The efficiency of soil hand-sorting in assessing the abundance and biomass of earthworm communities. Its usefulness in population dynamics and cohort analysis studies

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XIVth International Colloquium on Soil Zoology and Ecology

Session 2 – Soil biodiversity / Poster contribution

Abstract

Pit digging and manually revising soil blocks is a frequently used method used for field studies of earthworm communities. The aim of this study was to compare the efficiency of hand-sorting (HS) to extract small earthworms, ca. 0.2 g, and the usefulness in studies of population dynamics and cohort analysis. Many earthworms are not recovered when revising manually the soil. Factors include soil characteristics, i.e., moisture, texture, etc., and also a human factor, which is more relevant if the study is conducted in the long-term. We used data collected in a field study of earthworm communities during two years in the savannas of Colombia. Small soil blocks (20x20x20 cm) were dug out in order to collect the smallest earthworms by washing-sieving (WS) and compare the results with the standard HS of large monoliths (100x100 x 50 cm). In fact, this methodology has rarely been addressed in earthworm population field studies. Our results showed that HS efficiency varied owing to the species and ranged from 31.4% up to 100% in the savanna and from 44% to 80% in the pasture, for two small species, i.e *Aymara* n. sp. (epigeic) and *Ocnerodrilidae* sp (endogeic). In the case of the *Glossodrilus* n. sp. (endogeic) these values were similar, i.e. 51.7% and 58.1%, in the savanna and pasture, respectively. We also used frequency tables to calculate the average efficiency of hand-sorting 1m² soil cores for each weight class in each species in order to obtain a population density correction factor. This allowed us to make corrections in earthworm density in the histograms for population dynamics analysis. We conclude that this method should be the *modus operandi* in long-term earthworm demography studies.

**Keywords: Earthworms / Soil fauna / Methods / Population dynamics /
Demography**

1. Introduction

Methods for collecting earthworms are diverse, even ingenious sometimes, and have been described by many authors during the last century [15, 1, 18, 3, 13, 20]. Physical methods of extraction include hand-sorting (HS) and washing-sieving (WS). Hand-sorting yields satisfactory results for earthworms of more than 0.2 g live weight [16, 14], however, it has the disadvantage of low extraction efficiency for smaller worms [14]. Therefore, WS was developed to avoid and/or reduce such errors during HS earthworm extraction [1].

Since all methods tend to underestimate the actual earthworm population and we were interested in studying the demography of each species with life tables, HS was combined with WS of soil cores following the extraction method used by [12]. These are the most accurate techniques for extracting earthworms from soil cores [5]. The efficiency of HS against other extraction techniques has been addressed by many authors [15, 16, 14, 17, 4]. However, comparison of extraction methods according to earthworm weight has only been addressed in few studies [12, 21], although these have never been published. Therefore, our main objective was to compare the efficiency of both physical extraction methods and correct earthworm density and biomass accordingly, based on size class structure (age of population). It is a necessary step when complete studies on population dynamics and demography are pursued.

2. Materials and methods

2.1. Study site

The study was conducted at the “Centro Nacional de Investigaciones” CNI Carimagua (CIAT-CORPOICA) (4° 30'N, 71° 19'W and 150 m a.s.l.), on the Eastern Plains of Colombia. Rainfall averages 2,280 mm annually and occurs mainly from late March to mid-December. Mean annual air temperature is 26 °C. Soils are well-drained silty clay Oxisols (USDA) (Tropectic Haplustox, isohyperthermic), and are characterised by low pH (4.5, in H₂O), and a high Al saturation (>90%).

2.2. Earthworm sampling

Earthworms were collected during 17 months (from April 1994 to September 1995) in: i) a native savanna where *Andropogon bicornis*, *Gymnopogon* sp., *Panicum* spp., *Trachypogon* spp. and *Imperata* sp. were the most abundant plant species, and ii) a 2-ha, 17-year-old grazed pasture (*Brachiaria decumbens* + *Pueraria phaseoloides*). Two physical extraction methods were applied: field HS and WS in the lab. In each system earthworm sampling was performed by monthly digging five 100x100x50 cm³ monoliths and ten 20x20x20 cm³ soil cores, since small individuals may be overlooked. The small soil cores were dug 1 m away at both sides of the large monolith. Wet sieving consisted of brushing soil, with water, through a series of sieves of diminishing mesh size, i.e. 2.0, 1.0 and 0.5 mm. For further details of sampling design and procedure see [11].

Earthworms and cocoons were carried to the lab where they were separated into species. The main characteristics of the earthworm community (8 species) and their ecology can be consulted in [11, 10, 9] and [7]. Total body length (mm), maximum preclitellar diameter (mm) and weight (g) were measured. For the purpose of this

study we only used density and biomass data. Biomass values are given on 4% formaline solution, 16% lower than fresh weight on average [8].

