European Journal of Soil Biology 73 (2016) 59–68

Contents lists available at ScienceDirect

European Journal of Soil Biology

journal homepage: http://www.elsevier.com/locate/ejsobi

Original article

The efficiency of earthworm extraction methods is determined by species and soil properties in the Mediterranean communities of Central-Western Spain

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ARTICLE INFO

Article history:
Received 29 July 2015
Received in revised form
11 January 2016
Accepted 12 January 2016
Available online xxx

Keywords:
Earthworm sampling
Chemical expellants
Hand-sorting
Maturity stage
Ecological group
Mediterranean soils

ABSTRACT

Given the well-known role of earthworms in the functioning and health of soils and whole ecosystems, feasible and reliable studies of their abundance and diversity in agricultural lands are essential for the effective design of best agricultural practices. However, previous work has shown that the extraction efficiency of different methods proposed seems to depend on species and size of earthworms and presumably on soil type, which makes creating an earthworm inventory difficult. In the present study, we compare the efficiency of five earthworm extraction methods combining hand-sorting with chemical expellants (hand-sorting, formalin, allyl isothiocyanate (AITC), formalin + hand-sorting and AITC + hand-sorting) over a wide range of soil properties (depth, texture and water regime) in cultivated and semi-natural habitats found in a Mediterranean region (CW-Spain). Sampling efficacy was measured in terms of number of earthworms extracted, taking into account different species, ecological groups, development stages, size of individuals, and soil properties. We found 20 species, only 6 endogeic and 1 anecic species being abundant. The anecic *Aporrectodea trapezoides* responded reasonably to chemical expellants, as did certain soil surface dwelling endogeic species (*Microscolex phosphoreus and Microscolex dubius*), with above 50% of specimens of these species sampled after chemical application. For other endogeic species, such as *Allolobophora molleri* and *Aporrectodea rosea*, chemical expellants gave poor results (<15% and 5% of specimens, respectively), and combined methods produced similar results to hand-sorting alone. Hand-sorting appears necessary for sampling the total earthworm community in particular for endogeic species, but when only species richness is of interest, the application of a chemical expellant can be a time-efficient method. Response to different methods was irrespective of the earthworm size within species, but depended on the maturity stage of the specimens, habitat type and soil properties, making difficult the adoption of a simple sampling protocol for large surveys in highly fragmented Mediterranean earthworm communities.

1. Introduction

After more than 150 years of research in earthworm taxonomy, distribution and ecology, we still lack much information about the distribution of many earthworm species and the effect of habitat management and fragmentation on earthworm communities. Many studies warn of the negative impact of different agricultural practices and pollution on the abundance and diversity of earthworms [1–4], and there is increasing interest in using earthworms as bioindicators of the different impacts of farming practices as well as landscape structures and transformations. Indeed, project BioBio recently proposed the monitoring of earthworm diversity as a key direct indicator of biodiversity in agro-ecosystems in Europe [5]. Therefore, simplified and standardized methods are needed to conduct large earthworm surveys, which could enable the adoption of scientifically sound, best practices for farming and, consequently, better agrarian policy.

Mediterranean earthworms exhibit complex distribution

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http://dx.doi.org/10.1016/j.ejsobi.2016.01.005

patterns, high taxonomic diversity and great morphological variability [6–9]. They frequently exhibit clumped distributions, forming patches with small areas [10] because many species have narrow ecological requirements that are determined by the high spatial variability of soil and soil water in many Mediterranean landscapes [11]. All these factors pose challenges to any attempt at monitoring earthworm diversity and abundance in Mediterranean agricultural landscapes.

For years, researchers have been seeking optimal sampling methods to estimate earthworm populations, and although several reviews of this topic exist, the best collection technique remains controversial. The earliest reviews [12–15] distinguish between two types of methods: passive, or hand-sorting, and behavioural. The advantages and disadvantages of each have previously been discussed by several authors, and choosing the appropriate method for earthworm extraction depends on the purpose of the study and the soil conditions. The effectiveness of each earthworm extraction method can vary with species, age or activity as well as some soil parameters, such as soil water content, porosity and temperature. Coleman et al. [16] and Valckx et al. [17] summarized the most common earthworm extraction methods and their advantages and disadvantages.

Hand-sorting has long been the standard sampling method. However, it is very suitable for small and endogeic earthworms, which produce horizontal burrows, but not practical for sampling anecic earthworms, which can quickly escape to deeper layers of the soil profile [18–20]. Juveniles can also be underestimated by hand-sorting [21]. Furthermore, this method is extremely labour-intensive and time-consuming [22,23]; it requires extensive physical destruction of the soil [24] and is technically impossible in many places [17,25]. The use of chemical expellants, a behavioural extraction technique that induces earthworms to leave the soil, is faster and simpler. Originally described by Evans and Guild [26], who first used potassium permanganate and later formalin, mustard powder, household detergents and, more recently, an onion solution [27], the use of expellants has become the most popular technique for earthworm extraction. However, the efficiency of chemical expellants declines from epigeic (non-burrowing species that live in litter) to anecic (vertical burrows) to endogeic (horizontal, disconnected burrows) earthworms, due to differences in species behaviour and burrow orientation [18,21].

