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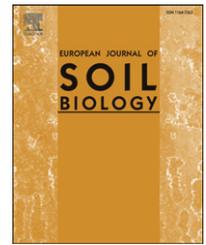


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Original article

Evaluating the efficiency of sampling methods in assessing soil macrofauna communities in arable systems

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ABSTRACT

The soil fauna is often a neglected group in many large-scale studies of farmland biodiversity due to difficulties in extracting organisms efficiently from the soil. This study assesses the relative efficiency of the simple and cheap sampling method of handsorting against Berlese–Tullgren funnel and Winkler apparatus extraction. Soil cores were taken from grassy arable field margins and wheat fields in Cambridgeshire, UK, and the efficiencies of the three methods in assessing the abundances and species densities of soil macroinvertebrates were compared. Handsorting in most cases was as efficient at extracting the majority of the soil macrofauna as the Berlese–Tullgren funnel and Winkler bag methods, although it underestimated the species densities of the woodlice and adult beetles. There were no obvious biases among the three methods for the particular vegetation types sampled and no significant differences in the size distributions of the earthworms and beetles. Proportionally fewer damaged earthworms were recorded in larger (25 × 25 cm) soil cores when compared with smaller ones (15 × 15 cm). Handsorting has many benefits, including targeted extraction, minimum disturbance to the habitat and shorter sampling periods and may be the most appropriate method for studies of farmland biodiversity when a high number of soil cores need to be sampled.

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1. Introduction

Over the past 20 years the importance of the biodiversity of the soil macrofauna for many ecosystem services has received increasing recognition, particularly within agronomy [5,10,14,25]. Despite this, accurate assessment of populations of soil fauna remains a challenge due to difficulties in extracting organisms efficiently from the soil matrix [1,10]. Consequently, the soil fauna remains a rather neglected group in many large-scale studies of farmland biodiversity (e.g.

[13,19]). This study aims to assess the relative efficiency of the simple and cheap sampling method of handsorting for evaluating soil macrofauna communities in farmland.

Extraction methods can be described as physical ('direct' *sensu* Andre et al., 2002) whereby invertebrates are separated from the soil using wet (e.g. washing) or dry methods (e.g. handsorting), or dynamic ('indirect' *sensu* Andre et al., 2002) which relies on behavioural response of invertebrates to certain stimuli (e.g. Berlese–Tullgren funnels, baited traps) [11]. Many methods are specific for certain target taxa or groups,

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for example, chemical or electrical extraction for earthworms [16,22,23] and surface or sub-surface pitfall traps for active species [18] and therefore are of limited use for studies examining whole assemblage dynamics and interactions. This is especially important in soil habitats where distributions tend to be aggregated in response to local patchiness of the soil environment [2,12]. These methods also fail to provide quantitative data, as the area covered by the chemical extraction or pitfall trap is unknown, and their efficiency depends on a number of factors including soil conditions, vegetation cover and behavioural responses of individual species, which may lead to biased estimates of community composition [4,23,26].

Handsorting of soil cores is a simple and inexpensive physical method, but it can be laborious and labour-intensive. However, Schmidt [24] found that limiting sorting of a 25 × 25 cm soil core to a 40 min period recovered up to 87% of earthworm numbers and 97% earthworm biomass in just 36% of the time needed for full sorting. One potential source of error is underestimation of smaller or cryptic individuals [22,24]. Therefore, this study tests the relative efficiency of time-limited handsorting for extracting soil macrofauna in comparison with the dynamic methods of Berlese–Tullgren funnel and Winkler bag extraction.

The Berlese–Tullgren funnel method uses a heat-producing light source suspended above the soil core to provide a temperature, moisture and light gradient to drive the soil fauna out of the soil and into a collection pot, and is most commonly used in studies of soil and litter microarthropods [9]. The Winkler extractor encloses mesh bags filled with the sample inside an outer cloth sack [21]. As the sample dries out, invertebrates leave the mesh bags and fall into a collecting pot at the base of the cloth sack [20]. The Winkler extractor is often used in studies of leaf litter communities and has the advantage over the Berlese–Tullgren funnel of not requiring an electricity source.

The size of the soil core sampled represents a trade-off between taking a greater number of smaller samples to account for within-habitat variation, or taking fewer large samples to reduce damage to specimens (especially earthworms) and the number of zero counts in the data. A soil core measuring 15 × 15 cm and 10 cm deep has a cut surface area to volume ratio of 0.37, compared with 0.26 for a 25 × 25 cm by 10 cm deep core.