Average efficiency of HS (expressed as percentage) was calculated for every sampling month by dividing the number of earthworms recovered with the HS method and those collected with WS technique. When the number of earthworms collected during HS was higher than those recovered with the WS method, we accepted as valid those numbers and hence the value of HS efficiency was 100%.

2.3. Density correction as a function of earthworm size

In total, 8 earthworm species are found in the savannas of Carimagua [11]. Hand sorting is not suitable for recovering earthworms from soil cores when these are smaller than 200 mg [14], so only three species were used in this study: *Glossodrilus* sp. (endogeic), *Aymara* sp. (epigeic) and *Ocnerodrilidae* sp. (endogeic), with adult mean weights of 90 g, 60 g, and 6 mg, respectively [11].

A correction factor was applied for each size class by using the formula:

$$N = a_w / a_h$$

where a_w is the density per m^2 of the size class a recovered in the WS of all 20x20x20 cm^3 soil samples, and a_h is the density of the same size class collected by HS of 1 m^2 soil monoliths.

2.4. Statistical analysis

Regression analysis was performed between the earthworm weight and efficiency of HS. The Pearson r coefficient indicates the fit of the data to the curve so significance of efficiency in regard to earthworm weight was tested with one-way

ANOVA. Both statistical analyses were performed with SigmaStat, and the graphs were plotted with SigmaPlot.

3. Results

In total 10,941 earthworms were counted and weighed and included in the analysis: *Aymara* sp. (340 and 158 individuals [ind.]-why the brackets here and not below? from the savanna and pasture, respectively), *Glossodrilus* sp. (3,043 and 3,832 ind. from the savanna and pasture, respectively), and Ocnerodrilidae sp. (1,456 and 2,112 ind. from the savanna and pasture, respectively).

3.1. Seasonal global efficiency of 1m² soil cores

Average HS efficiency varied owing to the species considered (Table I) and ranged from 30% in the case of the smallest species, i.e. Ocnerodrilidae n. sp. to 100% for *Aymara* n. sp. in the savanna and from 44%, to 80% in the pasture, again for the same two species, respectively. The HS efficiency for *Glossodrilus* n. sp. was similar in both systems and it ranged from 52% to 58%, in the savanna and pasture, respectively. The seasonality of global HS efficiency of 1m² soil monoliths is illustrated in Figure 1. The lowest values appeared during the dry season in both systems, and ranged from 10 to 30% of the amount of earthworms presumably present in the sample. The seasonality of global efficiency for Ocnerodrilidae is indicated in Figure 2. High fluctuations in efficiency values were obtained for this species, and no earthworms were recovered during the dry season.

Regarding the species *Aymara*, n. sp. WS of soil monoliths was not very efficient since 80-100% of individuals were collected in the 1m² soil samples. In the savanna,

where cattle depositions were also present the efficiency of the hand sorting of 1m² monoliths was constant across all the sampling periods.

3.2. Density correction related to earthworm size

Here, the age of the population, that is, the weight or size class structure is taken into account. The average efficiency of HS of 1m² soil cores was calculated for each weight class by using frequency tables (Tables II and III). Nonetheless, some indications must be highlighted at this point. For the endogeic *Glossodrilus* sp. regarding the HS of 1m² soil samples in the savanna, the percentage of individuals weighing more than 0.12 g was only 4% of the total number of individuals collected by that method, whereas in the pasture it was 6.3% of the total. During WS technique the percentage of individuals weighing more than 0.12 g was 10.6%. Therefore, those data associated to individuals larger than 0.12 g were not considered to assess the relationship between HS efficiency and data from WS technique. Therefore, it seems that some inconsistencies in efficiency owing to earthworm weight occurred. The higher the earthworm weight was the lesser the efficiency, and the opposite phenomenon should occur. Data were plotted and fitted to a non-linear type curve. The efficiency of HS method according to earthworm weight for the three species (average of both systems studied) is shown in Figure 3. Correlations were also significant (ANOVA, P<0.01) when each system was independently analyzed.

In *Aymara* sp. the percentage of individuals weighing more than 0.07 g was only 3.5% of the total regardless of the method. Consequently, and as we did for *Glossodrilus* sp. the relationship between the efficiency of HS against WS was only

based on those individuals with a weight less than 0.07 g. (Figure 3; ANOVA, $P < 0.01$).

For the smallest earthworm, *Ocnerodrilidae* sp., the percentage of individuals weighing more than 9 mg was, on average, 1.3% of the total, hence the same procedure was applied for this species (Figure 3; ANOVA, $P = 0.05$).