Pioneered by Raw [28], formaldehyde (or formalin) is the most commonly used chemical expellant. Although it does not physically destroy the soil, it is known to have toxic effects [29–31] and create health risks [32]. Hot mustard solutions may be a non-toxic alternative, but their efficiency depends on burrowing behaviour (more effective on deep-burrowing anecic species [18,25]), maturity stage (slightly more effective on adults than juveniles [19]) or body size [24]. Moreover, mustard is expensive, and protocols are difficult to standardize because of the variations in chemical composition [17,20,33].

Allyl isothiocyanate (AITC) is the active agent in mustard; it is a natural alkaloid produced through the enzymatic breakdown of the glucosinolates in mustard. However, it is found in many vegetables of the Cruciferae, and recent studies have explored the use of AITC as an earthworm expellant [17,20,31,34]. Its efficiency has been found to be similar to that of formalin, and it is more effective at expelling deep-burrowing anecic species than other ecological groups. Moreover, AITC is not toxic to humans or other organisms [35], and it even has potentially anti-carcinogenic properties [36]. Eisenhauer et al. [25] noted that expellant efficiency depends on soil type and soil moisture, so Pelosi et al. [33] recommended further testing of AITC in a wide range of soil types, cropping systems, and climate conditions.

Although there are standardized protocols for the extraction of earthworms [37], most trials have been performed in Central or Atlantic Europe, so there is a lack of data on the earthworm assemblies that are characteristic of Mediterranean agricultural landscapes under a different climate condition and whose populations are expected to be strongly fragmented. Therefore, in this study, we aim to assess the efficiency of AITC in the sampling of earthworm abundance and diversity compared to the use of formalin and hand-sorting. Specifically, we aim to answer the following questions. (i) Which method yields the most accurate results in terms of earthworm abundance and diversity? (ii) Does the efficiency of an earthworm extraction method depend on the species, the ecological group (epigeic, anecic, endogeic), the development stage (adult vs juvenile) or the size of the individual? (iii) Do soil properties affect method selection? To answer these questions, we first conducted a large earthworm survey to assess the efficiency of AITC across a range of soil types and habitats in a typical Mediterranean agrarian region, which experiences seasonal soil drying, with olive groves and wood pastures as the dominant land uses. A further intensive but smaller survey was performed in three study sites with species-rich earthworm fauna using different combinations of hand-sorting with two chemical expellants: hand-sorting, formalin, AITC, formalin + hand-sorting and AITC + hand-sorting. Different extraction methods have rarely been compared under the wide range of conditions and habitats (especially different water regimes from flooded to very dry) that are typical of Mediterranean landscapes.

2. Materials and methods

2.1. Study area and systems

This study was conducted at Tierras de Granadilla (~400 km^2; Cáceres province, CW Spain) near archaeological Roman ruins of Cáparra (40° 10′ 3″N – 6° 5′ 58″E Datum ED50; altitude ~ 400 m.a.s.l.). The climate is typical Mediterranean with warm dry summers and cold wet winters. Mean annual precipitation is 810 mm and mean annual temperature is 16 °C (Guijo de Granadilla weather station; 40° 13′N – 6° 8′W; www.ucm.es/info/cif/station/es-guijo.htm). Soils are acid, shallow and poor (mostly Distric Endoleptic Cambsiols; [38]), developed over granites and weathered slates, forming a gently undulating mosaic-like landscape, with olive groves and oak wood pastures (named dehesas: open woods with scattered *Quercus ilex* trees as overstory and native pasture as understory, usually farmed for extensive livestock breeding) as dominant land uses. According to Bunce et al. [39] classification for European habitats, 10 different habitats including General Habitat Categories and Linear Features were defined in olive groves and wood pastures. Briefly General Habitat Categories are defined primarily in terms of dominant plant life forms, tree and shrub species and cover, and management practices (e.g. cultivated, grazed, herbicides …) and include 6 types: perennial pastures, annual pastures, wood pastures, shrubs, woody crop and herbaceous crops. Linear Features are defined in function of structural elements and include 4 types: herbaceous strips, line of trees, line of scrubs, and water course.

2.2. Sampling locations

Sampling was conducted in two years during the spring (April 2010 and 2011), when optimal conditions (mild and wet soils under field capacity and saturation) occurred for earthworm sampling.