This study compares the efficiencies of the three methods in assessing the abundances and species densities of soil macroinvertebrates in semi-natural and cultivated soils in arable farmland. In addition, body size distributions of the Lumbricidae, and Coleoptera are compared across methods. Biases caused by the interaction of sampling method and vegetation type are also investigated. In order to identify the effect of soil core size on specimen damage, the proportions of damaged earthworms in handsorted cores measuring 15 × 15 cm and 25 × 25 cm (both 10 cm deep) are compared.

2. Materials and methods

2.1. Study site

This study was carried out in October 2004 on a calcareous clay/clay loam field, at ADAS Boxworth, Cambridgeshire, UK

(52°15'10"N, 0°01'54"W, 50 m a.s.l., mean annual rainfall 553 mm). Sown field margins had been established in 2001, using three seed mixes (grass only mix, tussock grass and forbs mix, fine grass and forbs mix, see Clarke et al. [6] for more details). Sampling took place in three plots (25 × 5 m with a 5 m buffer between adjacent plots) each sown with one of the seed mixes and subjected to a cut in the spring. Plots were a maximum of 125 m apart. Soil cores were also taken from the crop (winter wheat), at a distance of 50 m from the margin.

2.2. Experimental design

Nine soil cores measuring 15 × 15 cm and 10 cm deep were taken from the four vegetation types for each method (i.e. nine cores × three methods = 27 cores in each vegetation type). In the margins, these were arranged with a first row of three soil cores per method running parallel to and 1.5 m from an adjacent hedge, a second row at 3.0 m and the third row at 4.5 m from the hedge.

The sampling methods tested were handsorting, Berlese–Tullgren funnels and Winkler bags. Handsorted soil cores were examined for 20 min and all invertebrates encountered were extracted and preserved in 80% alcohol. For the Berlese–Tullgren funnels, each soil core was divided between four funnels, and a 25-W bulb was suspended 10 cm above the soil surface. For the Winkler bags, each soil core was divided into three mesh bags, which were then hung within a cloth bag. Samples were left for 7 days and invertebrates were extracted into 80% alcohol in both the Berlese–Tullgren funnels and Winkler bags.

The soil invertebrates extracted were sorted to class or order and abundance per soil core recorded. The Lumbricidae, Chilopoda, Diplopoda, Isopoda, Carabidae and Staphylinidae were identified to species as these groups represented the most abundant taxa. Larvae of Coleoptera, Lepidoptera and Diptera were also found in large numbers but were not identified to species due to taxonomic intractability. Abundance and species density data were summed for each sample of three replicate cores taken at the same distance from the boundary hedge.

To identify potential biases in the size of invertebrates extracted by the three methods, size distributions of the Lumbricidae, and Coleoptera were recorded. All alcohol-preserved specimens of the Lumbricidae were weighed individually and divided into four size categories (<0.10, 0.10–0.29, 0.30–0.49, >0.49 g). The body length of all Coleoptera individuals was measured to the nearest 0.1 mm and divided into three categories (<3.0, 3.1–6.0, >6.0 mm).

To compare the effect of soil core size on the proportion of damaged earthworms we recorded the numbers of damaged earthworms in the handsorted 15 × 15 cm cores. This was then compared with the proportion of damaged earthworms in handsorted soil cores measuring 25 × 25 cm by 10 cm deep taken from the same plots in October 2005.

2.3. Data analysis

To compare relative efficiencies of the three methods for extracting the soil macrofauna and to check for potential

biases in different vegetation types, an analysis of variance was carried out in MINITAB 14.1. The model analysed the effects of method, vegetation type and their interaction on the abundance and species density of the Lumbricidae, Chilopoda, Diplopoda, Isopoda and Coleoptera, and the abundance of larvae (Diptera, Coleoptera and Lepidoptera combined). Abundance data was log-transformed ($L_{10}(x + 1)$) and when significant differences were observed, post hoc comparisons were carried out using Tukey's HSD test. Comparisons of earthworm and beetle abundance (log-transformed, $L_{10}(x + 1)$) within the different size categories were made using an ANOVA with sampling method as the explanatory variable. An analysis of variance was also carried out on the proportion of damaged earthworms (arcsine transformed) per soil core, with core size (15 × 15 cm or 25 × 25 cm) as the explanatory variable.

