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Diversity and structure of litter-dwelling macrofauna (Oniscidea and Diplopoda) in upland forest fragments in the winter period

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Abstract. Winter activity of saprophagous soil macrofauna is a phenomenon not sufficiently studied yet. Shifts in the life cycle and activity during the cold, wet period of the year can be expected, especially in the context of climate change. During the winter of 2017–2018, 47 leaf litter samples and humus were obtained from upland forest fragments in South-Eastern Slovakia to analyze the community composition of Oniscidea and Diplopoda. 72% of samples contained terrestrial isopods; 613 individuals were assigned to 13 species, representing about one-third of species native to Slovakia. Common autochthonous Central European species of both groups were documented. Predominantly, *Porcellium collicola* (habitat generalist) and *Protracheoniscus politus* (strict forest dweller) were found. 85% of samples contained millipedes, with 647 specimens collected. Altogether 13 species from 5 orders and 10 families were determined; all were representatives of small-size forms of millipedes. *Haplogona oculodistincta* (Chordeumatida) was the dominant species; the most frequent was *Polyxenus lagurus* (Polyxenida). The representatives of the hemispherical morphotype (Glomerida) were completely absent. In general, large or large developmental stages of larger species were not present in the millipede material. Order Chordeumatida was the most diverse; five species found made up 36% of all collected millipede individuals. Nine species of millipedes belonged to the typical regional fauna in lowland forests, while four remaining species represented fauna rarely documented in Central Europe. A qualitative comparison of the species showed apparent differences in body size. Species active predominantly in the warm period are larger, while smaller body dimensions characterize the species collected in winter.

Keywords: winter, terrestrial isopod, millipede, Slovakia, assemblage structure.

For numbers of organismal groups, including Oniscidea and Diplopoda, species distribution, activity, and life cycles are affected by various factors, such as climate, soil chemistry, and habitat availability (Kime 1992, Tajovský 1997, Zimmer 2004, Parmesan 2006). Out of these factors, climatic conditions appear to be most significant in altering populations of invertebrates. Unsuitable weather can impact the reproduction, distribution, and continuity of populations. The diversity and abundance of soil-dwelling invertebrates are not only affected by the density of the humus layer but also by the depth of snow cover (Templer et al. 2012). The onset of harsh, low temperatures can influence the life cycles, depending on the species' ability to adapt (Richards 1995).

Climate changes are associated with the time of the year. For woodlice and millipedes, a humid microclimate tends to be more suitable (Hornung 1984). Therefore, the peak of their activity in the temperate climate could be expected in mild, wet weather of spring and autumn (Kania & Kłapeć 2012). Most soil arthropods are considered inactive in winter, burrowed deep in the soil, where they can escape bad weather and rough conditions on the surface. According to Gupta (1963), terrestrial isopods of temperate areas hibernate during winter. Some species migrate downward into the soil and aggregate on the base of the trees (Paris 1963). Sutton (1972) suggests that decreasing temperatures increase the activity of terrestrial isopods, resulting in transfer from the surface to deeper soil layers. However, very few studies have examined their depth migrations throughout the year (Rendoš et al. 2012, 2016, Rudy et al. 2018).

Millipedes are active most of the year; nevertheless, spring and autumn are typical for their mass occurrence, with activity and density peaking in spring (De Smedt et al. 2020, 2021). For isopods, spring and early summer represent the most common breeding season (Achouri & Charfi-Cheikhrouha 2002), with peak activity in summer (De Smedt et al. 2021). Furthermore, many millipede species can withstand low winter temperatures. For example, some representatives of the family Chordeumatidae tend to be active during mild winter and start their reproductive cycle early before spring during the first temperature increase (Tajovský 1996, Kocourek et al. 2017).

In our study, we focused on broader spatial coverage to test the potential of the winter period in mapping the soil macrofauna diversity. This study aimed to describe assemblages of terrestrial isopods and millipedes inhabiting lowlands and foothills of the South-Eastern Slovakia in the winter period, in the uppermost layers of soil and leaf litter near the soil surface. We also aimed to provide a population analysis of the species of the studied groups, which are potentially active throughout the winter.