In April 2010, a total of 237 sampling plots were selected for a large survey ("large-scale campaign") of all different habitats and linear features in the study area, which had previously been mapped (Table S1). In each sampling plot, earthworms were extracted from three 30 × 30-cm squares located 20 m from the border of the habitat/field plot and 10 m from each other, and to capture the linear elements, the 3 sampling squares were placed along a line in the middle of the habitat. The data from the three squares were pooled for a total of 2700 cm^2 sampled per plot. Given that each study plot was defined as representing a uniform habitat, this sampling method was considered to be sufficient to represent the plot.

In April 2011, a more intensive sampling effort ("small-scale campaign") was conducted in 27 sampling plots (two wood pastures, D6 and D9, and one olive grove, O8; Table S1) with contrasting hydric conditions, which were selected from the sites sampled in the large-scale survey based on the criteria of maximum abundance and number of species. In each sampling plot, six replicated subplots were established 20 m from the border of the habitat/field plot and 10 m from each other.

2.3. Sites and soil characterization

Based on a regional soil map [40] and a list of plant species (unpublished data), the 237 points sampled in 2010 as part of the large-scale survey were classified by soil depth (4 classes: shallow, medium, deep, and very deep), soil texture (5 classes: Loamy sand, Sandy loam, Loam, Sandy clay loam, and Clay loam) and water regime (3 levels: Dry, Mesic, and Wet based on the vegetation following an exhaustive inventory of the flora within a 100 m^2 area). Table S1 details the main characteristics of the uppermost soil layers in the study area in 2010, and Table S2 shows the main soil characteristics of the plots sampled in 2011 and analysed following Guitián and Carballas [41] and Anne [42].

2.4. Earthworm sampling methods

In the 2011 small-scale survey, five sampling methods were compared within each replicated subplot (one 50 × 50-cm square (2500 cm^2) per method); the methods combined hand-sorting with two different chemical expellants: (i) hand-sorting, (ii) formalin, (iii) AITC, (iv) formalin + hand-sorting and (v) AITC + hand-sorting.

(i) Hand-sorting (denoted HS): The vegetation and litter above the soil were removed and searched for epigeic earthworms before being discarded. A 20-cm deep soil core was extracted using a spade and placed on a white plastic sheet, and earthworms were carefully hand-sorted from the soil.

(ii) Formalin (denoted FORMOL): After carefully removing the vegetation at the ground level (avoiding soil tremor as much as possible and collecting epigeic earthworms if they emerged), 1.25 l of 0.55% formalin solution was poured onto the soil, and the earthworms that surfaced for 20–30 min (after which the movement of earthworms to the surface ceased) were collected. For sampling points on sloping ground, a 20-cm high metal frame was driven into the ground to a depth of approximately 1–2 cm to prevent the chemical from running off of the sampling square.

(iii) Allyl isothiocyanate (denoted AITC): We prepared the AITC solution shortly (not more than 4 h) before fieldwork to prevent the loss of irritating activity. The AITC was first dissolved in 70% ethanol to yield a 5 g/l solution, and this solution was then diluted with water to a concentration of 0.1 g/l [20,33]. Taking the same precautions noted for the formalin extraction, 5 l of AITC solution (0.1 g/l) were twice poured into the soil at 5 min intervals, and the earthworms appearing at the surface within a 20-min period were collected.

(iv) Formalin + Further Hand-sorting (denoted FORMOL + HS): After extraction with formalin as described in ii, the remaining earthworms were sampled by hand-sorting in soil squares as described in i. The chemical was first applied to avoid the earthworms that could escape both vertically and laterally.

(v) AITC + Hand-sorting (denoted AITC + HS): After extraction with AITC as described in iii, the remaining earthworms were sampled by hand-sorting in soil squares as described in i.

In the large-scale survey of 2010, samples were taken from each plot following methods iii and v (three 30 × 30-cm squares = 2700 cm^2 sampling area). For each 30 × 30-cm square, 2 l of AITC solution were used instead of 5 l, and hand-sorting by one person lasted for a maximum of 20 min for the 30 × 30-cm squares and for a maximum of 60 min for the 50 × 50-cm squares (the maximum time was used in the clay soils but not in the sandy soils).

2.5. Taxonomic identification

The collected earthworms were carefully washed with water and fixed and preserved in plastic containers filled with 96% alcohol, and once in the lab, the containers were refrigerated at 4 °C until identification. Earthworms were counted, weighed (conserved weight with gut contents) and identified to species following the taxonomic key by Álvarez [43] and Bouché [44] as well as a general key for Spanish earthworms (unpublished); the nomenclature followed was that of Blakemore [45]. Adults (with a clitellum), sub-adults (either without clitellum or tubercula pubertatis) and juveniles (without a clitellum and tubercula pubertatis) were separated. Only a few of the juvenile earthworms could not be identified to the species level.