To compare patterns of species accumulation, sample-based rarefaction curves for each of the three methods were constructed using EstimateS 7.5 [8] (50 randomisations, samples randomised without replacement). As the aim was to compare estimates of species density rather than species richness, the number of species was plotted as a function of the accumulated number of samples, not individuals [15].

3. Results

A total of 927 individuals and 46 species were extracted, of which the earthworms were the most abundant (51% of total) and the beetles were the most speciose (23 species). There were no significant differences between the three methods in the estimates of Lumbricid, Isopod, Chilopod, Diplopod and Coleoptera abundances, but handsorting and the Winkler bags were less efficient than the Berlese-Tullgren funnels at extracting Diptera, Lepidoptera and Coleoptera larvae (Table 1). Handsorting also appeared to underestimate the species densities of the Isopoda and the Coleoptera (Table 1). However, when the Bonferroni adjustment of significance levels was applied to account for the increased probability of

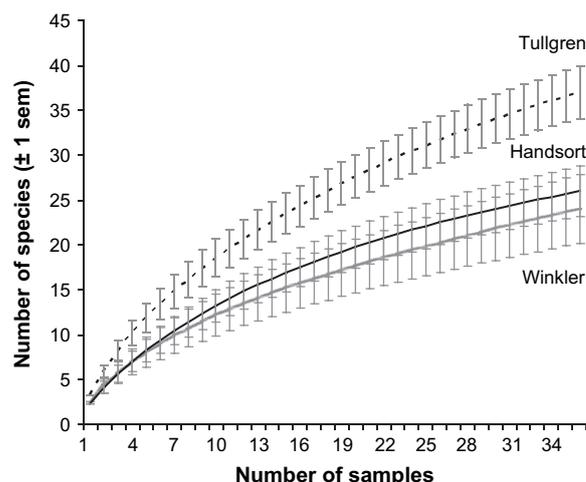


Fig. 1 – Species accumulation curves for the three sampling methods (±1SEM).

Type I error due to multiple testing, these differences became non-significant. The species accumulation curves show that the Berlese-Tullgren method accumulated species faster, and recovered more species in total, than the handsorting and Winkler method (Fig. 1).

While abundance and species richness of the macrofauna responded significantly to the vegetation type (Table 1), there was no significant interaction with sampling method, indicating that there were no obvious biases in the methods for any of these habitats. There also were no significant difference between the sampling methods in the abundances of earthworms and beetles in the different size classes (Lumbricidae biomass <0.1 g, $F_{2,33} = 0.60$; 0.1–0.2 g, $F_{2,33} = 0.86$; 0.3–0.4 g, $F_{2,33} = 1.67$; >0.5 g, $F_{2,33} = 0.26$; all $P > 0.05$. Coleoptera length <3.0 mm, $F_{2,33} = 0.86$; 3.1–6.0 mm, $F_{2,33} = 2.42$; >6.1 mm, $F_{2,33} = 0.71$; all $P > 0.05$). This suggests that handsorting is as efficient at recovering small individuals as the two dynamic sampling methods. Significantly smaller proportions of

Table 1 – The effect of sampling method and vegetation type on the abundance and species densities of soil macrofauna

	Mean (±SEM)			Method $F_{2,24}$	Veg type $F_{3,24}$
	Handsort	Tullgren	Winkler		
Abundance					
Lumbricidae	15.00 (2.55)	12.92 (1.90)	11.83(1.70)	0.26 ^{NS}	3.53*
Isopoda	1.42 (0.92)	2.33 (0.54)	2.33(0.68)	2.97 ^{NS}	6.75**
Diplopoda	2.92 (1.36)	3.08 (1.00)	1.42(0.48)	2.09 ^{NS}	14.54***
Chilopoda	0.42 (0.19)	1.33 (0.41)	0.75(0.22)	3.25 ^{NS}	7.20***
Coleoptera	2.00 (0.64)	3.17 (0.51)	1.58(0.51)	2.85 ^{NS}	2.36**
Larvae	0.50 (0.29)	11.42 (3.10)	2.83(0.46)	21.2***	3.64*
Species density					
Lumbricidae	1.83 (0.27)	2.00 (0.28)	2.08 (0.29)	0.23 ^{NS}	2.70 ^{NS}
Isopoda	0.50 (0.23)	1.42 (0.34)	1.17 (0.24)	4.41*	5.50**
Diplopoda	0.83 (0.24)	0.92 (0.29)	0.50 (0.19)	1.11 ^{NS}	5.53**
Chilopoda	0.42 (0.15)	0.92 (0.29)	0.17 (0.11)	2.60 ^{NS}	6.40**
Coleoptera	1.25 (0.46)	2.67 (0.38)	1.08 (0.36)	4.81*	1.69 ^{NS}

***P < 0.001; **P < 0.01; *P < 0.05; NS, not significant.

earthworms were damaged in the 25 × 25 cm soil cores compared to the 15 × 15 cm cores (mean ± SEM = 0.11 ± 0.0 and 10.26 ± 0.04, respectively; $F_{1,22} = 8.07$; $P = 0.01$).

