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SHORT COMMUNICATION

Short note on enchytraeid occurrence in deep layers of urban soils

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1. Introduction

Most soil organisms require aerobic conditions and access to carbon as a source of energy and building material for their bodies. Therefore, in soils of most terrestrial ecosystems animals are concentrated in the upper 10–15 cm layer and few individuals of few species occur below 50 cm depth. Where plant roots or earthworm tunnels reach further down, also other soil organisms follow along these rather thin structures, i. e. the rhizo- and drilosphere (Lavelle & Spain 2005). Exceptions where a more general colonization of soil into greater depth occurs are dry grasslands and arable fields. Ploughing relocates substantial amounts of dead organic matter into greater depth whereas the upper soil is more exposed to desiccation and freezing, resulting in a more even distribution of soil organisms along the vertical profile and their penetration in considerable numbers at least down to the plough pan. Also buried soil horizons with adequate dead organic matter content might host soil organisms as long as some exchange of soil air provides enough oxygen (Gurwick et al. 2008). Such buried horizons are typical of alluvial soils. Buried soil horizons or buried layers or pockets of humus-rich soil or of other organic materials can also be found in urban soils as a consequence of land reclamation, landscaping, construction activities, dumping of waste etc. However, most samplings for soil fauna have been limited to the upper soil layer of some 8 to 15 cm based on the general experience that further down numbers of soil fauna are negligible (and also due to the fact that extracting soil cores reaching further down can get difficult even in deeply weathered soils). This all applies also to the vertical distribution of potworms or enchytraeids (Annelida: Clitellata: Enchytraeidae), that are a numerically and functionally important part of soil mesofauna. Very few data are available about the occurrence of enchytraeids below 20 cm soil depth (see Discussion). Although enchytraeids reach only low densities in deep soil and their occurrence is clustered based on the availability of sources of organic matter (Dózsa-Farkas 1992), their occurrence still shows that they find food and participate in decomposition processes at substantial depth. Deeper layers might also host specialized species hitherto unknown to science (Dózsa-Farkas 1991).

As part of an environmental assessment project, samples of anthroposols were taken from several sites in the Swedish city of Malmö at three different depths down to 2 m below ground. Soil samples were examined to find out if they contained enchytraeids and if so what species and in what numbers were present. Unfortunately, only little information about the project and sampling sites was available to the author; presented background data are mostly based on Törneman (2014). Nevertheless this was a rare opportunity to investigate the possibility of soil animal live in urban soils at substantial depth.

2. Material and methods

In October 2012, five sites in the city of Malmö in southern Sweden were sampled, covering reclaimed land areas at the sea front as well as old industrial and residential land, currently used as urban greens of various types and sizes (Tab. 1). At each site one pit was dug and soil was sampled from layers defined by three depth ranges: 0.1–0.5 m, 0.6–1.0 m and 1.6–2.0 m (for the exact ranges actually sampled at the individual sites see Tab. 1). The individual samples were put into plastic

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containers (one sample per site and soil depth). From each container (= site × soil depth combination) three subsamples were taken, each of a volume of ca. 120 cm^3 and a fresh weight of 120–224 g (depending on soil texture and density). Larger stones were discarded. From these soil samples, enchytraeids were extracted by wet funnel extraction (without heating) over 48 hours, with retrieval of extracted specimens after the first 24 hours and at the end of the extraction period. The extraction method was based on Graefe's extraction method without heating while using a water-filled funnel as originally proposed by O'Connor (Dunger 1989, Kobetičová & Schlaghamerský 2003). The lower parts of the battery of funnels (in particular the attached collection tubes) were cooled by a cold water bath as ambient temperature was high. Soil samples prior to extraction and extracted enchytraeids in water-filled Petri dishes were stored at ca. 6 °C. Tap water was used for extraction and storage. Within three days after extraction all extracted specimens were sorted out and counted under a preparatory microscope. Subsequently they were identified alive under a compound microscope with Nomarski interference contrast (Olympus BX 51, magnification up to 500x).

In the given case reference to soil surface area was not possible (and would not make sense either as no continuous soil cores had been extracted). Abundance is therefore reported as mean numbers of individuals per volume (1 dm^3) and per 1 kg fresh weight of soil. The corresponding standard errors of the mean (SE) are provided as indicators of variance, however, one has to be aware that the three subsamples on which means and SE are based did not present independent samples.

3. Results

In total, 25 enchytraeid specimens of six species and three genera were obtained (Tab. 2). Many of the observed enchytraeids were of apparently poor health, with unusually thin bodies, complicating species identification. Some died shortly after extraction and one could not even be assigned to genus due to its partially decomposed state and coverage by soil particles. At two sites only one (Turbinkanalen) or three (Vendelsgången) enchytraeids were extracted, all from the uppermost soil layer sampled. At a single site, Gråsejen, one individual was also found in the deepest soil layer. This was also the site with the highest total enchytraeid number, with most specimens extracted from the uppermost soil layer. Abundances per soil volume and soil fresh weight with the corresponding standard errors of the mean (see Materials and Methods) are given in Tab. 3.

