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A method for efficient extraction of rotifers (Rotifera) from soils

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ABSTRACT

Rotifers are a group of soil animals which are not commonly quantified. The most recent data originated from soil studies focussed on nematodes and various modifications of the Baermann funnel, which is not suitable for rotifers. The present study developed a method for quantitative extraction of rotifers, which gives representative results describing rotifer populations and facilitates subsequent identification to the species level. The LC extractor recovered rotifer numbers that were two orders of magnitude higher than what was extracted from the Baermann funnel. The method involves spreading a soil sample on a plastic sieve and uses a very thin layer of cellulose submerged in distilled water in a Petri dish. The Petri dish is situated on a cooled panel and light from the top by fluorescent lamps. The temperature of the base panel which gave the best extraction results was determined experimentally to be 5 °C. Rotifers were not able to withstand any heating from the top, which drastically reduced extraction results.

Introduction

Soil and litter dwelling rotifer communities represent an important part of terrestrial soil fauna (Pourriot 1979). In order to study them, they must be separated from the soil. At the same time, the animals must remain fully viable for enumeration and species determination. To date, a suitable method, which allows for quantitative analysis of rotifer populations, has not been developed. Although Donner (1965) used the method of direct microscopic analysis of soil dispersed in water drops, such an approach is extremely laborious and not suitable for common analyses (Berthold et al. 1999). Some quantitative data of rotifer populations exist from studies concerning other groups of soil hydrobionts, such as nematodes (Sohlenius 1979, 1982; Anderson et al. 1984; Pouyat et al. 1994; Steiner 1994a, 1994b; Háněl 2000, 2001, 2004; Sohlenius 2004). In these papers, the Baermann (1917) funnel technique or some modification (Overgaard-Nielsen 1949; Hallas and Yeates 1972) was applied to extract animals from soil. Although a wet funnel may be adequate for nematodes or tardigrades, rotifers are able to adhere on the funnel walls and actively swim in the water. If they fall down the funnel they frequently die due to anoxic conditions and are then unidentifiable.

The aim of this study was to develop and test a suitable method for the quantitative extraction of interstitial dwelling rotifers, and for the collection of viable animals for identification.

Material and methods

Method description

LC extractor is the working name of the device – LC being the abbreviation for light-cooling extractor. The principle behind LC extraction is based on rotifer activity whereby the organisms spontaneously move from microspaces in a substrate with unfavourable conditions to nearby areas with greater water contents. If rotifers in question are hydrobionts, extraction is performed in a water environment. The extraction scheme is shown in Fig. 1. A soil sample is submerged in distilled water (Ricci 1984), until it is situated between the bottom of the Petri dish and the water surface. The Petri dish is then placed on a solid panel that is refrigerated to an appropriate temperature (5 °C). The soil sample and water are separated by a very fine cellulose layer, which allows for the passage of animals. The panel, measuring $32 \times 42 \times 5\,cm^3$, is cooled by a compressor situated on the floor below the extractor. The compressor is controlled by a thermostatic device adjusted to the appropriate temperature with a hysteresis of ±2 °C. The samples are illuminated by four fluorescent lamps (Osram Duluxstar®, Lumilux® Warm White, 24 W and 1600 lm in total) suspended from above at 20 cm from the samples. All is enclosed in a plexiglass box (Fig. 2).

Sample processing

Soil samples used for tests originated from the organic layer (decomposed and partially decomposed litter) of soil from an oak-hornbeam mixed temperate forest. The substrate was collected in

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a plastic bag and homogenized. Extractions were done in the lab as soon as possible. Ten grams of the substrate was weighed and placed on a very thin layer of cellulose (used in toilet paper). The sample in the paper was then placed into a plastic ring (3 cm hight, 7.5 cm in diameter) with a nylon sieve (mesh size 500 µm) installed 3 mm above the bottom. Thickness of the substrate did not exceed 5 mm. The plastic ring containing the sample was placed into a polystyrene Petri dish (85 mm in diameter), lined with a steel needle on the bottom and filled with distilled water. The Petri dish was then placed into the extractor for a 24 h exposure. Following this exposure, the plastic sieve was carefully removed from the Petri dish. Total rotifer numbers and morphological groups (such as bdelloids, *Adineta* sp., *Encentrum* sp., etc.) can then be counted directly in the Petri dish under light microscope (magnification 25 times). For detailed identification, live specimens must be transferred with a micropipette onto a slide. Counting and identification must not be done immediately, but over a couple of days after the plastic sieve is removed and the Petri dish is left on the cooled panel with the light switched off.

