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Short communication

A calibration of the efficiency of Winkler eclectors for extracting Collembola at different humidities

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Abstract

It is often desirable to collect Collembola from remote locations where the usual extraction systems based on thermal gradients are simply impossible, e.g. for lack of adequate energy supplies. Winkler funnels are often used in such situations as zero-energy extractors of soil arthropods, but have not been calibrated for the collection of Collembola. Here we report a series of trials comparing densities and species-richness of Collembola in leaf moulds using parallel Tullgren and Winkler extractions. Each litter was tested separately with the Winkler funnel environment at low and at high humidity. Our results showed that Winkler funnels are a poor substitute for Tullgrens, under-recording species by 30% and densities by 78%, suggesting that the basic Winkler funnel method is not suitable for objective quantitative Collembola population studies.

Keywords: Suspended soils, remote fieldwork, field sampling, Tullgren funnels, Winkler funnels

1. Introduction

Biodiversity inventories of soils or epiphyte communities should include Collembola, as these routinely exceed 10,000 animals per square metre in soils and are found in every canopy/epiphyte community, dominating in some (Hopkin 1997, Yanoviak et al 2004). Although the design and concepts have developed little from the original methods of Berlese (1905), Tullgren (1918) and Macfadyen (1953, 1961), the standard tool for extracting Collembola from litter and soil remains some variant of a high-gradient extractor (here called a Tullgren funnel). These have the drawback of needing a continuous source of electrical power. For remote field sites this requirement is critical; for instance, the power consumption of one array of Tullgren funnels was calculated to be greater than the entire solar array running the Imperial College Gashaka field site in Nigeria (D. Weaver pers. Comm.)

A zero-power alternative is the Winkler extractor (variously 'Winkler Eclector' or 'Winkler/Moczarski eclector'), which suspends litter in loose mesh above a collecting vessel and relies on natural dehydration and random movements to dislodge invertebrates from their substrate. Although this technology is a century old (Holdhaus 1910), and is a proven extraction method for Coleoptera (e.g. Besuchet et al. 1987) that has been calibrated for several groups of larger terrestrial invertebrates (Krell et al. 2005), there are no published estimates of the efficiency of this method for Collembola. Here we present preliminary calibrations of the extraction efficiency of Winkler funnels using paired trials against a standard Tullgren extraction. Since natural desiccation is suggested as one factor forcing animals' movement in Winkler funnels, we sought to investigate whether humidity affected them by calibrating the Winkler funnels in both a low- and high-humidity environment. Thus, the present examinations aimed at answering the following questions: (1) to what extent do data on Collembola collected using Winkler extractors agree (in species composition and density) with data from the same substrate using a Tullgren extractor, and (2) is there any evidence that humidity affects the efficiency with which Winkler extractors collect Collembola?

2. Sites and Methods

All sample collection and extraction work was performed in the UK, with both Winkler and Tullgren extractors being located in Whitelands College, Roehampton University. The Winkler extractors were used as supplied by Hildegard Winkler (http://www.entowinkler.at), and run in a controlled environment chamber with 16:8 hours light:dark, constant 20 °C and either standard (ca. 40%) or high (ca. 98%) humidity. The Tullgren funnels were 20 cm diameter funnels with 60 W bulbs.

Leaf litters were gathered fresh from 3 sites (three collections each). Site 1 was in Dorking, Surrey, 51° 12' 57" N, 0° 18' 58" W (TQ1759447696 in the UK grid system), on a domestic garage roof where *Parthenocissus* leaves have accumulated into a suspended soil. Site 2 was oak leaf mulch from Whitelands Garden's compost heap, 51° 26' 52" N, 0° 14' 42" W (TQ2194373603 in the UK grid system). Site 3 is an ancient oak/hornbeam woodland in the weald of Surrey, 51° 8' 7" N, 0° 14' 59" W (TQ2244838845). In all cases approximately 20 litres of the humus-rich substrate was collected by hand tools with minimal disturbance, returned to the laboratory and immediately placed into the Tullgren funnels (aliquots of ca. 15 g) or Winkler extractors (ca. 40 g). Tullgren funnels were extracted (1–4 days) into 70% IMS (Industrial Methylated Spirits, industrial ethanol), Winkler extractors (1–8 days) into an IMS/ethylene glycol mix. The durations used and replication differed between experimental runs and are listed in Table 1 along with other experimental details. All animals were recorded

Tab. 1 The experimental runs; each run involved a fresh collection from the field site.