2.6. Statistical analysis

For the large-scale survey, the mean values of the earthworm abundance data (expressed as the number of individuals extracted with the combined method, AITC + HS; n = 237) were compared by generalized linear models (GLZ) constructed for the species pool and for each of the seven main species individually. We tested whether the dependent variable (count data) was Poisson distributed (log-link), and habitat type, soil depth, soil texture and soil water regime and their respective two-level interactions were used as explanatory variables. The Pearson chi2 correction for data overdispersion was included in the model, according to McCullagh and Nelder [46]. In all cases, model validity was checked by visual examination of the residual plots and an assessment of the dispersion parameters [47].

The efficiency of AITC for earthworm extraction in the large-scale survey was examined with a chi-square test comparing the number of earthworms extracted with AITC (observed variable) with the total number of extracted earthworms (AITC + HS; expected variable). This test was run for each of the seven main species and on the pooled data for all 20 species; in each case, only the sampling plots containing earthworms were included. A cluster analysis of the earthworm community, computed using the Euclidean distance of individual abundance that accounted for only the earthworms extracted with AITC and all earthworms extracted with AITC + HS was performed to determine whether the two methods led to different earthworm community classifications.

To check the dependence of AITC efficiency on the characteristics of the study sites, GLZ models were constructed using the AITC efficiency index (defined as the percentage of individuals extracted with AITC with respect to the total (AITC + HS)) as the response variable and Habitat type, Soil depth, Soil texture and Soil water regime as fixed factors. GLZs were run for the seven main species and on the pooled data for all 20 species. We checked that the dependent variable (percentage) was Gamma distributed (log-link). Only cases (plots) with >3 individuals of the species under analysis were included, and within each factor, levels with >3 cases (repetition) were included.

Given the non-independence of earthworms extracted with certain methods from the same plot (e.g., AITC and AITC + HS), three sets of analyses were run on the data from the small-scale survey. First, we compared the AITC, FORMOL and HS methods by means of a generalized linear mixed model (GLMM) to test whether the species richness estimate from any of the chemical methods differed from the species richness value using the HS method. Again, we checked that the dependent variable (count data) was Poisson distributed. Method, Species and Maturity Class (adult, sub-adult and juvenile) and their respective interactions were used as explanatory variables, and the effect of site was considered to be a random effect. An observation-level random effect was included to account for the observed over-dispersion, and in all cases, model validity was checked by visual examination of the residual plots and an assessment of the dispersion parameters [47]. Second, the HS, AITC + HS and FORMOL + HS methods were compared using a similar GLMM to determine the extent to which HS-extracted the earthworms were present. Third, to test whether the chemical expellant extracted most of the earthworms, the number of earthworms extracted with AITC (defined as the observed variable) was compared with the expected number of earthworms (AITC + HS) by means of a chi-square test (the same was done for the comparison of FORMOL vs FORMOL + HS). These tests were run separately for every combination of Species and Maturity Class. Finally, to determine whether the efficiencies of the methods differed with earthworm size, a GLM was used to compare the mean weights of the earthworm species (g per individual) as a continuous dependent variable. The method of extraction (HS, AITC, FORMOL, and HS after AITC or FORMOL), species (3 main species: *Zophoscolex ibericus*, *Aporrectodea rosea*, and *Aporrectodea trapezoides*), Maturity Class (adult and juvenile) and the combinations of these factors were included as fixed factors. If a GLMM was found to be significant, further comparisons of the means were performed using the post hoc Duncan test.

All statistical analyses were run in STATISTICA 7.0 (StatSoft, Inc., Tulsa, OK, USA), except for the GLMMs, which were run with the "lme4" package in program R (R Core Development Team, R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1. Variation in earthworm abundance among habitats and soil properties

A total of 3992 earthworms belonging to 20 species were collected during the large-scale survey of 2010. The most frequently collected species were *A. rosea* (Savigny, 1826), *A. trapezoides* (Dugès, 1828), *Allolobophora molleri* (Rosa, 1889), *Microscolex dubius* (Fletcher 1887), *Microscolex phosphoreus* (Dugès, 1837), *Zophoscolex chitae* (Díaz Cosín, Mato and Trigo, 1988) and *Z. ibericus* (Trigo, Mariño and Diaz Cosín, 1988) (Table 1). All these belong to the endogeic ecological category except for *A. trapezoides*, which is very variable in its behaviour but is primarily considered to be anecic, and *M. dubius* and *M. phosphoreus*, which are considered to be epi-endogeic and soil surface-dwelling endogeic, respectively, as both burrow near the surface. Additional species were found but were in low abundance and could not be used for further analysis at the species level. A few juvenile specimens could not be identified because of the condition of their preservation or the lack of characteristics usually used for identification.