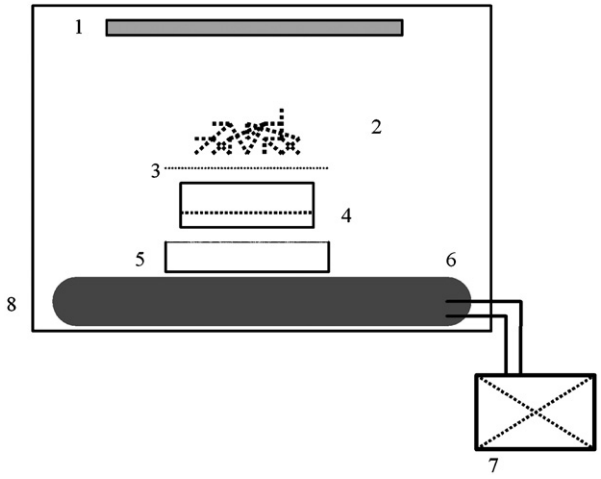

Fig. 1. Scheme of the LC extractor: (1) fluorescent lamp, (2) soil sample, (3) very thin layer of cellulose, (4) plastic ring with nylon mesh situated 3 mm above bottom, mesh size 500 µm, (5) Petri dish filled with distilled water, (6) solid stainless panel cooled by the vaporizer principle, (7) compressor, and (8) plexiglass box.

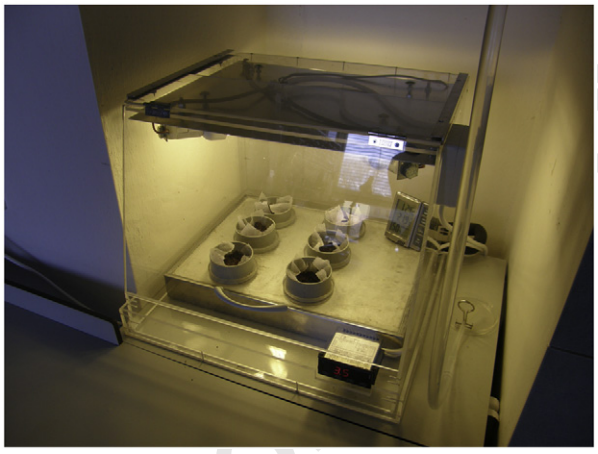

Fig. 2. LC extractor.

Baermann funnel method

Two methods were tested against LC extraction. The Baermann funnel method is well described by Baermann (1917). The funnel was filled with distilled water every time. High-gradient modification was developed by lighting from the top with incandescent (heat producing) bulbs up to air temperature of 25 °C. The lower part of the funnel was cooled to 10 °C. In both cases, substrate was submerged and extracted for 24 h.

Method testing

The temperature to which the base panel of the extractor should be refrigerated was analyzed without using six replicates in each treatment by one-way ANOVA (Dunnett's test). The same statistical method was used in the second test analyzing the particular role of cold- and heat-producing light and cooling (5 °C). A two-way ANOVA with interactions and six replicates was used to determine the effect of fluorescent (cold) light and the cooling of the base panel (5 °C) on extraction efficiency. In the comparison of the three extraction methods, five replicates were used in the non-parametric Kruskal–Wallis test followed by the *a posteriori* Tukey test. Data from direct counts were used and tested for normality in all cases. Non-normally distributed data were analyzed with non-parametric methods.