Run	Date Month-Year	Litter Source	Winkler humidity	Tullgren extraction (days)	Winkler extraction (days)	Replicates analysed
1	10-08	Dorking	L	1, 4	1, 4	4
2	11-08	Dorking	L	1, 4	1, 4	4
3	04-09	Whitelands	L	4	8	4
4	04-09	Whitelands	L	4	8	4
5	11-09	Faygate: litter	L	1, 4	1, 4, 8	3
6	11-09	Faygate:Moss	L	2, 4	2, 4, 8	3
7	06-10	Whitelands	H			4
8	07-10	Faygate: litter	H	2, 4	2, 4, 8	4
9	11-10	Dorking	H	1, 4	4, 8	4

Tab. 2 A comparison of Collembola species richness ('Spp', total number of species) and density estimates for Tullgren (T) and Winkler funnels (W) for each experimental run. Underlined species were dominant in the sample. *Desoria trispinata* is recorded here for the first time in the UK. It as well as the very rare (in the UK) *Sminthurinus trinotatus* appear to be exotic species imported with ornamental plants.

Run	Spp T	Spp W	Density 100g^{-1} T	Density 100g^{-1} W	Species
1	4	3	323	111	*Friesea mirabilis, Orchesella cincta, Parisotoma notabilis, Sminthurinus trinotatus*
2	3	3	231	121	*Dicyrtomina saundersi, Orchesella cincta, Sminthurinus trinotatus, Sphaeridia pumilis.*
3	9	6	193	45.4	*Desoria trispinata, Entomobrya multifasciata, Entomobrya nicoleti, Isotomurus* sp., *Lepidocyrtus cyaneus, Orchesella cincta, Parisotoma notabilis, Pseudosinella alba, Sminthurinus elegans, Tomocerus vulgaris.*
4	11	11	357	99.1	*Desoria trispinata, Entomobrya multifasciata, Entomobrya nicoleti, Isotomurus* sp., *Lepidocyrtus cyaneus, Neanura muscorum, Orchesella cincta, Parisotoma notabilis, Pseudosinella alba, Sminthurinus elegans, Tomocerus vulgaris.*
5	17	6	648	119	*Anurida granaria, Dicyrtomina saundersi, Entomobrya nivalis, Entomobrya albocincta, Folsomia quadrioculata, Friesea mirabilis, Hypogastrura burkilli, Isotoma viridis, Isotomurus palustris, Lepidocyrtus cyaneus, Lepidocyrtus lanuginosus, Lipothrix lubbocki, Neanura muscorum, Orchesella cincta, Orchesella villosa, Parisotoma notabilis, Protaphorura* sp., *Pseudisotoma sensibilis, Pseudosinella alba, Sminthurinus elegans, Tomocerus vulgaris.*
6	17	5	357	43.4	*Anurida granaria, Dicyrtomina saundersi, Entomobrya nivalis, Folsomia quadrioculata, Friesea mirabilis, Hypogastrura burkilli, Isotomurus palustris, Lepidocyrtus cyaneus, Lepidocyrtus lanuginosus, Neanura muscorum, Orchesella cincta, Parisotoma notabilis, Protaphorura* sp., *Pseudisotoma sensibilis, Pseudosinella alba, Sminthurinus elegans, Tomocerus vulgaris.*
7	8	8	602	160	*Desoria trispinata, Entomobrya nicoleti, Isotomurus* sp., *Lepidocyrtus cyaneus, Monobella grassei, Neanura muscorum, Parisotoma notabilis, Pseudosinella alba, Tomocerus vulgaris.*
8	10	9	1890	196	*Arrhopalites* sp., *Ceratophysella denticulata Folsomia quadrioculata, Friesea mirabilis, Lepidocyrtus lanuginosus, Lipothrix lubbocki, Orchesella cincta, Orchesella villosa, Parisotoma notabilis, Protaphorura* sp., *Pseudosinella alba, Tomocerus vulgaris.*
9	8	4	270	187	*Isotomurus* sp., *Lepidocyrtus cyaneus, Monobella grassei, Orchesella villosa, Sminthurinus trinotatus, Sphaeridia pumilis, Tomocerus vulgaris.*

(to order) and counted; Collembola were identified to species using Hopkin (2007) and Fjellberg (1998, 2007). After extraction was complete, the litter was dried at 105 °C; all densities are animals per 100 g.

