniej warstwie gleby były istotnie wyższe niż w wastwach głębszych. Najliczniej występowały gatunki *Proisotomodes bipunctatus* i *Isotomodes productus*.

Słowa kluczowe: dżdżownice, skoczogonki, różnorodność gatunkowa, monokultura trawiasta, trawiasta uprawa wielogatunkowa

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Introduction

Soil invertebrates play an important role in mineralisation processes (Bradford et al. 2002; Scheu 2002; Kreuzer et al. 2004.). They contribute to the circulation of nutrients in the soil and their availability to plants (Wardle 1999), so they can indirectly affect the plant communities (Haimi et al. 1992; Wardle 1999; Scheu, and Setälä 2002; Bonkowski and Scheu 2004; Salamon et al. 2004). This is because soil invertebrates, especially earthworms and springtails, modify the activity and spatial distribution of microorganisms and fungi in soil (Scheu, and Setälä 2002; Bonkowski and Scheu 2004). Earthworms are considered to be one of the most important soil organisms responsible for the nutrient cycle, especially in grassland ecosystems (Edwards, and Bohlen 1996; Spehn et al. 2000; Scheu 2003), while among arthropods springtails play an important role in soil processes (Hopkin 1997, Gange 2000).

Numerous works are confirming the role of soil invertebrates in stimulating plant development (Scheu et al. 1999; Schmidt and Curry 1999; Kreuzer et al. 2004). Earthworms living in deeper soil layers such as *Aporrectodea caliginosa* (Sav.), by excavating corridors, can affect both soil properties and the spread of soil organisms, including springtails (Wickenbrock and Heisler 1997). The influence of earthworms on soil mesofauna has been the subject of research in the last few decades, and yet the results are still ambiguous. The experience presented here is part of a comprehensive study on the impact of earthworms on soil fauna.

The purpose of the presented experiment was to determine whether and how the species differentiation of vegetation and the presence of earthworms affect the density and species diversity of springtail communities.

1. Methods

In 2004-2005, a pot experiment was carried out in which the influence of vegetation diversity and the presence of soil earthworms on soil animal communities was studied. The experiment was conducted in pots with a surface area of 450 cm^2 and 20 cm of height, filled with homogenous light soil. The experimental lysimeters, 600 in total, were dug into the ground in an experimental plot. In May 2004, one-half of the lysimeters were sown with *Festuca rubra* . (monoculture M) and the other half - with a mix of 8 grass species usually used in grassland science (polyculture P). In July 2005, earthworms were introduced into half of the experimental lysimeters with *Festuca rubra* and grass mix, 6 specimens to each *Aporrectodea caliginosa*, which corresponds to the average density of 130 specimens per square meter in the natural environment located on the same soil type as the one used in lysimeters, partially sown with *Festuca rubra* and partially - with grass mix (lysimeters with *Festuca rubra* were placed in the part of the plot sown with *Festuca rubra*, and the lysimeters with the grass mix - in the part sown with grass mix). Plants belonging to other species than those sown at the beginning of the experiment were successively removed from the lysimeters. The vegetation in the experimental plot and lysimeters was mown twice per season: in June and September. Windrow was removed from the plot and lysimeters. Studies on springtail communities covered some lysimeters of each variant of the experiment. Soil samples were collected from the experimental lysimeters and divided into the following layers: 0-5 cm (a), 5-10 cm (b), 10-15 cm (c) and 15-20 cm (d) in April (before the introduction of earthworms), July (60 days after the introduction of the earthworms), September (after 120 days) and November (after 180 days). Six or ten samples were collected each time from every experiment variant, from various experimental lysimeters. They were collected from the central part of the pot to avoid the effect of the margin. The springtails were flushed out from the soil samples in a Tullgren funnel, then the species were identified based on Stach (1955), and Fjellberg's (1998, 2007) keys.

