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Short Communication

Effect of *Solidago* Eradication Methods on Soil Invertebrates - Preliminary Studies

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Abstract

Evaluation of belowground impacts of methods of invasive plant eradication, especially in the context of grassland restoration, gives information about rates of ecosystem restoration. This study tested the hypotheses: (1) the method of seed application and plant species diversity used in grassland restoration impacts on mesofauna communities, (2) the mowing regime influences the abundance of soil invertebrates. A two-factorial experiment using: (1) different methods of seed introduction and composition (sowing the seed mixture of pasture grasses, pasture grasses with legumes, seed collected from a semi-natural meadow, and application of fresh hay), and (2) different frequencies of mowing (once, twice and three times per year), was established during the restoration of grassland which had been invaded by *Solidago* plants. The experiment was designed as a randomized complete block design with four replications. The results were revealed that mowing intensity decreased the abundance of Chilopoda and Isopoda, while the introduction of fresh hay used in grassland restoration positively affected nematodes. Also, the biological soil quality index based on arthropods (QBS-ar) indicated a decrease of soil biological quality in stands mown 2 and 3 times in comparison to mowing once. Further studies are needed to investigate the soil mesofauna dynamics exposed by mowing, and plant diversity.

Keywords: biological invasions, grassland restoration, *Solidago* eradication, soil mesofauna

Introduction

Plant invasions severely affect the size and species composition of both native plant and animal communities. It can be caused through disruption of biotic interactions or changes in abiotic ecosystem characteristics [1-2]. North American-originated *Solidago* spp., commonly called goldenrods, is one of the most significant invasive alien plants in Central Europe [3]. Due to prolific vegetative propagation, *Solidago* species form dense stands that decrease and alter the biodiversity of plants [4-5] as well as arthropods, e.g. pollinators or coleopterans [6-7]. Moreover, these invasive plants alter the physico-chemical and biological

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properties of soil, which causes a moderate effect on mesofaunal diversity, mainly concerning Collembola and Nematoda community composition [8-9] and soil microbial communities [10]. Shifts in the species composition of different organisms can occur through changes in habitat structure and abiotic properties of the soil [8]. However, the ecological consequences of a strong expansion of invasive plants, including *Solidago* on invertebrates, are not yet well understood, especially in relation to soil mesofauna.

Soil mesofauna are enormously diverse and play a decisive role in ecosystem functioning. They are common, abundant and functionally important in most ecosystems [11]. All soil organisms are responsible for nutrient cycling, formation of organic matter, soil structure and many other physical properties, but such groups as earthworms, nematodes and arthropods play a significant role in the majority of soil processes. It is understandable that the presence of plants is one of the important factors influencing their occurrence. Moreover, mesofauna respond relatively quickly to any changes in the soil environment and are commonly used as bioindicators of biological soil quality [12-13]. All of them are known for their sensitivity to disturbances associated with agriculture, including the disappearance or simplification of ground cover [14-15].

According to Weidlich et al. [16], primarily, prescribed fire and mowing followed by hand-pulling, cutting and harrowing were the most frequent non-chemical interventions used for controlling invasive plant species during ecological restoration. The chemical application method (glyphosate spraying) is also common. In recent studies, possibilities of restoration of *Solidago*-invaded lands were used individually or by the combination of multiple forms of control strategies such as grazing, mowing, manual removal, periodic flooding, scalping, rototilling, different seeding methods, and the use of herbicides [17-18]. In this preliminary study we analyzed the abundance of different mesofauna organisms collected from an invasive *Solidago* stand and subjected to various methods of restoration. We tested the following hypotheses: (1) the method of seed application, and plant species diversity used in grassland restoration have an impact on mesofauna communities, (2) the mowing regime influences the abundance of soil invertebrates.

Material and Methods

Study Site

The experiment was established in Wroclaw, Poland (N 51°09′42.57'', E 17°06′43.97'') on abandoned arable land, now dominated by the invasive North American *Solidago* species (*S. gigantea* Alton and *S. canadensis* Linnaeus). The study site was located at an altitude of 118 m a.s.l. in a small river valley surrounded by suburban buildings and extensively used meadows. The mean annual temperature in the region is 9ºC and mean annual precipitation is 578.2 mm per year (data for period 1968-2019). The soil type is Anthropic Regosol, loamy sand texture.