4. Discussion

4.1. Relative efficiencies and potential biases

Handsorting in most cases was as efficient at extracting the majority of the soil macrofauna as the Berlese–Tullgren funnel and Winkler bag methods. However, the abundance of Coleoptera, Lepidoptera and Diptera larvae was seriously underestimated by both the handsorting and Winkler bag methods. This may be because many of the larvae are small, or cryptically coloured, and therefore difficult to detect visually, but this does not account for the low abundance in the Winkler bags. Flies were observed being attracted to the warmth of the Berlese–Tullgren funnels and it seems likely that the higher number of diptera larvae in the Berlese–Tullgren samples (mean 10.5 compared to 1.9 in Winkler samples and 0.0 in handsorted samples) was due to eggs being laid by these flies, with a shortened incubation time under the lamps allowing the larvae to emerge within the week of extraction. We therefore recommend that the funnels are covered over during extraction or set up in a sterile room, to prevent contamination. The warmth from the lamps would also have accelerated the development of eggs and larvae already present in the samples, therefore increasing detectability and providing a biased representation of the population.

Handsorting also underestimated the species densities of the Isopoda and adult Coleoptera, although this difference was non-significant when a Bonferroni adjustment was made. The woodlice species *Haplophthalmus menzei* and *Trichoniscoides albidus* were found only in the Berlese–Tullgren and Winkler samples. These species do not have the rapid darting movement of most woodlice, but move slowly [17], therefore making detection by eye more difficult. The opposite may be true for the Coleoptera, many species of which move rapidly or can fly, so while they may be easier to detect, capture by hand is difficult. The species accumulation curves reflect these biases, with the Berlese–Tullgren method extracting more species in fewer samples than the other two methods (Fig. 1).

Comparison of different size classes of the earthworms and beetles showed that handsorting did not underestimate the abundance of the smallest individuals. However, in this study, there were only a few beetles smaller than 2 mm, and there is a possibility that handsorting may underestimate densities of the smallest beetles found in other habitats, such as the Ptiliidae which may be as small as 0.4 mm and are abundant in woodland.

There were no obvious biases among the three methods for the particular vegetation types sampled. However, if more diverse habitats were to be sampled, such as woodland and grassland, it is recommended to test again for biases. Similarly, this study was carried out on a single soil type (calcareous clay/clay loam), and if sampling was to take place in a number of different soil types, it would be necessary either to check for biases, or calibrate the chosen method in the

different soil types by introducing a known number of invertebrates into a defaunated core and comparing recovery rates [1]. Additionally, efficiency may be affected by seasonal and climatic variation.

4.2. Other considerations

The main disadvantage of the handsorting method, as with many sampling methods, is that it is labour-intensive and can be tedious, especially for a single fieldworker. Handsorting is particularly prone to variations in individual skill or experience, and if more than one person is involved, then there is a risk that sampling efficiencies will vary between individuals, thus introducing an additional bias. However, a study comparing the efficiencies of a ‘counting crew’ of 13 trained fieldworkers carrying out direct counts of soil microfauna found no significant differences between individuals [3].

Weather (hot, cold or wet) can also affect on-site handsorting, although this can be avoided by taking samples under cover. Although difficult to quantify, a benefit of handsorting is that the fieldworker may gain a better understanding of the environmental factors influencing soil assemblages through direct observations of correlations between, for example, changes in soil texture or moisture and invertebrate abundances. Such observations may inform future data collection or help develop new hypotheses.

An advantage of handsorting is that, unlike the other methods, extraction can be targeted at only those invertebrates required, and so reduces unnecessary mortality of unwanted invertebrates. This may be particularly important in habitats with a high number of rare species. The resulting pots containing the samples from the Berlese–Tullgren funnel and Winkler bag methods tend to have high numbers of mesofauna, and can be rather muddy, therefore requiring longer sorting time at the next stage compared with handsorted samples.