4. Discussion

All soil samples were taken below a depth of 10 cm. Soil fauna in general and enchytraeids in particular are rarely sampled at such depths. Even when sampling arable soil, only few authors sampled into greater depth than 20 cm, for instance Röhrig et al. (1998) down to 25 cm, Ryl (1977) down to 30 cm and Didden (1990) down to 40 cm. Persson & Lohm (1977) sampled enchytraeids in grassland soil down to 20 cm depth in summer and 28 cm in winter. Willard (1974, in Dózsa-Farkas 1992) to 70 cm, Nielsen (1955) down to 75 cm and Kurt (1961, in Dózsa-Farkas 1992) even to a depth of 100 cm below ground. The only study reporting enchytraeids from a depth below 100 cm was that of Dózsa-Farkas (1991, 1992). Within that study, soil layers reaching down to 145 cm below ground were sampled in a Hungarian oak-hornbeam forest and a species new to science, *Fridericia profundicola* Dózsa-Farkas,1991, was found exclusively between 40 and 125 cm depth. Dózsa-Farkas (1992) also reported several individuals of *Fridericia paroniana* and some of *Enchytraeus buchholzi*, *E. norvegicus* and *Enchytronia parva* from an earthworm tunnel in a depth of 60–65 cm. Also Persson & Lohm (1977) found a *Fridericia ratzeli* individual in an earthworm tunnel sampled at 40–50 cm depth. Even within the uppermost 10 cm of soil, enchytraeids show usually a marked decrease in numbers with increasing depth. If it were not for the study of Dózsa-Farkas (1991) in an undisturbed forest soil, the fact that enchytraeids were found down to a depth of almost 2 m could be considered very surprising. On the other hand, only a single specimen (at Gråsejen) was extracted from the deepest soil layer sampled at the five sites. At most sites the soil included humus also at medium depth, often also in the deepest one. This might have been the consequence of some topsoil that had been buried in the course of historic construction activities or landscaping. Judging from the appearance of the soil, soil at Gråsejen and Rundelen was most humous, even down to the deepest layer sampled. Soil respiration was measured by another member of the contractor's team and these were the two sites where soil respiration did not markedly differ between the layers, whereas it decreased with depth elsewhere, reaching in the lowest layer approximately half the value of the upper one (Jílková 2013, unpubl.). These two were also the sites with highest enchytraeid abundance, including deeper soil layers. However, even at Gråsejen 8 of the 10 enchytraeids were extracted from the upper-most of the investigated soil layers, which had been sampled closer to the soil surface (0.1–0.2 m) than at the other sites (Tab. 2). Where some of the sampled soil layers consisted of dense clay or rocky moraine, no enchytraeids were found. Such material was apparently lacking organic matter that could have served as a food source as well as larger soil pores allowing access of the worms. However, the sample size (extracted soil volume) was still too limited to exclude the possibility of enchytraeid occurrence at very low densities. Soil contamination could have contributed to low densities and the observed bad state of health or malnourishment observed in many specimens. Contamination by heavy metals was measured. Detailed data are not to the author's disposal, but zinc and mercury concentrations in the deeper layers were reported as high, i.e. exceeding benchmark values for the protection of the soil environment and of human heath, respectively (Törneman 2014). In Sweden this means above 250 mg Zn per kg soil and 0.25 mg Hg per kg soil for sensitive land use, such as in the case of residential areas (Naturvårdsverket – Swedish Environmental Protection Agency 2009).

Species of the genus *Buchholzia* as well as the species complex of *Enchytraeus buchholzi* s.l. are known to occur rather frequently in urban and other disturbed soils (Schulte et al. 1989, Schlaghamerský & Pižl 2009). *Enchytraeus buchholzi* had been found in substantial depth also by Dózsa-Farkas (1992). On the other hand, the extraction of *Buchholzia fallax* from a depth of 0.3–0.5 m was not typical as the species of this genus (at least the closely related and more common *B. appendiculata*) prefer the litter layer and uppermost mineral soil rich in humus (Dózsa-Farkas 1992; Graefe & Schmelz 1999).

Both genera avoid too acidic soils, as do *Fridericia* species, many of which have also been found in urban greens (Schlaghamerský & Pižl 2009). Although the pH of the soil was not measured, they were clearly not acid, the presence of calcareous sand and limestone indicating slightly acidic or even slightly alkaline conditions. It is difficult to assess the found abundances in the context of known densities of soil-dwelling enchytraeids as a clear connection to soil surface area is lacking. Taking into

Table 1. Characterisation of the five sampling sites in the city of Malmö (Sweden).

