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1 **Species assemblages and diets of Collembola in the organic matter accumulated**
2 **over an old tar deposit**

3
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11
12 **Running title:** Collembola over tar

13
14 **Abstract** An oil refinery was abandoned in 1964 at Merckwiller-Pechelbronn (Alsace,
15 France). We investigated the food diets and species assemblages of Collembolan
16 communities living in a thick pasty tar deposit overlaid by an organic soil, by
17 comparison with an adjacent unpolluted plot. At the polluted plot the species richness
18 of the springtail community was low (9 species, versus 27 at the unpolluted plot) but its
19 total abundance was at the same level in both plots. Most springtails consumed more
20 fungi than bacteria at the polluted plot, contrary to the unpolluted plot. Changes in
21 habitat features and food resources might explain the observed decrease in local
22 biodiversity, rather than direct PAH toxicity.

23
24 *Keywords:* Collembolan communities; Gut contents; Polycyclic Aromatic Hydrocarbons

25 **1. Introduction**

26

1 The direct toxicity of pollutants to soil animals has been widely reported and
2 indirect effects, for instance through changes in habitat and food quality, are probable
3 [3,11].
4

5 Polycyclic Aromatic Hydrocarbons (PAHs), such as naphthalene, phenanthrene,
6 or benzo[a]pyrene, are toxic aromatic compounds [2,24] which are nonpolar,
7 hydrophobic and neutral molecules composed of two or more fused benzene rings in
8 linear, step, or cluster arrangement. Nowadays, their presence in the soil results mainly
9 from industrial activities such as coke production, petroleum refining and other high-
10 temperature industrial processes, which induce pollution by accidental oil spilling and
11 waste deposition, even long after site abandonment. Field studies revealed that the
12 presence of PAHs decreases the biodiversity of soil animal communities and changes
13 their vertical distribution [1,10]. Laboratory bio-assays have demonstrated toxic and
14 anaesthetic effects of PAHs on soil animals [7,5,8], as well as bioaccumulation [17] and
15 avoidance [18].
16

17 Our study site was a petroleum refinery abandoned in 1964. It still displays a
18 patchy pollution of soil and ponds by PAHs. The aim of our work was to study the
19 impact of this type of pollution on soil fauna by a comparison between a plot polluted by
20 PAHs and a nearby unpolluted control plot. In a parallel study on the composition of
21 humus profiles, we observed that the organic matter which accumulated over the 40-
22 year-old tar deposits was permeated by a large population of mycorrhizal fungi, in
23 contrast to the adjacent unpolluted plot which was characterized by the rapid
24 incorporation of litter to mineral matter through earthworm activity [12]. We used a
25 community-level approach to evaluate the impact of PAHs on the saprophagous
26 compartment of the ecosystem. The collembolan community (Arthropoda: Hexapoda)
27 was chosen for its great abundance and diversity in a wide variety of soil types [19,20]
28 and the flexibility of its alimentary habits [11,15,21]. Population indices and food bolus

1 analyses were used to follow changes in function and biodiversity of collembolan
2 populations under the influence of PAH pollution.

3

4 **2. Materials and methods**

5

6 *2.1. Study site*

7

8 The study was conducted at the site of the petroleum refinery of Merckwiller-
9 Pechelbronn, about 50 km north of Strasbourg (Alsace, France). The refinery had an
10 intensive activity until 1964, afterwards it was progressively dismantled then totally
11 abandoned. The site is located on the Merckwiller-Pechelbronn oil field (bituminous
12 sand) on the western edge of the Rhine rift valley. The superficial soil (5 m) is
13 composed of recent fluvial deposits overlaying 1400 m thick sediments above the
14 granitic substratum [26].