Logistically, the Berlese–Tullgren method is the most demanding, as it requires a lot of space under cover and an electricity supply. In this study, each 15 × 15 cm soil core filled four funnels, which limited the number of soil cores processed in a week by the number of funnels available. In this case, only 12 soil cores could be processed each week, compared to the 15 cores handsorted per day. If a large number of cores were to be sampled, which is often the situation in factorial experiments, the Berlese–Tullgren funnel sampling would cover several weeks, which could result in temporal variation in invertebrate abundances. In addition to incubating diptera eggs, we observed that the high temperatures from the lamps resulted in the mortality of several small earthworms on the sides of the funnels before they reached the sample pots. This highlights the difficulty in balancing the temperature to create enough of a gradient to force movement of arthropods but to avoid scorching soft-bodied invertebrates such as earthworms.

Samples were left in the Winkler bags for a week, as recommended by Krell et al. [20]. However, this recommendation was for sampling leaf litter, and we found that one week was insufficient for the soil to dry out. It is likely that invertebrates moved into the core of each net bag where the soil remained moist rather than outwards into the pot. Longer

Table 2 – Summary for cross methods comparison

Method	Time	Skills needed	Equipment requirements	Advantages	Disadvantages
Hand-sorting	15 samples/day	Good observational skills	Low: spade; tray; forceps	Targeted extraction minimises bycatch; minimal long-term disturbance; shorter sampling periods; cleaner samples	May underestimate cryptic or fast-moving species; monotonous and laborious; variation in sampling efficiency between field workers
Tullgren funnels	12 samples/week; limited by number of funnels	None	High: electricity; funnels; under cover	Good for cryptic and fast-moving species; good for larvae?	Contamination by diptera if left uncovered; Earthworms susceptible to desiccation
Winkler bags	20 samples/week limited by number of bags	None	Medium: Winkler bags; under cover	Good for leaf litter or soils with high levels of organic matter	Sandy soils likely to fall through mesh invertebrate extraction incomplete if soil stays moist

extraction times are necessary but this risks the inclusion in the data of a second generation of some invertebrates [20]. Additionally, while the mesh bags held the clay soil in this study, sandy soil is more likely to fall through. A finer mesh could be used, but this may restrict extraction of the larger invertebrates.

For both the Berlese–Tullgren funnel and Winkler bag methods, the soil cores must be removed from the field, which is time-consuming and laborious. Also, it is unlikely that the cores will be returned to their original location following extraction, which may have implications if the study site is used by other researchers. The handsorting method can be carried out in the field, and the core returned directly back into its hole following extraction, therefore minimising long-term disturbance.

4.3. Soil core size

Disadvantages common to all methods reflect the choice of soil core size. By sampling only to a depth of 10 cm, anecic earthworms, which retreat into burrows up to 1 m below the surface during the day, will be underestimated. This is one reason why chemical and electrical extraction methods are often used for sampling earthworms, but as mentioned earlier, these methods do not give accurate quantitative data, as extraction efficiencies and area sampled vary with soil type, moisture and vegetation [23]. We found that the use of soil cores measuring 15 × 15 cm resulted in a higher proportion of damaged earthworms, compared to 25 × 25 cm cores, the minimum size most earthworm researchers use [7]. The trade-off with increasing the core size is the longer time needed for processing (handsorting) or more equipment needed (Berlese–Tullgren funnels/Winkler bags) unless the number of cores taken is reduced, in which case there would be a less powerful estimate of spatial variation within a habitat/treatment.

5. Conclusion

Table 2 provides a summary to allow comparison of the three methods. In conclusion, extracting invertebrates by handsorting is as efficient as dynamic extraction for the majority of the

soil macrofauna, although woodlouse and beetle species densities may be slightly underestimated. Although it may be monotonous for the fieldworker, handsorting has many benefits, including targeted extraction, minimum disturbance to the habitat and shorter sampling periods. The use of the Winkler bag method seems to be more suited to assessment of leaf litter invertebrates than soil populations, although it may work well for soil with a high proportion of organic matter or for extracting invertebrates from root mats. Berlese–Tullgren funnel extraction is the most efficient in terms of recovering the highest abundances and species densities of the macrofauna, but is logistically demanding. Therefore, this method may be the first choice for studies requiring only low numbers of soil cores or small core sizes. However, if an experiment demands a high number of soil cores to be sampled, handsorting may be the most appropriate method.

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