The collection of material was conducted during winter from November 2017 to the middle of March 2018. Studied locations were situated in lowlands and foothills of five orographic units, at the interface of the three biogeographic regions, the Western and the Eastern Carpathians and Pannonian Lowland (Carpathian Basin), namely Košická kotlina Basin, Slanské vrchy Mts, Eastern Slovak Upland, Eastern Slovak Plain, and Vihorlatské vrchy Mts.

Winter 2017/2018 in Eastern Slovakia was characterized by only a few freezing days. The average depth of snow reached 5.3 cm. Average day air temperatures for all the collecting sites reached 4°C. The macrofauna material was collected on cold days, always with temperatures above the freezing point. During all the 15 collecting days, the lowest day temperature was 1°C, while the highest day temperature reached 13°C. Sampling was conducted exclusively at sites without permanent snow cover throughout this study.

Overall, 47 samples of soil containing leaf litter and upper layers of humus (to ca 5 cm of depth) were sifted using an entomological sieve. Every sample represented 1 m^2 of the sown substrate (ca 0.05 m^3). Collected soil was air-dried in a Berlese-Tullgren apparatus, and dried soil was thoroughly controlled under a stereomicroscope. Invertebrates were preserved in containers with 96% ethanol. Adult individuals were distinguished from juveniles and males from females based on the presence of male gonopods and other secondary sexual characters. The presence of marsupium with offspring was controlled in females of the terrestrial isopods.

A total of 1260 representatives of terrestrial isopods and millipedes were collected. We distinguished 30 taxa on the species level; however, only 26 were determined to species level due to the absence of adults or clear characteristics. Forty-six samples (97.9%) contained at least one of the two studied groups, while 28 samples (59.5%) were positive for terrestrial isopods and millipedes. The mean abundance of all individuals per square meter was 26.8 (±1.5). Positive samples were characterized by the presence of up to 7 species.

Terrestrial isopods were present in 33 samples (72.3%). A total number of 613 individuals of 6 families and 13 species was collected. One sample contained 1 to 177 individuals, while the relative abundance of individuals per m^2 was 13.0 (±2.05). In positive samples, a maximum of five different species were present (with an average of 1.9 species), while the samples mainly contained only one species. The majority of collected individuals belonged to the family Trachelipodidae (59%), represented by four species, where *Porcellium collicola* had the highest dominance (57.1%) (Table 1). Most of the species were represented by adult individuals (69%); 47% of adults were females, while males made up 22%. All female specimens were without marsupium.

Table 1 Overall assemblage structure of terrestrial isopods collected in winter. Abbreviations: number of individuals (n_i), frequency (C), dominance (D), mean abundance of specimens per square meter (A); minimum and maximum number of individuals per sample/square meter (n_{min} - n_{max}), males (m), females (f), juveniles (juv.).

Family	Species	n_i	C (%)	D (%)	A	n_{min} - n_{max}	m	f	juv.
Armadillidiidae	*Armadillidium vulgare*	1	2.1	0.2	0.02	0–1	1	-	-
Agnaridae	*Protracheoniscus politus*	158	32	26	3.4	0–28	30	37	91
Ligiidae	*Ligidium hypnorum*	2	4.3	0.3	0.04	0–1	-	-	2
Philosciidae	*Lepidoniscus minutus*	21	15	3.4	0.4	0–6	-	7	14
Porcellionidae	*Porcellio spinicornis*	2	2.1	0.3	0.04	0–2	1	1	-
Trachelipodidae	*Porcellium collicola*	350	40	57	7.4	0–172	102	186	62
	Trachelipus nodulosus	6	2.1	1	0.1	0–6	-	-	6
	Trachelipus rathkii	4	4.3	0.7	0.1	0–3	-	-	4
	Trachelipus ratzeburgii	4	4.3	0.7	0.1	0–2	1	3	-
Trichoniscidae	*Haplophthalmus mengii*	3	4.3	0.5	0.1	0–2	2	-	1
	Hyloniscus riparius	7	2.1	1.1	0.02	0–1	-	3	4
	Trichoniscus carpaticus	8	4.3	1.3	0.2	0–6	1	5	2
	Trichoniscus pusillus	47	11	7.7	1.1	0–27	-	45	2
Total		613	100	100	13.0	0–177	138	287	188