From the density of each weight class obtained with the two extraction methods a correction factor was then calculated for the three species (Tables II and III). Data from the hand sorting refer to the first 20 cm depth, so that comparisons with data from washing sieving extraction were valid. Once the correction factor was calculated both density and biomass of each size class were corrected and used in the histograms for population dynamics analysis during all the study periods (Figure 4; Jiménez, unpubl.).

4. Discussion

The performance of direct HS of soil monoliths during a rather continuous period resulted in high fluctuations of efficiency. Hand-sorting is widely assumed to be subjected to error and inefficiency, which are likely to vary between season and taxonomic group and with body size and soil type [6]. For example, the seasonal global efficiency for *Ocnerodrilidae* was highly irregular and no clear pattern was observed, except for the dry season. The soil was very hard to dig and therefore earthworms were barely collected during this period. The efficiency of HS may also be dependant on the number of Colepteran nests in the soil. A high number of individuals were found around these rich-organic rounded structures, what means

there is a high spatial autocorrelation in this species [9]. In this case WS of soil samples allowed us the recovery of a high number of individuals.

On the other hand, *Aymara n. sp.* is a small native epigeic species that is normally found inside the cattle depositions in the grazed natural and non-natural pastures. Its movements are fast, a self-defensive strategy typical of species living in high risk environments, so it was easily observed during sampling.

Root density is another factor that may result in low efficiency values for some species. In fact, roots were very abundant in the introduced grass-legume pasture. On the other hand, there is also a human factor of almost equal or greater importance than the physico-chemical properties of soil. This includes the training and encouragement of field assistants [2, 12]. Some correlation analysis has been used to explore relationships between some variables related to sorting efficiency, disregarding temporal trends [19]. In opposition to the general accepted theory, soil moisture content was unrelated to the extraction efficiency.

The physical HS method cut the earthworms and hence requires careful handling of samples and data derived from them [3]. Estimation of earthworm weight is necessary [11, 8] and can be used to relate efficiency of the HS method to the WS techniques since the former mainly misses the smaller worms.

Nevertheless, HS is necessary in earthworm field studies since it recovers earthworms in good conditions (it seems that wet sieving damages large and thin worms), and WS acts as a check or validation on HS efficiency among people working in the field [22]. It is recommended that two or more 20x20 cm soil monoliths are taken for washing-sieving.

During the wet sieving process, a 1 mm mesh size was appropriate for retaining even the smallest cocoons [22]. The underestimates of cocoon extraction during HS compared with washing-sieving, seem to be due to difficulties in manually collecting those cocoons from the abundant grass root system. Even cocoons of some species were not recorded when soil samples were washed through 0.5 mm and 0.125 mm sieves (data not shown). These were probably destroyed by water stream.

Finally, these studies on HS efficiency, although tedious and back-breaking, must be considered when performing population dynamics and cohort analysis of earthworm communities. By so doing more reliable data about the demography of smaller earthworm species will be obtained.

Acknowledgements

This study was supported by the EU Macrofauna project (STD-2 programme). We also wish to thank CIAT (International Center for Tropical Agriculture) for human and technical support and the different directors of Carimagua research station Jaime Triana and Darío Cárdenas (CORPOICA). Thanks to Jay Martin for kindly checking the English. Finally, our most sincere gratitude to the field staff for their excellent work and friendship and to the local warm people in the “Llanos”.

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Tables

Table I

Variations of monthly earthworm density¹ (average $\pm$ standard deviation) due to the type of sampling employed and the global efficiency of hand-sorting earthworms in 1m² soil monoliths.

Species	Native savanna			Introduced pasture		
	Density	Density	Efficiency	Density	Density	Efficiency
	HS	WS	%	HS	WS	%
<i>Aymara</i> sp.	3.8 $\pm$ 4.9	0.6 $\pm$ 2.5	100	1.8 $\pm$ 2.3	0.3 $\pm$ 0.3	87.5 $\pm$ 25.1
<i>Glossodrilus</i> sp.	30.8 $\pm$ 26.3	50.4 $\pm$ 36.5	58.1 $\pm$ 30.3	41.9 $\pm$ 37.6	68.6 $\pm$ 50.6	51.7 $\pm$ 21.7
Ocnerodrilidae sp.	7.7 $\pm$ 8.4	25.8 $\pm$ 31.3	31.4 $\pm$ 24.6	16.3 $\pm$ 18.8	37.0 $\pm$ 40.6	44.0 $\pm$ 22.9

HS: hand sorting; WS: washing-sieving

¹ Average value of 17 months (all values are indicated in figures 1 and 2 for *Glossodrilus* sp. and Ocnerodrilidae sp., respectively).