A non-parametric test – Wilcoxon rank-sum test – was used to analyse the mean concentrations, and to determine the effect of the presence of earthworms and their time of activity, the type of grassland cultivation, and the soil layer – a non-parametric ANOVA rank Kruskal-Wallis test. The Shannon-Wiener diversity index (1963) was used to analyse the species diversity of Collembola communities. The Hutcheson t-test (1970) was used to analyse the differences. Moreover, the share of epigeic, hemiedaphic and euedaphic species according to Christiansen (1964) was distinguished in Collembola communities.

2. Results

Taking into account the duration of the experiment and the material collected during the experiment, it was found that the density of springtails depended on the type of cultivation (N=432, H=14.59, P=0.001), soil layer (N=432, H=18.26, P=0.0004) and the sampling period (N=432, H=24.24, P=0.0000). The presence of earthworms did not significantly affect the density of springtails (N=432, H=1.99, P=0.16). However, if one considers the density of springtails in individual crop types with a division into lysimeters with and without earthworms, it turns out that higher densities were recorded in pots without earthworms than with earthworms both in the monoculture (M) and in the polyculture (P) (Table 1).

Table 1. The average density of springtails (Collembola) and standard deviations (SD) for the whole period of the experiment, together for all soil layers from which the samples were taken (the densities are given in thousands of individuals/m^2).

Type of crop	Pots without earthworms N±SD	Pots without earthworms N±SD
Grassland monoculture (M)	7.8±3.3[a, b]	2.8±1.0[c]
Grassland polyculture (P)	8.7±1.2[a]	5.6±1.6[b]

a) statistically significant differences between the number of springtails in the monoculture with and without earthworms, P<0.05;
b) statistically significant differences between the number of springtails in a polyculture with and without earthworms, P<0.05;
c) statistically significant differences between the number of springtails in a monoculture and the number in a polyculture in case of lysimeters with earthworms, P<0.05.

The density of springtails in individual experimental lysimeters differed in subsequent dates. Before the introduction of earthworms significantly higher densities of the examined invertebrates were observed in the topsoil layer, both in the monoculture and in the polyculture, i.e. up to the depth of 5 cm, than in deeper layers (fig. 1A). In the initial period after the introduction of earthworms into the experimental lysimeters, higher numbers of Collembola were recorded in the lysimeters with earthworms than without earthworms, but only in the topsoil layer in the monoculture (fig. 1B). Over time, the influence of earthworms was ambiguous and was visible in the soil layer from 5 to 15 cm (fig. 1C and D). After the introduction of earthworms, Collembola densities were higher in polycultures in the majority of experimental vases. However, significantly higher densities of springtails were recorded in the monoculture after 180 days, in the topsoil layer, while in the remaining variants the densities were similar (fig. 1D).

In the monoculture of red fescue (M), 11 species of springtails were found, and in the polyculture (P) -16 species (Table 2 and 3). In the species composition of the springtail communities, there were no clear differences associated with the presence of earthworms or vegetation diversity. In the springtail communities in both crops, the most numerous ones were the representatives of *Proisotomodes bipunctatus* and *Isotomodes productus* (Table 2 and 3). Moreover, in the monoculture (M and M+E) *Proisotoma minuta* and *Schoetella ununguiculata* were numerous, and in the polyculture with earthworms (P+E) - Friesea mirabilis (Table 2 and 3)

Hemiedaphic and euedaphic species prevailed in the springtail communities, while epigeic species were present mainly in the lysimeters with grassland polyculture (fig. 2C and D). In this culture, the presence of epigeic species was noted both in the topsoil layer but also in the deeper layers of the soil (fig. 2D). In the lysimeters with earthworms, even at a depth of up to 20 cm (fig. 2D).