Millipedes were recorded in 40 samples (85.1%), where 647 representatives were obtained, belonging to 10 families (5 of which represent order Chordeumatida) and 5 orders. A total of 17 species of millipedes were determined. The relative abundance of individuals per square meter was 13.8 (±0.97). Up to 122 individuals and 1–5 species per sample were recorded (with an average of 2.1 species). 36% of all collected millipedes belonged to the order Chordeumatida (5 families represented each by one species), with Verhoeffiidae being the most dominant family. The highest dominance was achieved by the species *Haplogona oculodistincta* (26.3%). The frequency of occurrence, even of the most numerous species, was low. The most constant species in samples was *Polyxenus lagurus*, present in almost one-fifth of samples with an average abundance of 3 individuals per m^2 (Table 2). Adult individuals prevailed (62%), consisting of 44% of females and 18% of males. 38% of all collected millipedes were juveniles.

The structure of winter communities of both studied groups was uneven in this study if we compare the results at individual localities. Individuals of various species were present mostly separately in the collected samples, except for a few species. Some species were characterized by aggregated occurrence, namely *Haplogona oculodistincta*, *Brachydesmus dadayi,* and *Polyzonium germanicum*. We may interpret it as one of the characteristics of winter collecting and the result of the lower activity of macrofauna. There has been little cold tolerance work done on the studied groups (Duman & Newton 2020). However, isopods and millipedes were confirmed to withstand hypothermia, and some species can survive temperatures between -4° and -5°C (Tanaka & Udagawa 1993, David et al. 1996). We recorded the species with higher cold tolerance and cold hardiness. Species richness and diversity for both studied macrofauna groups were relatively low. It could be explained by the used sampling method, rather similar characteristics of sampling locations, just as well as climatic specifics of the winter period.

The occurrence of both studied groups in samples showed a reverse trend; the higher the number of millipedes in one sample, the lower the number of isopods and *vice versa*. The same trend could be observed in the species richness of both groups in the samples. Both terrestrial isopods and millipedes are detritivores using the same food resources and inhabiting the same habitat. Thus, they probably compete for the basic ecological demands to some extent (Benckiser 1997).

Table 2 Overall assemblage structure of millipedes collected in winter. Abbreviations: same as in the Table 1.

Order Family	Species	n_i	C (%)	D (%)	A	n_{min} - n_{max}	m	f	juv.
Polyxenida									
Polyxenidae	*Polyxenus lagurus*	116	23.4	17.9	2.5	0–65	7	19	90
Polyzoniida									
Polyzoniidae	*Polyzonium germanicum*	44	4.3	6.8	0.9	0–42	7	9	28
	Polyzonium sp. juv.	7	8.5	1.1	0.15	0–2	-	-	7
Julida									
Julidae	*Enantiulus nanus*	27	4.3	4.2	0.6	0–19	7	8	12
	Julidae sp. juv.	40	42.6	6.1	0.85	0–10	-	-	40
	Leptoiulus sp. juv.	3	4.3	0.5	0.06	0–2	-	-	3
	Megaphyllum sp. juv.	21	10.6	3.2	0.4	0–9	-	-	21
Blaniulidae	*Proteroiulus fuscus*	2	2.1	0.3	0.04	0–2	1	1	-
Chordeumatida									
Craspedosomatidae	*Craspedosoma transsilvanicum*	1	2.1	0.2	0.02	0–1	1	-	-
Verhoeffiidae	*Haplogona oculodistincta*	171	19.1	26.3	3.6	0–108	51	120	-
Hungarosomatidae	*Hungarosoma bokori*	11	12.8	2	0.3	0–5	-	11	-
Mastigophorophyllidae	*Mastigona bosniensis*	5	8.5	0.8	0.1	0–2	-	5	-
Chordeumatidae	*Melogona broelemanni*	45	4.7	6.9	0.95	0–37	9	34	2
Polydesmida									
Polydesmidae	*Brachydesmus* sp. juv.	1	2.1	0.2	0.02	0–1	-	-	1
	Brachydesmus dadayi	95	6.4	14.6	2.1	0–76	31	63	1
	Brachydesmus superus	19	4.3	2.9	0.4	0–13	4	12	3
	Polydesmus sp. juv.	37	14.9	5.7	0.8	0–19	-	-	37
	P. transylvanicus	2	2.1	0.3	0.04	0–2	1	1	-
Total		647	100	100	13.8	0–122	119	283	245