Statistical comparisons of the mean earthworm abundance values revealed significant differences among habitats and soil types (Table 1). On average, earthworms were more abundant in grasslands (e.g., perennial pasture with 36.1 ± 6.9 individuals/ 0.27 m^2) than in woody and cultivated habitats (e.g., herbaceous crops with 7.1 ± 6.8 individuals). Earthworm abundance was higher in loamy sand soils (32.5 ± 5.2) than in clay loam soils (11.5 ± 2.6) with greater differences between soil types in crops and grasslands than in woody habitats (significant Habitat × Texture interaction; Table 1). Abundance was also higher in wet than in mesic and dry soils (27.2 ± 3.9, 21.0 ± 3.2 and 12.4 ± 1.3, respectively), with greater differences in grasslands than in woody habitats (significant Habitat × Water regime interaction; Table 1), and in coarse-textured than in fine-textured soils (significant Texture × Water regime interaction; Table 1). The soil depth results were more surprising with maximum values observed in the deepest and shallowest soils and lower values in soils at depths between 50 and 100 cm. The effect of soil depth was more significant in mesic and dry soils than in wet soils (significant Soil depth × Water regime interaction; Table 1).

The response to these ecological factors varied among species (Table 1). For instance, although *A. trapezoides and A. molleri* were more abundant in wet and/or shallow soils, *Z. ibericus* and *A. rosea* were more abundant in drier and/or deeper soils. *A. trapezoides, A. rosea* and *Z. ibericus* were found more often in coarse-textured soils, but *A. molleri and M. dubius* were more commonly found in finer-textured soils. Finally, *A. trapezoides* and *A. molleri* dominated Annual and Perennial pastures and Herbaceous strip habitats; *A. rosea* was mostly found in Lines of scrub, *Z. ibericus* in Lines of trees and Z. *chitae* in Shrub-dominated areas.

3.2. Efficiency of AITC and its dependence on soil properties

The percentage of earthworms extracted with AITC with respect to AITC + HS (%AITC) was low, except for *A. trapezoides* and *M. dubius* whose efficiencies were greater than 60% (Table 2). The number of earthworms extracted with AITC was significantly lower than the total number of earthworms (AITC + HS) for all species except *M. dubius* and *M. phosphoreus*, and the biomass results were similar (data not shown). The results of the cluster analysis (Figure S1) demonstrated that when the earthworm community was characterized with the use of only AITC, *A. trapezoides* abundance was overweighted, whereas the abundances of the other species were underweighted (especially those of *A. rosea* and *A. chitae*) compared with the results of the full sampling approach (AITC + HS).

Considering all species together, AITC efficiency varied marginally among habitats (Table 3). In general, efficiency was lower in woody habitats (lines of trees, lines of scrubs and shrubs) and water courses but higher in annual and perennial pastures and herbaceous crops. For some species, efficiency varied significantly among habitats. For instance, *M. dubius* varied from 92.6% to 81.8% in perennial pastures and woody crops, respectively, to 11.1% and 14.3% in wood pastures and water courses.

For certain species, AITC efficiency depended on soil properties, most notably for *A. rosea*, *A. molleri*, *Z. chitae* and *Z. ibericus* (Table 3). For instance, *A. molleri* efficiency was maximized in dry soils, but it was higher for *A. rosea* in wet soils. AITC efficiency for *A. molleri* was higher in coarse-textured than in fine-textured soils, but AITC efficiency for *Z. chitae* was surprisingly higher in deep soils than in shallow soils.

Table 1
Results of GLZ models applied to the large-scale survey data to test differences in earthworm abundance in relation to species and habitat (HT: 10 categories) or soil type, the latter characterized in terms of soil depth (D: 4 categories), soil texture (T: 5 categories) and soil water regime (WR: 3 categories). Results for data of 20 species pooled are also shown. (N = 237).