15

16 Nowadays, the site area (20 ha) is characterised by the presence of old
17 buildings now included in a great variety of naturally established ecosystems
18 (woodland, grassland, ponds) with zones polluted by hydrocarbons, in particular by tar
19 deposits. Two plots (polluted versus unpolluted used as control), were sampled in
20 October 2002. They were considered to be representative of the patchy occurrence of
21 tar, as ascertained by the examination of a number of adjacent (polluted and
22 unpolluted) soil profiles. The **polluted plot**, approximately 20 m², was characterised by
23 a thick pasty tar patch overlaid by a 3-5 cm organic layer, a discontinuous field layer
24 composed of *Hedera helix*, *Geranium robertianum*, *Carex pilosa*, *Solidago canadensis*
25 and *Taraxacum officinale*, a shrub layer composed of *Fraxinus excelsior*, *Rubus*
26 *fruticosus* and *Salix capraea* and a tree layer composed of *Acer campestre*, *Betula*
27 *pendula* and *Quercus robur*. The unpolluted, **control plot** was located 15 m south of
28 the polluted plot, and its surface was arbitrarily fixed to 20 m², for the sake of

1 comparison, although it did not differ from the rest of the site. It was characterised by a
2 much greater plant biodiversity and a good earthworm mull humus. The field layer was
3 composed of *Hedera helix*, *Arum maculatum*, *Carex pilosa*, *Fragaria vesca*, *Geranium*
4 *robertianum*, *Geum urbanum*, *Potentilla reptans*, *Stachys sylvatica*, *Solidago*
5 *canadensis* and *Taraxacum officinale*, the shrub layer was composed of *Acer*
6 *pseudoplatanus*, *Carpinus betulus*, *Cornus mas*, *Cornus sanguinea*, *Crataegus*
7 *monogyna*, *Fraxinus excelsior*, *Ligustrum vulgare*, *Prunus avium*, *Rosa canina* and
8 *Rubus fruticosus*, and the tree layer was composed of *Acer campestre*, *Prunus avium*
9 and *Quercus robur*.

10

11 2.2. Sampling and identification of animals

12

13 Collembola were sampled on the organic matter accumulated over the tar
14 deposit and in the adjacent unpolluted plot. At each plot, five soil cores, 5 cm diameter
15 and 10 cm depth (litter included), were collected with a core sampler and, after
16 discarding tar pieces, stored in plastic bags. Springtails were extracted from soil/litter
17 by the dry-funnel method [9] and preserved in 95% (v/v) ethyl alcohol until sorting and
18 identification. The animals were sorted under a dissecting microscope, then mounted in
19 chloral-lactophenol (25 ml lactic acid, 50 g chloral hydrate, 25 ml phenol). They were
20 identified to species level using a phase contrast microscope at 500x and several keys
21 and diagnoses [4,6,13,16,23,27,28,29]. We calculated in each sample the total
22 abundance, the the species richness, the Shannon Index and the equitability. We also
23 calculated the total species richness of the five samples taken at each plot.

24

25 2.3. Observation of gut contents

26

1 Gut contents from each mounted Collembola were observed under phase
2 contrast at 500x magnification and classified in twelve categories:

- 3
- 4 • Melanized hyphae
 - 5 • Hyaline hyphae
 - 6 • Fungal spores
 - 7 • Bacteria
 - 8 • Mineral particles
 - 9 • Holorganic humus (amorphous organic matter)
 - 10 • Hemorganic humus (mixture of organic matter and mineral particles)
 - 11 • Micro-algae
 - 12 • Pollen grains
 - 13 • Empty guts
- 14

15 The proportion in volume of the different categories was visually estimated to the next
16 5% in each animal gut then averaged by species and by site.

17

18 *2.4. Chemical analyses*

19

20 Samples were collected in April 2003 to determine the amount of PAHs from the
21 EPA list [14] in (1) the 10 top cm of the A horizon of the control soil (2) the tar deposit
22 at 10-20 cm depth and (3) the OF horizon overlying the tar deposit. To check the
23 validity of our control plot, samples were collected in May 2003 in the park of the
24 laboratory (Brunoy, Ile-de-France, France), in the 10 top cm of a similar soil (rich
25 earthworm mull at neutral pH) under deciduous woody vegetation. Composite samples
26 were taken from each plot, then kept in glass jars and rapidly transported to the
27 laboratory. Then, soil samples were homogenised and sieved at 1 cm then kept in

1 glass jars at -18C° until analysis. Each sample was defrozen, dried, then sieved at 2
2 mm.