Experimental Design

The field experiment concerning *Solidago* species eradication was established in April 2020, with five various methods of seed introduction and composition. Three different seed mixtures were sowed: grasses (G), grasses with legumes (L), seeds collected from a semi-natural meadow (M). Another method of seed application was spreading of fresh hay obtained from high-biodiversity meadow (F). Control (C) was the plots without seed application. The species composition of seeds mixtures, as well as fresh hay is presented in Supplementary Table S1. Second factor of the experiment were different mowing frequencies: once (in June), two times (in June and August), and three times (in June, August and September). The experiment was arranged in a 5 × 3 factorial completely randomized design with four replications. In total, 60 plots, arranged

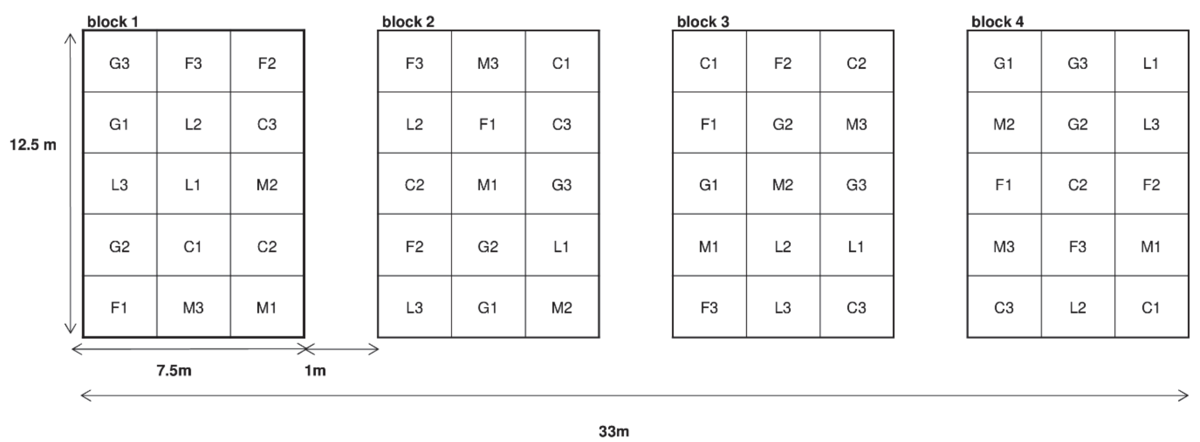

Fig. 1. Plan of the experiment. Abbreviations: F – fresh hay, M – meadow seed mixture, G –grass seeds, L – grasses with legumes, C – control, 1 – mowing one time per year, 2 – mowing two times per year, 3 – mowing three times per year.

in four blocks located at a distance of 1 m from each other, were established. The size of every plot was 2.5 × 2.5 m, and thus the whole experiment covered a rectangle of 12.5 × 33 m. The plan of the experiment is presented in Fig. 1.

The observations were performed on plots sized 2 × 2 m, and therefore the distance between observation plots was 1 m. The experiment was established on abandoned arable land, covered by a dense *Solidago* stand. Before seed and fresh hay application, the area was mown, and biomass was removed from the experiment site. Next, soil was prepared using a power harrow and compacted with a roller. The plots were mown according to the planned scheme once, twice and three times per year.

Data Collection and Processing

Soil was sampled with the use of a circular sampler of 10 cm diameter from the depth of 10 cm. One sample was taken from the center of each plot in September 2020, and so 60 samples were taken altogether. Then soil samples were illuminated in Tullgren funnels (light bulb 25 W) for 24 hours. Soil invertebrates extracted from the soil were kept in 75% ethyl alcohol. They were identified to different taxa which are used for the determination of QBS-ar [19]. Also, other taxa (not arthropods) were counted. The abundance of the taxa identified in the study is presented in Supplementary Table S2. The principle of the QBS-ar index (biological soil quality index based on arthropods) was used to evaluate the traits of sampled arthropods according to their specialization to edaphic life. The maximal number of scores for one taxa is 20 (e.g. Pseudoscorpiones or Acari), while the minimal number is 1 (e.g. adults of holometabolous insects). Some arthropods (mainly Collembola) needed to be accessed according to certain traits. The scores associated with each taxa are also included in Supplementary Table S2. The sum of EMI (Ecomorphological index) scores was counted in each sample. The QBS-ar index is the mean from those sums within certain treatments.