To estimate density and richness from the time series for each collection of litter for each extraction method, we used the mean of all replicates at the greatest number of days (4 for Tullgren, 8 days for Winkler) unless this was lower than the value for the second-greatest number of days (2 or 4), when the combined mean over both days was used. The efficiency was estimated by regressing density on Collembola in a litter estimated from the Winkler extractors against density estimated from the Tullgren funnels, finding the best zero-intercept line. In this regression one data point is one litter type from one collection. Comparisons across all sample dates were performed using the non-parametric Mann-Whitney U test.

3. Results

The litter types from the three different sites held distinct Collembola communities (Tab. 2). Although the design controlled for variable extraction times, these made surprisingly little difference; only for Winkler-estimated density for runs 1 and 9 (hence 2 tests out of 28) was there a significant increase with numbers over time. The Tullgren- and Winkler-derived richness and density showed a consistent trend for Winklers to underestimate Collembola (Tab. 2). Pooling data over the whole trial, species richness and density were both significantly lower when estimated by the Winkler funnels ($U = 5.3$ and 6.5 respectively, both $p < 0.001$). The (zero-intercept) regression equations predicting Tullgren-derived values from Winkler-funnel estimates give multiples of *1.4 (species richness) and *4.6 (density per unit mass), but with wide scatter around the regression lines. Neither species richness nor density gave any indication that humidity reduced efficiency; density estimates at high humidity were in fact slightly (but non-significantly) higher than at low humidity. Table 3 lists some species-level efficiencies, showing considerable variation but a tendency for the larger epiedaphic species (*Tomocerus, Orchesella*) to be extracted more efficiently than others.

Tab. 3 Species-level estimates of Winkler funnel efficiency (compared to Tullgren funnelsw) for selected Collembola. Values close to 1 show high efficiency (1 = Tullgren-estimated density).

Species	**Efficiency**
Ceratophysella spp	0.68
Folsomia quadrioculata	0.13
Isotomurus spp	0.30
Lepidocyrtus lanuginosus	0.09
Neanura muscorum	0.12
Orchesella cincta	0.38
Protaphorura spp	0.08
Tomocerus vulgaris	0.53

4. Discussion

There is unlikely ever to be a perfect method for estimating field densities of soil arthropods, and the Tullgren-derived estimate we use as baseline is itself only an estimate that will under-record many small animals (Southwood & Henderson 2000). The distribution of Collembola is notoriously clumped, apparently due to aggregation pheromones (Usher 1969, Joose et al. 1977, Shaw & Usher 1996), so that estimates of means have large confidence intervals. The two replicate collections of the same compost pile in April 2008 (runs 3 and 4) gave rather different estimates of richness and density, despite having near-identical species composition (Tab. 3). Given these limitations, it remains hard to avoid the conclusion that Winkler bags are greatly inferior to Tullgren funnels for sampling Collembola. Not only do they underestimate densities and species richness, but their longer extraction times and consequent use of ethylene glycol leads to loss of specimen quality compared to rapid extraction into IMS. In our tests, the fact that Winkler-derived densities and species richness were underrepresented by a factor of 4.2 and 1.5, respectively (compared to Tullgren funnel results) suggest that this method will never be a satisfactory tool for quantitative studies of Collembola populations. For remote sites researchers should continue to collect by pitfall trapping and hand sorting, although solvent flotation methods may be worth considering (McSorley & Walter 1991, Ducarme et al. 1998, Querner & Bruckner 2010). It is undoubtedly best to use a combination of different methods to compile a picture of Collembola community composition for a habitat. For instance, Querner & Bruckner (2010) used both pitfall traps and soil cores to build up landscape-scale information on Collembola in Austria, finding that each method averaged 13 species per site, but in combination yielded an average of 25 species per site.

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