Factors		20 species pooled	A. *rosea*	A. *trapezoides*	*A. molleri*	*M. dubius*	*M. phosphoreus*	*Z. chitae*	*Z. ibericus*
Habitat type	Wald χ^2	175	199	937	305	50.4	2.73 (7)	73.4	215
(HT: 10)	P_{value}	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.909	**<0.001**	**<0.001**
Depth	Wald χ^2	37.0	153	567	169	8.25	4.65	0.884	301
(D: 4)	P_{value}	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.041**	0.199	0.829	**<0.001**
Texture	Wald χ^2	109	166	315	49.6	74.5	5.42	8.11	277
(T: 5)	P_{value}	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.247	0.088	**<0.001**
Water Regime	Wald χ^2	13.5	169	312	398	14.6	7.56	1.32	56.4
(WR: 3)	P_{value}	**0.004**	**<0.001**	**<0.001**	**<0.001**	**0.001**	**0.023**	0.515	**<0.001**
HT × D (24)	Wald χ^2	159	131 (12)	91.3 (12)	47.2 (12)	0.589 (4)	2.70 (12)	0.017 (12)	26.7 (12)
	P_{value}	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.964	0.997	1.000	**0.008**
HT × T (12)	Wald χ^2	231	32.3 (6)	40.0 (6)	7.99 (6)	0.844 (6)	1.96 (6)	0.118 (6)	14.9 (6)
	P_{value}	**<0.001**	**<0.001**	**<0.001**	0.238	0.991	0.924	0.999	**0.021**
HT × WR (18)	Wald χ^2	168	14.1 (4)	6.29 (4)	4.26 (4)	5.55 (12)	4.11 (4)	0.171 (4)	1.01 (4)
	P_{value}	**<0.001**	**0.007**	0.178	0.372	0.937	0.391	0.997	0.909
D × T (8)	Wald χ^2	2.06	2.71 (1)	0.350 (1)	0.001 (1)	0.001 (1)	0.001 (1)	0.001 (1)	0.013 (1)
	P_{value}	0.151	0.100	0.554	0.997	0.995	0.999	0.988	0.909
D × WR (11)	Wald χ^2	44.4	69.3 (4)	21.2 (4)	22.6 (4)	3.14 (4)	19.3 (4)	15.8 (4)	24.1 (4)
	P_{value}	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.535	**0.001**	**0.003**	**<0.001**
T × WR (12)	Wald χ^2	142	15.4 (2)	57.9 (6)	92.0 (6)	0.124 (6)	5.15 (6)	4.02 (6)	44.9 (6)
	P_{value}	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.999	0.524	0.674	**<0.001**

Table 2
Mean number of earthworms (±S.E.) extracted in the large-scale survey by the AITC (allyl isothiocyanate) method, by subsequent hand-sorting (HS) and total (AITC + HS). Mean values refer to 0.27 m^2 (pooling the three 0.3 × 0.3 m sampling squares per plot). %AITC means the percentage of earthworms extracted with AITC with respect to the total. AITC efficiency was checked by comparing earthworms extracted with AITC with respect to the total by means of Chi–Square tests for seven main species and for data of 20 species pooled. P < 0.05 means that HS after AITC provided a significant number of additional EW. N: number of cases included in the model in each case (sampling plots where the earthworm species were found).

Species	Mean abundance (standard error)					
	AITC	HS after AITC	Total	% AITC	χ^2	P
All species	7.39	11.80	19.2	38.5	1706	**<0.001**
(N = 208)	(±0.67)	(±0.91)	(±1.40)			
Z. chitae	0.61	4.52	4.7	12.8	93.5	**<0.001**
(N = 23)	(±0.20)	(±0.85)	(±0.9)			
Z. ibericus	1.25	5.07	6.1	20.6	302	**<0.001**
(N = 71)	(±0.93)	(±0.82)	(±0.9)			
A. molleri	1.15	7.59	8.1	13.7	359	**<0.001**
(N = 54)	(±0.31)	(±1.11)	(±1.2)			
A. rosea	0.22	6.22	6.1	3.62	528	**<0.001**
(N = 91)	(±0.83)	(±0.84)	(±7.9)			
A. trapezoides	6.44	3.92	10.4	62.2	359	**<0.001**
(N = 177)	(±0.71)	(±0.47)	(±1.1)			
M. dubius	2.85	1.50	4.4	65.5	20.3	0.378
(N = 20)	(±0.94)	(±0.42)	(±1.1)			
M. phosporeus	1.21	1.32	2.5	47.7	36.4	0.315
(N = 34)	(±0.27)	(±0.27)	(±0.4)			

3.3. Method efficiency in small-scale sampling of 2011

Five species were found in the small-scale sampling of 2011: *A. trapezoides, A. rosea*, *A. molleri*, *M. phosphoreus* and *Z. ibericus*, all of which had been found in the large-scale sampling of 2010 (Table 4). *A. rosea* and *A. trapezoides* were the most abundant species, and *M. phosphoreus* was the least abundant. Juvenile was the most abundant development class for most species except for *M. phosphoreus*, for which adults were dominant (significant Maturity class × Species interaction; Table 4).

The number of earthworms extracted varied significantly among the three tested methods (AITC, FORMOL and HS), and the differences depended on the earthworm species (significant Method × Species interaction; GLMM 1 of Table 4). Both chemicals yielded similar results for most species, but AITC was better for *A. rosea* than FORMOL. However, for *A. molleri*, FORMOL provided better results. Both AITC and FORMOL extracted significantly fewer earthworms than the HS method, except for *M. phosphoreus*, for which the result was the reverse. The lowest chemical expellant efficiency was found for *A. rosea*. Except for *M. phosphoreus*, the use of chemical expellants alone yielded poor results, and the number of earthworms extracted with FORMOL or AITC alone was significantly lower with respect to the number of earthworms extracted using the combined methods (Chemical + HS) (Table 5). Among the methods that included HS, extraction was maximized by combining AITC with subsequent HS (AITC + HS), which was followed closely by FORMOL + HS. Both of the combined methods produced better results than HS alone, except for *A. trapezoides* (significant Method × Species interaction; GLMM 2 of Table 4).