Statistical Analysis

Statistical analysis was done using the Scheirer–Ray–Hare test, which is a nonparametric test used for a two-way factorial design. Post-hoc tests were done using Dunn [20] Kruskal-Wallis multiple comparison and p-values adjusted with the Benjamini-Hochberg method for each significant factor using R software. The values of QBS-ar had normal distribution. Therefore, for those data ANOVA was used.

Results

In total, 11 825 specimens of soil fauna were collected (Supplementary Table S2). The high number

Table 1. Results of the Scheirer-Ray-Hare test (H and p values) showing the effects of experimental factors on soil fauna abundance.

Treatment		The most abundant soil fauna groups									
		Collembola	Acari	Hemiptera	Formicidae	Coleoptera larvae	Diptera larvae	Chilopoda	Isopoda	Symphyla	Nematoda
Seed introduction method	H*	5.80	2.26	5.49	3.77	5.34	8.11	2.39	1.18	3.31	22.03
	p	0.21	0.69	0.24	0.44	0.25	0.09	0.66	0.88	0.51	**0.001**
Mowing regime	H	1.91	1.28	3.60	0.48	1.77	1.87	6.21	9.36	3.26	2.57
	p	0.38	0.53	0.17	0.79	0.41	0.39	**0.04**	**0.01**	0.20	0.28
Seed introduction method x mowing regime	H	5.38	10.11	3.48	7.34	6.80	4.90	3.15	5.79	6.18	4.38
	p	0.72	0.26	0.90	0.50	0.56	0.77	0.92	0.67	0.63	0.82

*H – sum of squares of the ranks, p = significance value of soil organisms allows analysis of the effects of experimental factors. When considering the abundance of the fauna in general, the highest number of individuals was presented for Collembola (67.37%), Acari (23.26%), and Nematoda (4.42%). The number of remaining mesofauna was less than 1%. The abundance of the most numerous ten taxa was analyzed, while the number of each of this group was higher than 40.

The results of the Scheirer-Ray-Hare test showed that there were significant effects of the seed introduction method and mowing regime on selected taxa. Additionally, there were no interactive effects of the seed composition and introduction method and mowing regime for soil fauna abundance (Table 1). Considering the mowing regime, significant effects were found on Isopoda and Chilopoda abundance (p = 0.04 and p = 0.01, respectively). The average number of Isopoda individuals decreased with increasing mowing regime (the lowest numbers were in stands mown twice a year and the highest in stands mown once per year). For Chilopoda, the post hoc test did not show significant differences between treatments (Fig. 2).

Considering the seed composition and introduction method, only Nematoda differed significantly between treatments (p = 0.001). The average number of Nematoda was higher in the fresh hay application method in comparison to the remaining treatments (Fig. 3).

For QBS-ar index calculation, the abundance of all taxa was taken into account (Supplementary Table S2). QBS-ar varied from 80 to 105 in different treatments (Fig. 4). It was found that mowing significantly affects the QBS-ar value, which indicates significant effects of mowing on biological soil quality (p = 0.03). The value of the index was significantly higher in the plots mown once in comparison to plots mown two or three times. Thus, there was a similar trend to Isopoda abundance. Considering the seed composition and introduction method (Fig. 4a), there was no significant effect observed (p = 0.13).

Discussion

Generally, invasive plants can cause negative impacts on both native biodiversity and ecosystem functioning, including productivity, nutrient cycling, and soil organic matter [21-22]. Therefore it is clear that the consequence may also apply the occurrence of soil organisms. Van Hengstum et al. [2], synthesized 56 studies that compared invaded vs. non-invaded habitats. They estimated that invaded habitats have a median of 29% lower abundance and 17% lower species richness compared with non-invaded habitats. Several other case studies also reported significant changes in arthropod abundance following plant invasions [23]. The ecological consequences of plant invasion on soil biota have been much less studied to soil mesofaunal taxa. In riparian wet meadows this problem was reported by Sterzyńska et al. [8]. For the described reasons it is expected that invasive plants, especially those as expansive as goldenrods, will significantly alter the abundance and species structure of various mesofauna representatives, and treatments restoring the natural appearance of a given surface will also have a positive effect on soil fauna.