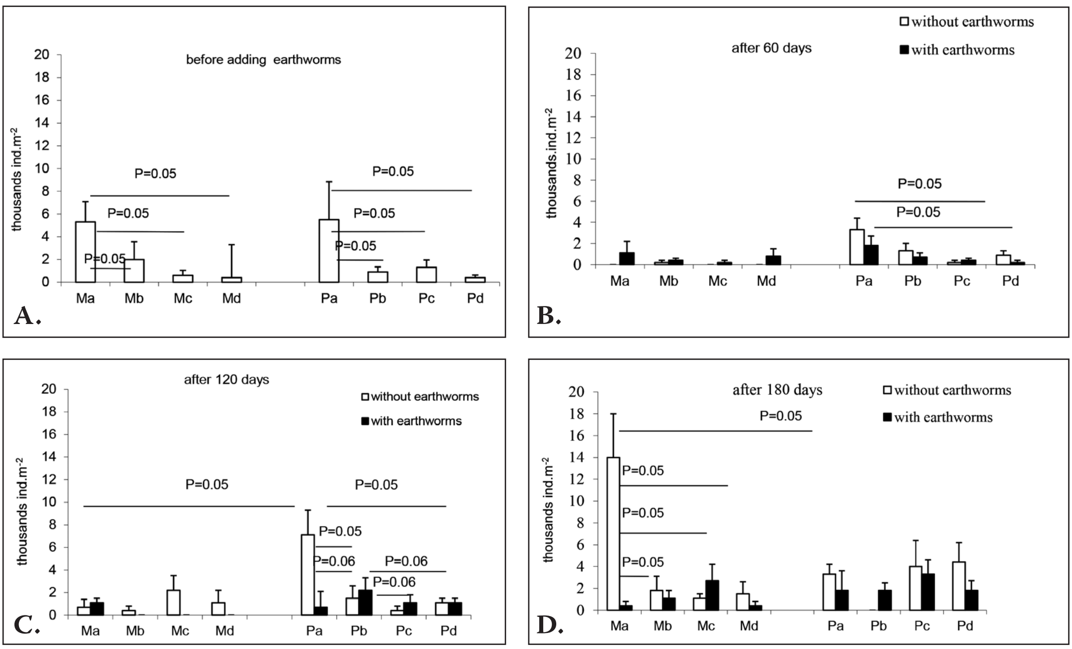

Fig. 1. Dynamics of the number of springtails in the grass monoculture (M) and polyculture (P) without and with earthworms, in different soil layers (a – 0-5 cm, b – 5-10 cm, c – 10-15cm, d – 15-20 cm).

Table 2. Collembola's dominance structure in grass monoculture without (M) and with earthworms (M+E), in different soil layers: a – 0-5 cm; b – 5-10 cm; c – 10-15 cm; d – 15-20 cm (based on the whole material).

Species	Ma	Mb	Mc	Md	Ma+E	Mb+E	Mc+E	Md+E
Proisotomodes bipunctatus	4.8	0	6.7	20	40	33.3	0	0
Hemisotoma thermophila	4.8	7.1	0	0	6.7	0	25	60
Entomobrya arborea	1.6	0	0	0	0	0	0	0
Friesea mirabilis	3.2	7.1	6.7	0	40	0	0	0
Isotomiella minor	0	0	6.7	30	0	0	0	0
Isotomodes productus	1.6	57.1	80	40	6.7	33.3	0	20
Isotomurus palustris	1.6	0	0	0	6.7	0	0	0
Parisotoma notabilis	1.6	0	0	0	0	0	0	0
Proisotoma minuta	46	28.6	0	0	0	0	0	20
Pseudachorutella assigillata	6.3	0	0	0	0	0	0	0
Schoetella ununguiculata	28.6	0	0	0	0	33.3	75	0

Table 3. Collembola's dominance structure in polyculture without (P) and with earthworms (P+E), in individual layers: a – 0-5 cm; b – 5-10 cm; c – 10-15 cm; d – 15-20 cm (based on the whole material).