Results

Efficiency of LC extractor and Baermann funnel

Extraction results given by the LC extractor and both modifications of the Baermann funnel are compared in Table 1. It is clear that results obtained from both modifications of the Baermann funnel differed significantly from the new method presented, recovering less than 2% of the active bdelloid animals obtained from the LC extractor. Monogonont rotifers recovered from the Baermann funnel represent less than 5% of those obtained from the LC extractor and decrease to zero in some genera (*Encentrum*, *Wierzejskiella*) and for the HG modification.

Table 1
Results comparing three extraction methods.

$N = 5$	Mean ± (SD)			Kruskal–Wallis		Tukey test
	Baermann funnel	HG Baermann	LC extractor	*H*	*P*	Sign. diff.
Bdelloids active	1.0 ± 0.4	0.9 ± 0.5	54.8 ± 3.9	9.82	0.007	LC–HG, LC–Baer.
Bdelloids inact.	0.5 ± 0.4	0.3 ± 0.2	2.8 ± 1.3	9.78	0.007	LC–HG
Monogononts	0.3 ± 0.2	0.0	7.8 ± 3.9	12.23	0.002	LC–HG
Tardigrada	5.7 ± 3.3	1.0 ± 0.5	13.0 ± 5.4	10.79	0.005	LC–HG

Non-parametric Kruskal–Wallis and *a posteriori* Tukey test were used.
Turkey test was significant if $P < 0.05$.

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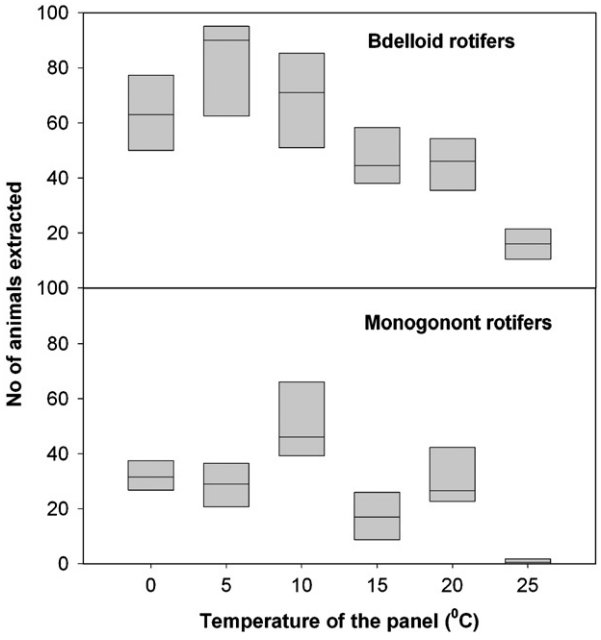

Fig. 3. Extraction results (per 10 g of soil) under different combinations of light and temperature gradients. Filled box are active, open inactive animals. Cooling (5 °C) no–yes, light no-cold–warm, respectively. Stars show the significance of Dunnett's test of all animals extracted versus control.

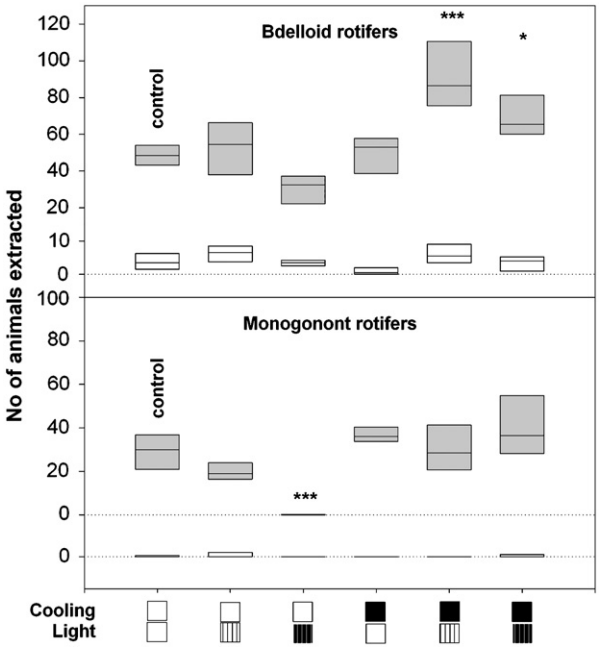

Fig. 4. Numbers of animals (per 10 g of soil) extracted in different temperatures of the base panel. Bars show median and 5–95 percentiles. Fluorescent light, 6 replicates.