Quantitative data on the species composition of terrestrial isopods and millipedes in the studied area for the warm period are insufficient and require further investigation. However, data on the regional species spectrum of lowland forests in the summer season are available (Bogyó et al. 2012, 2015). A comparison of the data indicates that season is linked to the body size of litter-dwelling macrofauna. According to De Smedt et al. (2021), the activity of isopods and millipedes in winter is generally lower; however, there is a shift in the active species to smaller and more drought-sensitive species. Concerning millipedes, testing of difference in mean body size between the species collected in the winter and the species commonly observed in the vegetative season resulted in statistical significance. Species active predominantly in the warm period are larger, while the species collected in winter are characterized by smaller body dimensions (Table 3). Based on these observations, we can hypothetically assume that cold resistance depends on phylogenetic relationships, latitudinal relationships, life forms, and body size. The body-size differences in species spectrum between winter and vegetation season were not statistically significant in terrestrial isopods (Table 3). The distribution of smaller and larger forms is similar in winter and summer; however, the number of species active in the cold season is lower.

Table 3 Body size comparison of regional isopod and millipede species. Oniscidea species pool according to Frankenberger (1959) and Flasarová (1994). Diplopoda species pool according to Haľková et al. (2021) and Mock et al. (2021). Species marked in grey: collected in winter. (*see Hauser, Voigtlander (2019) and original data; **see Frankenberger (1959) and Tabacaru (1974))

	Regional species pool	L (mm)	
		mean	mean (winter)
	Diplopoda	*	
Polyxenida	*Polyxenus lagurus* (Linnaeus, 1758)	2.5	2.5
Glomerida	*Glomeris hexasticha* Brandt, 1833	11.5	
	Glomeris tetrasticha Brandt, 1833	11	
Polyzoniida	*Polyzonium germanicum* Brandt, 1837	10.5	10.5
Chordeumatida	*Craspedosoma transsilvanicum* Verhoeff, 1897	11	11
	Haplogona oculodistincta (Verhoeff, 1893)	9	9
	Hungarosoma bokori Verhoeff, 1928	6.5	6.5
	Mastigona bosniensis (Verhoeff, 1897)	14	
	Melogona broelemanni (Verhoeff, 1897)	8	8

(Table 3 – continued next page)

(Table 3 – continuation)

	Regional species pool	L (mm)	
		mean	mean (winter)
	Diplopoda	*	
Julida	*Leptoiulus proximus* (Němec, 1896)	25	
	Megaphyllum projectum Verhoeff, 1894	28.5	
	Julus terrestris Linnaeus, 1758	18	
	Julidae complex of species (juveniles)	5	5
Polydesmida	*Brachydesmus dadayi* Verhoeff, 1895	7	7
	Brachydesmus superus Latzel, 1884	8.5	8.5
	Polydesmus complanatus (Linnaeus, 1761)	19	
	Polydesmus denticulatus Koch, 1847	13.5	
	Polydesmus transylvanicus Daday, 1889	6	6
	Strongylosoma stigmatosum (Eichwald, 1830)	19	
Mean		**12.69**	**7.67**
Isopoda	**Oniscidea**	**	
	Armadillidium versicolor Stein, 1859	11.5	
	Armadillidium vulgare (Latreille, 1804)	9	
	Haplophthalmus mengii (Zaddach, 1844)	3	
	Hyloniscus riparius (Koch, 1838)	4.5	4.5
	Hyloniscus transsilvanicus (Verhoeff, 1901)	6	
	Lepidoniscus minutus (Koch, 1838)	8	
	Ligidium germanicum Verhoeff, 1901	7	
	Ligidium hypnorum (Cuvier, 1792)	8.5	
	Porcellium collicola (Verhoeff, 1907)	5	5
	Porcellium conspersum (Koch, 1841)	7	
	Protracheoniscus politus (Koch, 1841)	8	8
	Trachelipus rathkii (Brandt, 1833)	12.5	
	Trachelipus ratzeburgii (Brandt, 1833)	12	12
	Trichoniscus carpaticus Tabacaru, 1974	2.5	2.5
	Trichoniscus pusillus Brandt, 1833	3.5	
Mean		**7.2**	**6.4**

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