301 Table II
302 Mean density and correction factor for each size class of the three species in the savanna. Data from hand sorting method refers only to the
303 first 20 cm in order to assess valid comparisons between the two methods used. HS = hand sorting; WS = Washing-sieving; CF =
304 correction factor.
305

Species											
<i>Glossodrilus</i> sp.				<i>Aymara</i> sp.				<i>Ocnerodrilidae</i> sp.			
Weight	HS	WS	CF	Weight	HS	WS	CF	Weight	HS	WS	CF
class ¹				class ¹				class ²			
0.01	0.5	1.7	3.3	0.01	0.72	7.1	9.9	1	0.04	0.43	10.8
0.02	1.7	3.3	1.9	0.02	0.98	2.98	3.0	2	0.36	1.56	4.3
0.03	2.4	5	2.1	0.03	0.31	1.14	3.7	3	1.25	5.97	4.8
0.04	3.4	5	1.5	0.04	0.34	0.71	2.1	4	1.95	6.39	3.3
0.05	1.5	2.8	1.9	0.05	0.75	1.42	1.9	5	2.15	5.4	2.5
0.06	3.8	6.5	1.7	0.06	0.41	0.85	2.1	6	1.1	3.4	3.1
0.07	4.9	7	1.4	0.07	0.24	0.43	1.8	7	0.44	2.27	5.2
0.08	3.6	5.1	1.4	0.08				8	0.25	0.28	1.1
0.09	4.5	5.3	1.2	0.09				9	0.16	0.42	2.6
0.10	1.5	2.1	1.4								
0.11	2.1	4.3	2.1								

306 ¹ grams
307 ² milligrams

308

309

310 Table III

311 Mean density and correction factor for each size class of the three species in the pasture. Data from hand sorting method refer only to the
312 first 20 cm in order to assess valid comparisons. Same abbreviations as in table II.

313

Species											
<i>Glossodrilus</i> sp.				<i>Aymara</i> sp.				<i>Ocnerodrilidae</i> sp.			
Weight	HS	WS	CF	Weight	HS	WS	CF	Weight	HS	WS	CF
class ¹				class ¹				class ²			
0.01	0.3	1.8	7.2	0.01	0.32	4.97	15	1	0.09	0.43	4.8
0.02	0.9	1.7	1.8	0.02	0.20	0.71	3.5	2	0.86	0.85	1.0
0.03	1.7	3.3	1.9	0.03	0.22	0.14	0.6	3	1.53	4.55	3.0
0.04	3	4.7	1.6	0.04	0.24	0.57	2.4	4	2.83	5.54	2.0
0.05	2	1.7	1.0	0.05	0.35	0.71	2.0	5	3.66	10.23	2.8
0.06	5.2	8	1.5	0.06	0.20	0.28	1.4	6	3.66	9.23	2.5
0.07	5.6	8.9	1.6	0.07	0.18	0.28	1.6	7	2.16	4.12	1.9
0.08	5.6	8.2	1.5	0.08	0.01	0.14	14.1	8	1.01	1.42	1.4
0.09	6.6	10.5	1.6					9	0.44	0.71	1.6
0.10	1.9	2.4	1.2								
0.11	4.7	9.5	2.0								
0.12	2.3	4.8	2.1								

314 ¹ grams

315 ² milligrams

316

Figure legends

Figure 1. Seasonality of global efficiency of HS of 1m² soil monoliths for *Glossodrilus* sp. in both systems studied.

Figure 2. Seasonality of global efficiency of HS of 1m² soil monoliths for *Ocnerodrilidae* sp. in both systems studied.

Figure 3. Efficiency of HS method according to earthworm weight for the three species studied in both systems. Correlations were adjusted to the function $y = ax^b$ and tested with ANOVA. ■ *Glossodrilus* sp. ($y = 123.79x^{0.244}$, $P < 0.05$), □ *Aymara* sp. ($y = 305.18x^{0.607}$, $P < 0.001$) and ▲ *Ocnerodrilidae* sp. ($y = 157.09x^{0.429}$, $P < 0.05$). The weight of *Ocnerodrilidae* was multiplied by 10 in order to plot the data in the same graph.

Figure 4. Distribution of size classes (age) in *Glossodrilus* sp. after field data from the native savanna were corrected. a) April 1994, b) October 1994. Coc = cocoons.

Jiménez J.J., Lavelle, P. & Decaëns, T. – Figure 1

Jiménez J.J., Lavelle, P. & Decaëns, T. – Figure 2

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346 Jiménez J.J., Lavelle, P. & Decaëns, T. – Figure 3

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354 Jiménez J.J., Lavelle, P. & Decaëns, T. – Figure 4

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