3.4. Method efficiency with respect to earthworm maturity class and size

The methods worked differently for adults and juveniles (significant Method × Maturity Class interactions; χ^2 (2) = 14.0; P = 0.0008; Table 4). Although HS alone extracted most of the juvenile earthworms (88% with respect to the combined methods (chemical expellant + HS)), HS extracted only 68% of the adult earthworms. However, the efficiencies of the chemical expellants were similar for adults and juveniles (Fig. 1), and these patterns were not affected by the species (non-significant Method × Species × Maturity Class interactions; Table 4).

As expected, earthworm size (weight) varied significantly among species and among maturity stages (adults > juveniles, Fig. 2). The size of the extracted earthworms were independent of the method used, regardless of the maturity class (non-significant Method × Maturity Class interaction: Fig. 2) and the species (non-significant Method × Species interaction; Table 6).

4. Discussion

In the large-scale survey of 2010, extraction with AITC was generally low, collecting less than 40% of the earthworm

Table 3
Percentage of earthworms extracted with AITC with respect to the total (AITC + HS) in the large-scale survey and results of GLZ models to detect the effect of site factors (habitat type, soil water regime, soil texture and soil depth) on the efficiency of the AITC method for earthworms extraction. Results are presented for seven main species and for data of 20 species pooled. For analysis plots with less than 3 individuals (for species under analysis) were excluded; within each factor, levels with less than 3 cases (repetition) were also excluded (blank cells). (n.a. = Not available).

		All species	*A. rosea*	*A. trapezoides*	*A. molleri*	*M. dubius*	*M. phosphoreus*	*Z. chitae*	*Z. ibericus*
Habitat type	**Perennial pastures**	49.7	7.5	68.7	14.4	92.6	n.a.	n.a.	36.0
	Herbaceous crops	57.4	n.a.	77.8	n.a.	54.5	n.a.	n.a.	n.a.
	Tree-dominated systems	46.3	0.0	67.9	n.a.	11.1	37.5	9.1	34.8
	Herbaceous strips	32.7	0.0	63.6	5.7	n.a.	n.a.	n.a.	n.a.
	Line of scrubs	21.9	0.0	57.5	0.0	n.a.	n.a.	n.a.	15.3
	Line of trees	8.8	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	4.2
	Shrub-dominated systems	30.2	7.8	57.9	0.0	n.a.	n.a.	5.7	12.5
	Annual pastures	47.4	3.0	63.6	17.7	n.a.	41.4	14.3	21.3
	Water course	28.4	10.6	39.9	17.2	14.3	n.a.	n.a.	n.a.
	Woody crop	31.5	0.0	60.9	n.a.	81.8	50.0	18.5	17.4
	Wald χ^2	14.8	53.8	5.73	23.2	16.1	1.177	8.13	8.25
	n	208	90	177	54	17	34	21	65
	P_{value}	**0.096**	**<0.001**	0.767	**0.001**	**0.003**	0.940	**0.043**	0.311
Water regime	**Dry**	41.8	2.1	64.9	20.5	57.6	30.2	9.9	18.9
	Mesic	35.7	3.6	59.6	9.6	83.3	73.3	n.a.	20.5
	Wet	41.2	5.3	62.2	12.8	58.8	n.a.	11.1	0.0
	Wald χ^2	1.05	8.55	0.423	6.51	0.177	2.320	4.326	1.66
	n	208	91	177	54	20	34	23	71
	P_{value}	0.590	**0.014**	0.809	**0.039**	0.916	0.314	**0.038**	0.437
Texture	**Loamy-Sand**	35.5	3.4	56.5	14.4	n.a.	n.a.	n.a.	22.3
	Sandy-Loam	43.8	3.5	66.0	14.0	63.8	46.2	13.2	15.4
	Loam	28.6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	Sandy–Clay Loam	45.4	5.6	63.9	4.5	90.5	n.a.	n.a.	n.a.
	Clay-Loam	30.7	0.0	64.5	n.a.	n.a.	n.a.	0.0	n.a.
	Wald χ^2	2.29	16.9	3.52	8.98	0.016	n.a.	0.060	14.3
	n	208	91	177	54	18	n.a.	23	69
	P_{value}	0.683	**0.002**	0.475	**0.011**	0.898	n.a.	0.806	**0.003**
Depth	**<50 cm**	58.3	3.4	80.1	7.8	n.a.	n.a.	n.a.	n.a.
	50–100 cm	41.1	5.0	64.7	20.4	71.0	63.6	12.0	12.9
	100–150 cm	39.7	2.7	61.7	3.4	62.8	30.3	8.3	18.2
	>150 cm	35.5	3.4	56.5	14.4	n.a.	n.a.	n.a.	22.3
	Wald χ^2	2.25	3.59	1.23	3.56	0.077	0.221	5.09	25.0
	n	208	91	177	54	19	34	23	71
	P_{value}	0.522	0.309	0.746	0.313	0.782	0.895	**0.024**	**<0.001**