There is therefore a need for action to reduce the importance of plant invasions. The removal of above-ground biomass by grazing or mowing could be beneficial for grassland biodiversity [24]. Mowing is widely used as a restoration method to replace invasive plants [25]. In our experiment, after one season we found the effect of mowing frequency on 2 from 10 analysed invertebrates groups. Only Chilopoda and Isopoda were significantly negatively affected by increased mowing intensity. The QBS-ar index, which

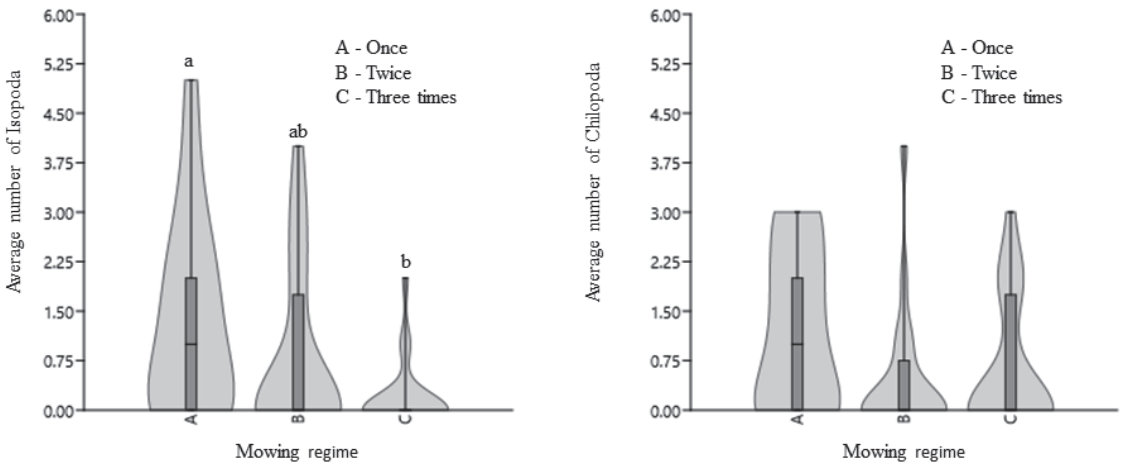

Fig. 2. Number of Isopoda and Chilopoda individuals with mowing regimes. Values followed by the same letter are not significantly different as determined by the Dunn test (P<0.05). Boxplots represent: median (horizontal line), interquartile range (box), minimum and maximum (vertical line), while the violins represent kernel density plot.

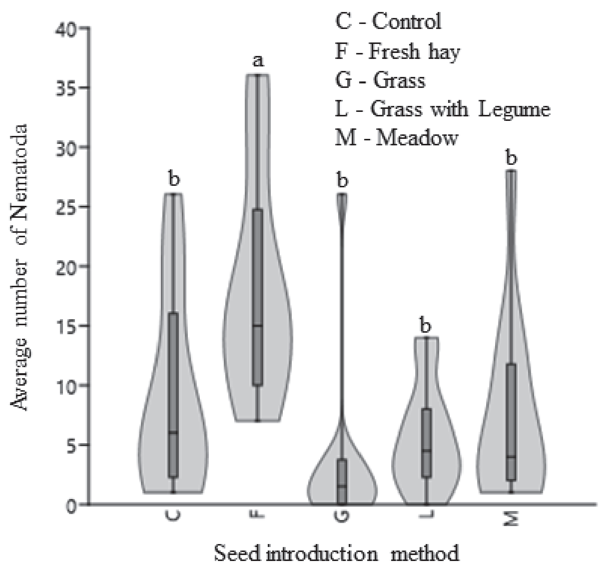

Fig. 3. Number of Nematoda responses to seed introduction methods. Values followed by the same letter are not significantly different as determined by the Dunn test (P<0.05). Boxplots represent: median (horizontal line), interquartile range (box), minimum and maximum (vertical line), while the violins represent kernel density plot.

evaluates the traits of all sampled arthropods according to their specialization to edaphic life, also indicated a similar trend. Therefore, the QBS-ar index seems more sensitive in the evaluation of soil quality in comparison to soil arthropods abundance itself. The mean value of QBS-ar was similar to values obtained by other authors [26-27]. For instance, the QBS-ar index calculated for post-mining areas varied from 40 to 140, and increased with the succession stage [26]. Mowing regimes change plant species composition. In the study of Józefowska et al. [28], mowing positively affected soil properties and enchytraeids abundance. Considering mowing frequency, it did not change the conservation value of seminatural grasslands [29]. In our study, frequent mowing negatively affected soil fauna. Gruss et al. [30] and Twardowski et al. [31], found negative effects of too frequent grazing on soil as well as epigeal fauna, which was explained by disturbance caused by cattle trampling. In the case of mowing, the explanation could be the changes in plant coverage, which have not yet been studied in this experiment.