3

4 PAHs were extracted from each sample with the automatic system ASE 200
5 (Accelerated Solvent Extraction) DIONEX, using a mixture of dichloromethane and
6 acetone (50/50) for soil and acetonitrile for tar. The extract was concentrated under
7 forced air with a TuroVap LV (Zymark), then PAHs were separated by HPLC (High
8 Power Liquid Chromatography) with UV detection (alliance 2690 chain, PDA 996
9 detector, column LC-PAH Supelco). After sieving the contaminated soil, which was
10 mainly made of badly decomposed tree litter, only a small amount of fine matter was
11 available for analysis, and only 7 major PAHs could be analysed on this material:
12 naphthalene, phenanthrene, fluoranthrene, pyrene, benzo(a)anthracene, chrysene and
13 benzo(ghi)perylene, the others being under detection level ($0.02\ \mu\text{g/g}$ for
14 uncontaminated soil, $0.04\ \mu\text{g/g}$ for contaminated soil, $0.1\ \mu\text{g/g}$ for tar). All
15 measurements were done in triplicate.

16

17 After extraction of fauna, air-dried soil was stored in plastic bags for pH
18 measurement. The soil was suspended in deionized H_2O and 1M KCl (1:5 soil:water
19 v:v) for pH H_2O and pH KCl, respectively. Each suspension was shaken for five
20 minutes, then pH was measured in the supernatant after sedimentation for 2 h with a
21 glass electrode. The difference between these two measurements (δpH) was taken as
22 an estimate of exchange acidity.

23

24 *2.5. Statistical treatment*

25

26 Non-parametric Mann-Whitney tests were performed to compare chemical
27 parameters (pH) and population parameters (abundance, species richness, diversity

1 and equitability of collembolan communities) between sites, using samples as
2 replicates (five in each site).

3

4 **3. Results**

5

6 *3.1. Chemical analyses*

7

8 The distribution of the 16 PAHs of the EPA list and the total amount of PAHs
9 were quite similar in the control soil from the Merkwiller-Pechelbronn site and in the soil
10 from the park of the laboratory (Table 1). As a consequence we estimated that the plot
11 chosen as a control at Merkwiller-Pechelbronn was valid. The total amount of the
12 seven PAHs analysed in the soil over the tar deposit at Merkwiller-Pechelbronn (5.95
13 $\mu\text{g/g}$) was six times higher than the corresponding amount at the control plot (0.93
14 $\mu\text{g/g}$). The concentration of benzo(ghi)perylene was eleven times higher than the
15 control, while the amount of fluoranthrene was only three times higher.

16

17 The pH measured in water and potassium chloride was neutral and did not
18 differ between both sites at Merkwiller-Pechelbronn (Table 1). However, mann-Whitney
19 U test revealed that exchange acidity, expressed by δpH , was higher on the unpolluted
20 site.

21

22 *3.2. Collembolan communities*

23

24 At the unpolluted plot 27 species were found whereas only 9 were found in the
25 polluted zone. The species richness per sample, the Shannon Index and the
26 equitability were higher at the control than at the contaminated plot (Table 2). However,
27 the total abundance of springtails did not differ between plots.