Species	Pa	Pb	Pc	Pd	Pa+E	Pb+E	Pc+E	Pd+E
Proisotomodes bipunctatus	61	28.6	29.4	25	12.1	50	14.3	20
Hemisotoma thermophila	0	0	5.9	0	0	0	0	0
Entomobrya arborea	3.4	0	0	0	12.1	0	7.1	10
Friesea mirabilis	11.9	0	0	0	36.4	0	0	0
Isotomiella minor	0	7.1	0	10	3	0	0	0
Isotomodes productus	3.4	57.1	29.4	60	15.1	40	57.1	40
Isotomurus palustris	10.2	0	0	0	0	10	0	0
Lepidocyrtus lanuginosus	1.7	0	0	0	0	0	0	0
Mesaphorura macrochaeta	1.7	0	0	5	0	0	21.4	10
Mesaphorura sp.	0	0	17.6	0	0	0	0	0
Orchesella cincta	0	0	5.9	0	0	0	0	0
Pseudachorutella assigillata	0	0	0	0	6.1	0	0	0
Schoetella ununguiculata	3.4	0	0	0		0	0	0
Sminthurinus aureus	0	0	0	0	12.1	0	0	0
Sminthurus viridis	3.4	0	11.8	0	0	0	0	0
Sphaeridia pumilis	0	0	0	0	3	0	0	0
Tomocerus albinus	0	7.1	0	0	0	0	0	20

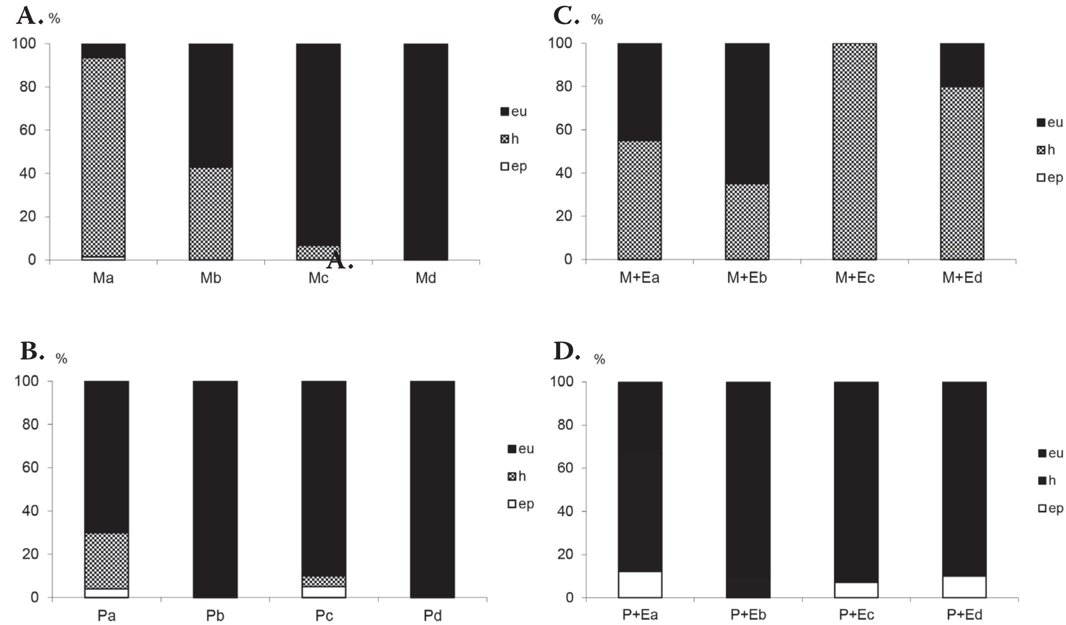

Fig. 2. Share of epigeic, hemiedaphic and euedaphic species found in different soil layers in the grass monoculture (M) and in the grass polyculture (P) without and with earthworms (M+E and P+E, respectively), in different soil layers: a – 0-5 cm; b – 5-10 cm; c – 10-15 cm; d – 15-20 cm (ep – epigeic species, h – hemiedaphic species, eu – euedaphic species).

The species diversity of the springtails was slightly higher in the pots with earthworms, regardless of the type of grassland culture, but these differences were not statistically significant (Table 4).