Table 2
Results of two-way ANOVAs showing the effect of light and temperature gradients on rotifer extraction.

Extraction effect	Cooling		Light		Interaction	
	F	*P*	*F*	*P*	*F*	*P*
Active animals						
Tardigrades	30.74	0.000	0.19	0.820	9.82	0.000
Bdelloid rotifers	33.60	0.000	9.77	0.000	7.64	0.002
Monogonont rotifers	45.06	0.000	7.27	0.003	13.27	0.000
Inactive animals						
Tardigrades	4.94	0.034	1.50	0.239	2.34	0.114
Bdelloid rotifers	1.48	0.234	9.34	0.000	1.49	0.241
Monogonont rotifers	0.15	0.700	0.15	0.460	1.97	0.157

Fluorescent bulbs and cooling at 5 °C were used.

The LC extractor also gave the best results for tardigrades, with the Baermann funnel extracting 44% of them. The HG modification proved to be totally unsuitable for tardigrade recovery.

Parameters of LC extraction

LC extraction results (Fig. 3) show, that for the control, good data can be generated without use of light and cooling. Fig. 3 indicates an interactive effect of light and cooling on extraction results for two main groups of rotifers. Although bdelloids exhibited the best extraction results (almost twice that of the control) under the simultaneous effect of cooling and lighting by fluorescent bulbs, monogononts were not positively affected. Analysis using a two-way ANOVA (Table 2) showed that both lighting and cooling have a highly significant effect on extraction results in both rotifer groups. Nevertheless, lighting also increased the number of inactive bdelloids, which were too degraded for subsequent detailed species determination. Warm light without cooling totally eliminated monogononts from the recovered populations (Fig. 4). Incandescent bulbs, which produce important amounts of infrared radiation, extremely limit extraction and must not be used. Light used during extraction had no significant effect on tardigrades, but the interaction was significant when light was used simultaneously with cooling.

Temperature of the extraction panel, which gave the best results, was tested experimentally. Fig. 4 shows that the highest numbers of bdelloids were obtained if refrigerated to 5 °C. The results were less evident for monogononts, but clearly the temperature must not exceed 20 °C.

Discussion

Results of the study show that LC extraction is a much more efficient method for extracting the rotifer community from soil. Modifications of the Baermann funnel technique, commonly used for obtaining data about soil rotifers as a by-product of studies focused on nematodes are not suitable for rotifer quantitative analysis. Efficiencies of the Baermann funnel technique and its high-gradient modification, performed at room temperature, were only around 2% of LC extraction. Rotifer abundances in such studies are probably highly underestimated. This is likely due to the ability of rotifers to adhere on surfaces with the adhesive glands on their toes and their active swimming in water. These data contradict the opinion of Hallas and Yeates (1972) that the Baermann funnel is highly efficient for rotifers (and tardigrades).

Use of LC extraction also simplified subsequent analysis, by keeping the animals in good condition for a few days. It also enabled analysis of total numbers or morphological groups on the same Petri dish, which prevents loss during transfer.

Application of any type of light may reduce extraction efficiency if not associated with effective cooling. Application of light and temperature gradients (in HG modification) had no significant positive effect on extraction in the Baermann funnel. In the extraction, cooling by refrigerated panel was found to be a more important factor than light in improving extraction results (Fig. 3).

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First, the new approach for rotifer extraction from soils is a much more efficient method than the methods based on the Baermann funnel. The new approach prolongs the time when animals are suitable for processing and allows gentle handling of animals, as well as saving animals in stages suitable for species determination. The Baermann funnel method is entirely unsuitable for rotifers. Second, a temperature of 5 °C proved to be the best for base panel cooling as it resulted in the highest extraction results for both bdelloids and monogononts. Third, samples can be illuminated but not heated from the top during extraction. Warming strongly degraded the results. Finally, although the Baermann funnel is applicable for tardigrades, the proposed method give better results for them as well.

Q1 Uncited reference

Phillips et al. 1999.

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