1
2 Only five species were common to both polluted and unpolluted plots: *Folsomia*
3 *candida*, *Parisotoma notabilis*, *Sminthurides malmgreni*, *Sminthurides parvulus* and
4 *Sminthurinus aureus*. Among these species, *S. aureus* was significantly more abundant
5 in the control soil (x 4). Over the tar deposit, 83% of the total abundance was
6 represented by *Proisotoma minuta* (43%) and *Parisotoma notabilis* (40%). *P. minuta*
7 was present in all samples taken at the polluted plot while it was absent from all
8 samples taken at the control plot. The abundance of this species (together with that of
9 *Parisotoma notabilis* in most samples from the tar deposit) explains why the total
10 abundance of Collembola did not differ between plots in spite of a much lower number
11 of species at the polluted plot (Table 2).

12 13 3.3. Diets of Collembola

14
15 The food diet of the three species *Parisotoma notabilis*, *Folsomia candida* and
16 *Sminthurinus aureus* was compared between plots (Figure 1). Fungal mycelium was
17 more common at the polluted plot, while the contrary was observed for bacteria, except
18 in *F. candida* guts which showed bacteria in equal proportion in both sites but more
19 hyaline hyphae and hemorganic humus over the tar deposit. The proportion of empty
20 guts was higher at the polluted plot for *P. notabilis* and *S. aureus*.

21
22 The diet of *S. aureus* was more diverse at the unpolluted plot, containing micro-
23 algae, pollen, humus, bacteria and fungal spores and mycelia, but only fungal
24 mycelium in the polluted plot. However the latter observation could be an artifact due to
25 the low number of specimens (3) collected over the tar deposit. The gut of the
26 bacteriophagous species *M. minimus* contained 68% bacteria, and this species was
27 only present at the unpolluted site, where it was found in all samples. The isotomid *P.*

1 *minuta*, which was present only at the polluted site where it dominated the community,
2 showed a high proportion of empty guts (67%), 21% mycelium and few bacteria (2%).

3

4 **4. Discussion**

5

6 In spite of a slight distance between plots and a low concentration of PAHs over
7 the tar deposit (Table 1), a great dissimilarity in collembolan communities was
8 observed between the organic soil accumulated over the tar deposit and the adjacent
9 control soil (Table 2). It should be noticed that sieving of the soil to 2 mm before PAH
10 extraction (see Materials and methods) could have led to underestimate PAH
11 concentration in the soil over tar. The toxicity of crude oil for *Collembola* was shown by
12 Sendstad [25] who found 2% of total *Collembola* in a polluted plot compared to a
13 control plot. Three years after application, toxicity was still present given the absence of
14 recolonisation by fauna of the polluted zone. In our study, the collembolan community
15 could be affected by i) the presence of pollutants in the soil, through direct effects of
16 PAHs or other toxic compounds on the most sensitive species and/or ii) indirect effects
17 such as changes in food resources. Like in a previous study on a site polluted by heavy
18 metals [11], the species richness of springtail communities was observed to decrease
19 under the influence of pollution, at constant or even higher total abundance. In both
20 cases, this could be explained by an increase in free habitats liberated by most
21 sensitive species. Isotomids seemed to be less sensitive to PAH pollution, as was also
22 observed by Blakely et al. [3]. Here this group was represented by the two dominant
23 species *Proisotoma minuta* and *Parisotoma notabilis*.

24

25 In our study, changes were observed in the diet of springtails under the
26 influence of pollution by hydrocarbons. This shift can be explained by a change in the
27 use of food resources, since fungi were favoured to the detriment of bacteria. This
28 possibility reflected a change in the composition of microbial communities in the favour

1 of fungi, which could explain why the bacteriophagous *Megalothorax minimus* was only
2 present at the unpolluted plot. Micromorphological analyses showed a high amount of
3 ectomycorrhizal fungal hyphae in the organic matter accumulated over the tar deposit
4 with a poor earthworm activity, while a rapid incorporation of organic matter to mineral
5 matter through earthworm activity was registered in the control soil [12]. Shifts in
6 microbial communities in favour of fungi could be better explained by a change in
7 humus form (and associated foodwebs) than by the direct toxicity of PAHs to
8 microorganisms [3].