Sampling site and soil depth	Character of soil layer	Current land use	Historic land use
Korvetten			
0.2–0.4 m	humous sand	parking lot	industry (shipyard), later reclaimed (landfilled)
0.7–1.0 m	humous sand and gravel		
1.7–2.0 m	sand (probably from the seabed)		
Gråsejen			
0.1–0.2 m	humous sand	urban green	cement industry
0.7–1.0 m	humous, calcareous sand with gravel and stones (limestone)		
1.7–1.9 m			
Vendelsgången			
0.2–0.3 m	humous sand	residential area: green space between houses	no information
0.7–0.9 m	calcareous sand		
1.6–1.7 m	rocky moraine, very hard		
Turbinkanalen			
0.2–0.3 m	humous sand	large urban green	reclaimed land (landfilled)
0.7–0.9 m	humous, gravelly sand, brick rubble and clay		
1.7–1.9 m	clay, sandy gravelly clay, brick rubble, moist		
Rundelen			
0.3–0.5 m	humous sand, traces of coal	small urban green neighboured by commercial and apartment buildings	urban: old part of the city centre, current state is the result of landscaping
0.6–0.9 m	humous sand, brick rubble, traces of coal		
1.7–2.0 m	sand, traces of coal		

Table 2. Enchytraeid species and numbers of individuals obtained from soil samples taken at three different depths at five sites in the city of Malmö (Sweden).

Site	**Korvetten**			**Gråsejen**			**Vendelsgången**			**Turbinkanalen**			**Rundelen**			**All sites**		
Species / Depth [m]	0.2–0.4	0.7–1.0	1.7–2.0	0.1–0.2	0.7–1.0	1.7–1.9	0.2–0.3	0.7–0.9	1.6–1.7	0.2–0.3	0.7–0.9	1.7–1.9	0.3–0.5	0.6–0.9	1.7–2.0	0.1–0.5	0.6–1.0	1.6–2.0
Buchholzia appendiculata (Buchholz, 1862)	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	5	-	-
Buchholzia fallax Michaelsen, 1887	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	2	-	-
Enchytraeus buchholzi s.l. Vejdovský, 1879	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1	1	-
Enchytraeus spp.	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
Fridericia isseli Rota, 1994	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-
Fridericia cf. *waldenstroemi* Rota & Healy, 1999	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	2	-
Fridericia spp.	-	2	-	2	-	1	3	-	-	-	-	-	1	2	-	6	4	1
Enchytraeidae, not identified	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-
***Enchytraeidae,* total**	**2**	**2**	**-**	**8**	**1**	**1**	**3**	**-**	**-**	**1**	**-**	**-**	**3**	**4**	**-**	**17**	**7**	**1**

Table 3. Mean enchytraeid abundances per volume and fresh weight of soil (means with their standard errors, based on three equal portions of the corresponding soil sample) taken at three different depths at five sites in the city of Malmö (Sweden).

Site / density unit	**Abundance ± SE**	**Abundance ± SE**	**Abundance ± SE**
Korvetten	**0.2–0.4 m**	**0.7–1.0 m**	**1.7–2.0 m**
(ind./dm^3)	5.33 ± 2.67	5.33 ± 5.33	0
(ind./kg)	4.36 ± 2.18	3.88 ± 3.88	0
Gråsejen	**0.1–0.2 m**	**0.7–1.0 m**	**1.7–1.9 m**
(ind./dm^3)	21.33 ± 17.49	4.36 ± 2.18	4.36 ± 2.18
(ind./kg)	19.33 ± 15.84	2.60 ± 2.60	2.12 ± 2.67
Vendelsgången	**0.2–0.3 m**	**0.7–0.9 m**	**1.6–1.7 m**
(ind./dm^3)	8.00 ± 4.62	0	0
(ind./kg)	7.14 ± 4.12	0	0
Turbinkanalen	**0.2–0.3 m**	**0.7–0.9 m**	**1.7–1.9 m**
(ind./dm^3)	2.67 ± 2.67	0	0
(ind./kg)	2.51 ± 2.51	0	0
Rundelen	**0.3–0.5 m**	**0.6–0.9 m**	**1.7–2.0 m**
(ind./dm^3)	8.00 ± 4.62	10.67 ± 5.33	0
(ind./kg)	5.95 ± 3.44	8.33 ± 4.17	0

account the fact that the soil pH must have been rather high, which usually means lower enchytraeid densities but species-richer assemblages than at sites of strong acidity (Beylich & Graefe 2007), the numbers of individuals obtained from the extracted volume of soil at Gråsejen were low but still in the range of what one might find at comparable sites when extracting the upper 10 cm of soil (Schlaghamerský & Pižl 2009). Elsewhere numbers were low, but taking into account the sampling depths nothing else could have been expected.

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