Table 4. Species diversity of the Collembola communities (Shannon index) in the pots sown with red fescue (monoculture – M) and 8 grass species (polyculture – P) (based on the whole material).

Variant	Monoculture - M	Polyculture – P
Without earthworms	2.5	2.5
Earthworms	2.7	2.8

3. Discussion

Springtails are relatively numerous in meadow soils, where their densities range from 1000 to 100000 individuals per m^2 (Petersen and Luxton 1982). In the experiment in which the influence of earthworm coprolites on the springtails clusters was studied, in two types of grass cultivation: *Dactylis* monoculture and a mixture of 6 species of grasses, the density of springtails ranged from 32900 to 48600 individuals per m^2 (Olejniczak 2000). In their research on grassland ecosystems, Salamon et al. (2004) recorded Collembola densities ranging from 6637 to 21717 individuals per m^2. In the experience presented, the density of springtails ranged from 2800 to 8700 individuals per m^2, which is close to the minimum values noted by other authors.

In earlier lysimetric experiment concerning the simplification of grassland cultivation, Olejniczak (2000) found no clear differences between the densities of springtails in grassland monoculture and the densities in polyculture grassland cultivation. Similar results have been obtained by Salamon et al. (2004), although they suggest that the plant species composition may affect the density of springtails.

In the presented experiment, no influence of the species richness of grassland cultivation on the density of springtails was observed either, although only in the case of lysimeters without earthworms. In pots with earthworms, the density of springtails was higher in polyculture than in monoculture. Some authors (e.g. Salamon et al. 2004) suggest that there is a relationship between springtails and the species diversity of vegetation and the presence of earthworms. Both earthworms and springtails affect the rhizosphere, usually causing a decrease in root biomass (Partsch et al. 2006). By eating microorganisms and fungi, they reduce the competition for nutrients between plants and microorganisms and fungi (Partsch et al. 2006). Partsch et al. (2006) found that earthworms are more active in environments with diverse vegetation. Perhaps the higher plant diversity and the associated increased earthworm activity contributed to the faster development of microorganisms and fungi in polyculture, providing springtails with a richer food source, which in turn resulted in their increased densities.

It should be mentioned here that earthworms can also eat microorganisms and fungi, so they compete for food with springtails (Brown 1995; Kreuzer et al. 2004). Perhaps the lack of competition caused that the amount of food available for the springtails in the lysimeters without earthworms was higher and thus higher densities of these animals were recorded. Partsch et al. (2006) believe that there is functional feedback between plants and organisms responsible for matter decomposition. They believe that earthworms and springtails play a key role here.

Apart from the food supply, the factors that significantly influence the densities of springtails are environmental conditions, especially soil moisture, acidity, temperature (Christiansen 1964; Butcher et al. 1971; Alvarez et al. 1997). This is reflected in the seasonal dynamics of the densities of Collembola. In natural and semi-natural ecosystems (forests, meadows, pastures), two peaks of density are observed (Christiansen 1964; Butcher et al. 1971). Thus, the populations of springtails were greater in wet periods with moderate temperatures (here, spring and autumn). These natural cycles of abundance could therefore overshadow the influence of earthworms and vegetation diversity.

In the presented experiment, pioneering species rapidly colonising new environments prevailed (Dunger 1991). This may explain the poor reaction of the springtails to the experimental conditions. The presence of epigeic species in deeper soil layers may indicate the influence of earthworms on the distribution of soil organisms, including springtails.

Conclusions

It seems that earthworms and their engineering activities may:

- affect not so much the density of Collembola, but their distribution in the soil, allowing them to use more space, modifying soil moisture and properties. (Earthworms can stimulate or inhibit the activity of springtails at different times in the season. They contribute to soil drying);
- contribute to the availability of food supplies.

It seems that the influence of earthworms on Collembola clusters is stronger than the species diversity of vegetation; however, both of these factors may jointly influence the springtail clusters.

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