Plant diversity is an important driver of soil biota abundance [32]. In our experiment we found significant effects of applying different methods of seed introduction and seed composition restoration on nematodes. The greatest abundance of this group was noticed in the stand where fresh hay was applied. This treatment has the greatest plant species richness (47 species). This is in line with the studies of other authors. In meta-analyses, increasing plant species richness positively affected decomposer activity, which is related with soil fauna abundance [33]. Birkhofer et al. [34] stated for example that the presence of legumes in the plant mixture positively affects soil biota.

The main findings of these preliminary studies are:

1. Mowing 3 times and 2 times per season negatively affected the abundance of Chilopoda and Isopoda in comparison to mowing once.
2. Introduction of fresh hay used in grassland restoration positively affected nematodes in comparison to the *Solidago*-dominated stand, and other seed introduction methods and seed composition treatments.
3. The biological soil quality index based on arthropods (QBS-ar) indicated a decrease of soil biological

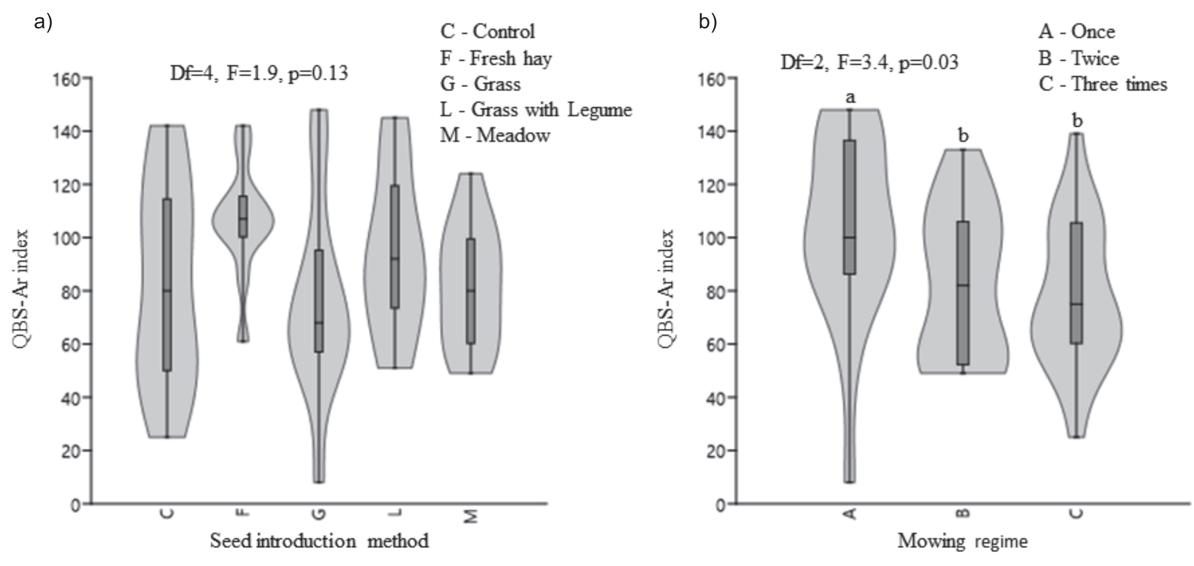

Fig. 4. Effects of seed introduction method a) and mowing times b) on QBS-ar index. Values followed by the same letter are not significantly different as determined by the Dunn test (P<0.05). Boxplots represent: median (horizontal line), interquartile range (box), minimum and maximum (vertical line), while the violins represent kernel density plot.

quality in stands mown 2 and 3 times in comparison to mowing once.