9

10 We found strong changes in invertebrate communities and alimentary habits of
11 soil animals forty years after abandonment of industrial activity. Despite a low PAH
12 concentration in the soil accumulated over the tar deposit (Table 1), the inability of
13 earthworm populations to colonize pollution spots, and the development of a superficial
14 ecto-mycorrhizal root mat caused significant changes in the environment of animal as
15 well as microbial communities. Given the shift from mull (dominated by earthworm
16 activity) to moder (dominated by enchytraeid activity) ascertained by
17 micromorphological analysis [12], the observed decrease in biodiversity and change in
18 the food diet of the Collembolan community can be ascribed, at least partly, to a
19 change in humus form [22].

20

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22

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27

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26

1 **Figure legends**

2

3 **Fig 1.** Composition of the food bolus of *Parisotoma notabilis*, *Folsomia candida*,
4 *Sminthurinus aureus*, *Megalothorax minimus* and *Proisotoma minuta* in the soil
5 over tar (T) and the control soil (C) from Merkwiller-Pechelbronn site. The
6 percentage contribution of each species to the total population is indicated
7 between brackets

8

Table 1

Chemical features of tar, organic matter accumulated over tar, control soil from Pechelbronn site and laboratory soil. Concentration of PAH (Polycyclic Aromatic Hydrocarbons) is expressed in µg/g dry soil. Means of three replicated measures on pooled samples (PAHs) or of five replicate samples (pHs) are followed by standard errors. Significant differences among groups at 0.05 level (for pH only) are indicated by different superscript letters (Mann-Whitney test). d.l. = detection limit

	Tar	Soil over tar	Control soil	Laboratory soil
pH (water)		6.7±0.3	7±0.4	
pH (potassium chloride)		6.3±0.3	6.2±0.4	
δ pH		0.4±0.04	0.8±0.05	
Naphthalene	52.7±1.0	0.493±0.022	0.096±0.003	0.064±0.005
Acenaphthylene	< d.l.	< d.l.	< d.l.	< d.l.
Acenaphthene	< d.l.	< d.l.	0.038±0.002	0.023±0.000
Fluorene	< d.l.	< d.l.	< d.l.	< d.l.
Phenanthrene	151.1±2.8	0.776±0.048	0.102±0.006	0.119±0.019
Anthracene	< d.l.	< d.l.	0.025±0.001	0.024±0.008
Fluoranthrene	< d.l.	0.655±0.012	0.238±0.020	0.230±0.024
Pyrene	< d.l.	1.551±0.136	0.178±0.020	0.152±0.014
Benzo(a)anthracene	< d.l.	0.633±0.033	0.107±0.011	0.080±0.009
Chrysene	35.7±0.4	0.694±0.051	0.108±0.005	0.105±0.013
Benzo(b)fluoranthrene	< d.l.	< d.l.	< d.l.	< d.l.
Benzo(k)fluoranthrene	< d.l.	< d.l.	0.057±0.004	0.048±0.004
Benzo(a)pyrene	< d.l.	< d.l.	0.157±0.019	0.147±0.011
Dibenzo(a,h)anthracene	< d.l.	< d.l.	0.023±0.003	0.016±0.001
Benzo(ghi)perylene	27.2±2.0	1.146±0.031	0.103±0.012	0.074±0.003
Indeno(123cd)pyrene	< d.l.	< d.l.	0.130±0.008	0.083±0.006
Σ 13 PAHs in uncontaminated soil			1.363±0.102	1.165±0.094
Σ 7 PAHs in contaminated soil		5.950±0.290	0.934±0.070	0.824±0.083

1

2

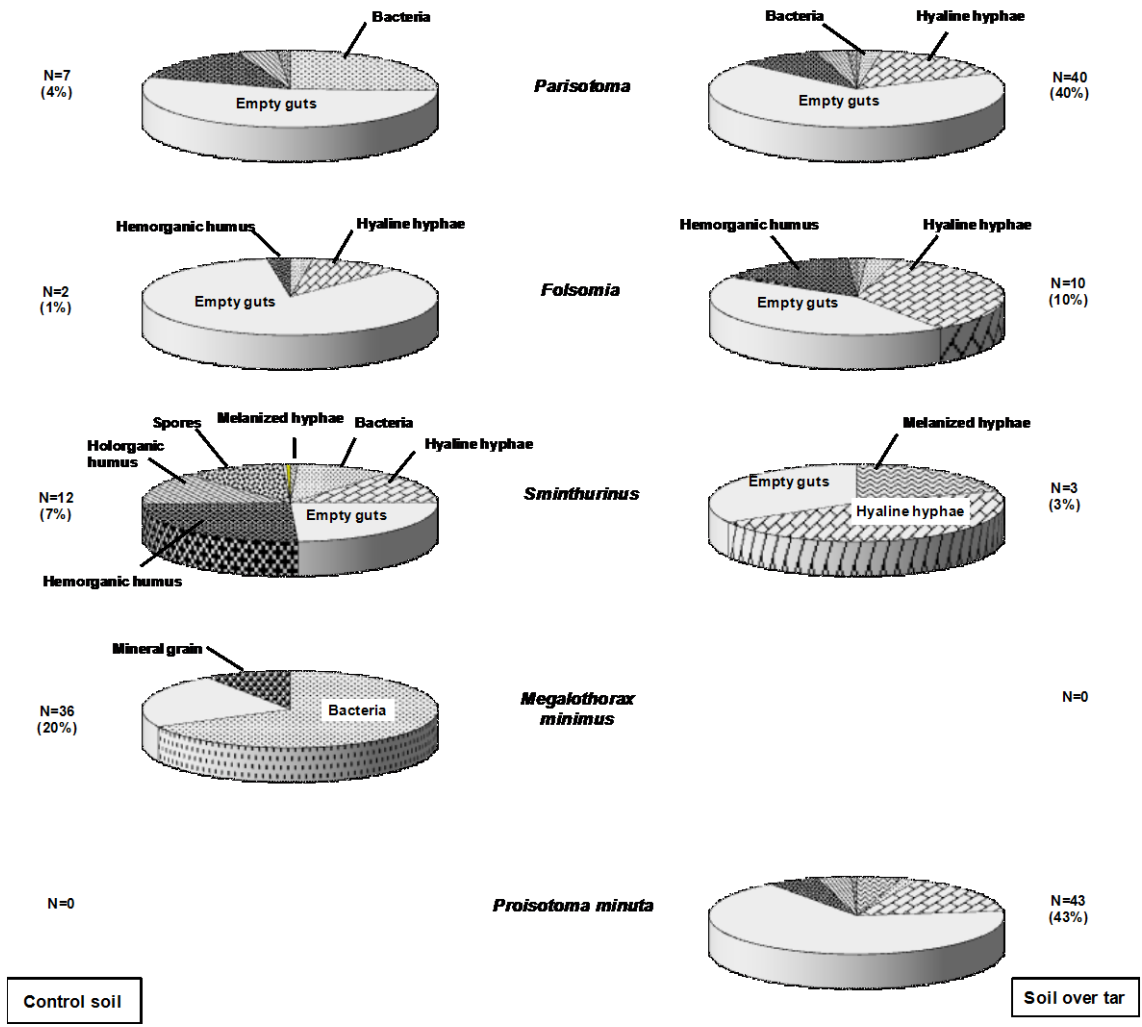
Table 2

Collembolan densities per sample (20 cm²) at unpolluted and polluted plots. Significant differences between groups (Mann-Whitney U test, P = 0.05) are indicated by stars