To conclude, we found that mowing once per year and fresh hay applied as a grassland restoration method were most beneficial for soil fauna. When planning strategies for the restoration of *Solidago*-invaded land, it is important to take into account the impact on belowground diversity of soil mesofauna. Further observations are needed to check their dynamics and taxonomic structure during subsequent years.

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Conflicts of Interest

The authors declare no conflicts of interest.

Funding Declaration

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Supplementary Material

Table S1. Plant species composition in seed mixtures and fresh hay used for grassland restoration in the experiment.

Treatment	Plant species
Meadow seed mixture (37 species)	*Achillea millefolium, Anthriscus sylvestris, Centaurea cyanus, C. jacea, Festuca pratensis, Daucus carota, Galium album, G. wirtgenii, Heracleum sphondylium, Knautia arvensis, Leontodon hispidus, Leucanthemum ircutianum/vulgare, Lotus corniculatus, Lychnis flos-cuculi, Papaver rhoeas, Plantago lanceolata, Prunella vulgaris, Anthoxanthum odoratum, Sanguisorba officinalis, Scorzoneroides autumnalis, Silene dioica, Silene vulgaris, Tragopogon pratensis, Trifolium pratense, Agrostis capillaris, Alopecurus pratensis, Rumex acetosa, Arrhenatherum elatius, Bromus hordeaceus, Cynosurus cristatus, Dactylis glomerata, Helictotrichon pubescens, Festuca rubra, Crepis biennis, Lolium perenne, Poa angustifolia, Trisetum flavescens*
Grass seeds (4 species)	*Poa pratensis, Lolium perenne, Festuca pratensis, Phleum pratense*
Grasses with legumes (6 species)	*Poa pratensis, Lolium perenne, Festuca pratensis, Phleum pratense, Trifolium repens, T. pratense*
Fresh hay (47 species)	*Achillea millefolium, Agrostis capillaris, Alchemilla monticola, Alopecurus pratensis, Veronica chamaedrys, Arrhenatherum elatius, Centaurea jacea, Festuca rubra, Dactylis glomerata, Dianthus deltoides, Festuca pratensis, Chaerophyllum aromaticum, Lotus corniculatus, Holcus lanatus, Hypericum maculatum, Knautia arvensis, Lathyrus pratensis, Leucanthemum vulgare, Lolium perenne, Heracleum sphondylium, Phleum pratense, Plantago lanceolata, Poa pratensis, Ranunculus acris, R. repens, Rumex acetosa, Senecio jacobaea, Stellaria graminea, Tragopogon pratensis, Trifolium pratense, T. repens, Anthoxanthum odoratum, Vicia cracca,V. hirsuta, V. sepium, Anthriscus sylvestris, Cerastium fontanum* ssp. *triviale, Taraxacum officinale, Trisetum flavescens, Campanula patula, Pimpinella saxifraga, Elymus repens, Solidago virgaurea, Galium mollugo, Lychnis flos-cuculi, Aegopodium podagraria, Geum urbanum*

Table S2. Soil animal abundance and EMI scores assigned to the taxa.

Taxa			No. individuals	Relative abundance (%)	No. samples	EMI scores
Arthropoda	Insecta	Formicidae (A)	51	0.44	4	5
		Other Hymenoptera (A)	17	0.14	12	1
		Hymenoptera (L)	1	0.01	1	10
		Coleoptera (A)	29	0.24	19	5
		Coleoptera (L)	67	0.57	35	5
		Diptera (A)	11	0.09	5	1
		Diptera (L)	93	0.79	36	10
		Thysanoptera (A)	2	0.01	2	1
		Lepidoptera (L)	3	0.03	3	10
	Enthognata	Collembola	7967	67.37	60	1-20**
		Diplura	1	0.01	1	20
	Myriapoda	Symphyla	81	0.68	32	10
		Chilopoda	45	0.38	23	20
		Diplopoda	19	0.16	17	20
	Crustacea	Isopoda	46	0.40	21	10
	Arachnida	Acari	2750	23.26	58	20
		Aranae	11	0.09	9	5
Nematoda			523	4.42	55	-
Annelida		Lumbricidae	20	0.17	8	-
Total			11825	100.0		

*A – adults, L – larvae

**There are 7 levels of EMI scores for Collembola (from 1 to 20), which depend on several traits. For instance, epigeic Collembola get 1, 2, or 4 scores, while euedaphic Collembola get 10 or 20 scores.