	Control soil					Soil over tar					Mann-Whitney
<i>Dicyrtoma fusca</i> (Lucas, 1842)	0	0	1	0	0	0	0	0	0	0	
<i>Dicyrtomina minuta</i> (O. Fabricius, 1783)	0	0	0	0	0	0	1	0	0	0	
<i>Folsomia candida</i> (Willem, 1902)	2	0	0	0	0	8	0	0	1	1	
<i>Folsomia manolachei</i> Bagnall, 1939	0	2	3	3	4	0	0	0	0	0	*
<i>Heteromurus nitidus</i> (Templeton, 1835)	0	0	0	0	1	0	0	0	0	0	
<i>Isotomiella minor</i> (Schäffer, 1896)	2	5	3	0	2	0	0	0	0	0	*
<i>Lepidocyrtus lanuginosus</i> (Gmelin, 1781)	10	2	0	3	8	0	0	0	0	0	*
<i>Lepidocyrtus lignorum</i> (Fabricius, 1781)	7	1	0	2	0	0	0	0	0	0	
<i>Megalothorax minimus</i> Willem, 1900	17	4	2	3	10	0	0	0	0	0	*
<i>Mesaphorura florum</i> Simón et al., 1994	0	1	1	2	1	0	0	0	0	0	*
<i>Mesaphorura hylophila</i> Rusek, 1979	1	5	8	7	5	0	0	0	0	0	*
<i>Mesaphorura macrochaeta</i> Rusek, 1971	1	1	3	2	0	0	0	0	0	0	*
<i>Micranurida sensillata</i> (Gisin, 1953)	0	0	0	0	1	0	0	0	0	0	
<i>Neanura muscorum</i> (Templeton, 1835)	0	0	0	0	0	1	0	0	0	0	
<i>Onychiurus jubilarius</i> Gisin, 1957	0	1	0	0	0	0	0	0	0	0	
<i>Orchesella flavescens</i> (Bourlet, 1839)	0	1	0	0	0	0	0	0	0	0	
<i>Parisotoma notabilis</i> Schäffer, 1896	3	0	0	2	2	34	5	0	0	1	
<i>Proisotoma minuta</i> (Tullberg, 1871)	0	0	0	0	0	12	5	3	17	6	*
<i>Pseudosinella alba</i> (Packard, 1873)	4	0	1	2	2	0	0	0	0	0	*
<i>Pseudosinella decipiens</i> Denis, 1925	0	0	1	0	0	0	0	0	0	0	
<i>Sminthurides malmgreni</i> (Tullberg, 1871)	0	0	1	0	0	1	0	0	0	0	
<i>Sminthurides parvulus</i> (Krausbauer, 1871)	0	1	0	1	0	0	0	0	1	0	
<i>Sminthurinus aureus</i> (Lubbock, 1862)	4	2	3	2	1	2	0	0	1	0	*
<i>Sphaeridia pumilis</i> (Krausbauer, 1898)	1	0	0	0	0	0	0	0	0	0	
<i>Stenophorura denisi</i> (Bagnall, 1935)	1	0	0	0	1	0	0	0	0	0	
<i>Tomocerus minor</i> (Lubbock, 1862)	0	0	0	0	0	0	0	1	0	0	
<i>Tomocerus vulgaris</i> Tullberg, 1871)	0	0	0	0	2	0	0	0	0	0	
<i>Vertagopus cinereus</i> (Nicolet, 1841)	0	0	0	0	1	0	0	0	0	0	
<i>Willemia budenbrocki</i> Hüther, 1959	0	0	0	2	0	0	0	0	0	0	
<i>Willemia intermedia</i> Mills, 1934	0	1	1	0	0	0	0	0	0	0	
<i>Xenylla tullbergi</i> Börner, 1903	0	1	1	0	1	0	0	0	0	0	*
Total abundance	53	28	29	31	42	58	11	4	20	8	
Species richness	12	14	13	12	15	6	3	2	4	3	*
Shannon Index	3.0	3.5	3.3	3.4	3.4	1.7	1.3	0.8	0.8	1.1	*
Equitability	0.8	0.9	0.9	1.0	0.9	0.7	0.9	0.8	0.4	0.7	*

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2 Fig. 1