Table 4
Mean number (±S.E.) of each earthworm species collected in the small-scale sampling in a surface area of 0.5 × 0.5 m with each extraction method (n = 18). Results of two GLMMs comparing earthworm number collected with different methods are also presented, with degrees of freedom in brackets. First GLMM compares mean values of earthworms extracted with AITC, FORMOL and HS (Hand-sorting) methods (capital letters denote significant differences at p < 0.05 level). Second GLMM compares mean values of earthworms extracted with HS, AITC + HS and FORMOL + HS methods (lowercase letters denote significant differences at p < 0.05 level among HS and each of the combined methods).

Species	AITC	FORMOL	HS	AITC + HS	FORMOL + HS
Z. ibericus	5.28 ± 2.16 AB	3.56 ± 1.87 B	8.11 ± 1.88 A − a	10.06 ± 3.22 b	8.28 ± 2.64 a
A. rosea	4.83 ± 2.06B	1.44 ± 0.69 B	13.94 ± 3.31 A − a	17.72 ± 6.52 b	15.33 ± 5.99 a
A. molleri	1.28 ± 0.73 B	3.67 ± 1.84 AB	7.06 ± 2.73 A − a	8.78 ± 3.81 a	8.61 ± 3.46 a
A. trapezoides	6.61 ± 1.14 B	5.50 ± 1.01 B	12.22 ± 2.1 A − a	12.61 ± 1.80 a	11.17 ± 1.61 a
M. phosphoreus	0.78 ± 0.57 A	0.89 ± 0.42 A	0.22 ± 0.15 B − a	0.94 ± 0.58 b	1.94 ± 1.03 b
Not identified	2.50 ± 1.10 A	1.56 ± 0.65 A	1.83 ± 1.72 A − a	3.05 ± 1.03 b	1.89 ± 1.04 a

Factors	GLMM 1(AITC, FORMOL, HS)	GLMM 2 (HS, AITC + HS, FORMOL + HS)
Methods (MT)	χ^2 (2) = 14.6; **P = 0.0007**	χ^2 (2) = 13.1; **P = 0.0014**
Species (SP)	χ^2 (5) = 5132; **P < 0.0001**	χ^2 (5) = 210.5; **P < 0.0001**
Maturity Class (MC)	χ^2 (1) = 105.4; **P < 0.0001**	χ^2 (1) = 165.5; **P < 0.0001**
SP × MC	χ^2 (5) = 66.0; **P < 0.0001**	χ^2 (5) = 81.5; **P < 0.0001**
MT × SP	χ^2 (10) = 22.6; **P = 0.0124**	χ^2 (10) = 19.2; **P = 0.0422**
MT × MC	χ^2 (2) = 16.8; **P = 0.005**	χ^2 (2) = 14.0; **P = 0.0008**
MT × SP × MC	χ^2 (10) = 9.04; P = 0.9824	χ^2 (10) = 9.06; P = 0.9822

community (slightly above 40% in terms of biomass). Similar results were found during the small-scale sampling of 2011 with only 37%

Table 5
Chi–Square test to compare the number of earthworms extracted with the chemical expellant with respect to the total number extracted with the combined method in the small-scale sampling. Tests were run for 5 species and two maturity classes, including in each test only subplots where the species under analysis was present. In brackets are given the degrees of freedom. (n.a. = Not available).

Species	AITC vs AITC + HS		FORMOL vs FORMOL + HS	
	Adult	Juvenile	Adult	Juvenile
Z. ibericus	χ^2 (8) = 18.2 **P = 0.0452**	χ^2 (9) = 38.0 **P < 0.0001**	χ^2 (8) = 34.1 **P < 0.0001**	χ^2 (8) = 34.6 **P < 0.0001**
A. rosea	χ^2 (6) = 74.5 **P < 0.0001**	χ^2 (10) = 106.9 **P < 0.0001**	χ^2 (7) = 69.4 **P < 0.0001**	χ^2 (13) = 140.4 **P < 0.0001**
A. molleri	n.a.	χ^2 (5) = 110.6 **P < 0.0001**	n.a.	χ^2 (5) = 55.7 **P < 0.0001**
A. trapezoides	χ^2 (15) = 25.7 **P = 0.0413**	χ^2 (15) = 41.8 **P = 0.0002**	χ^2 (15) = 25.5 **P = 0.0437**	χ^2 (15) = 47.1 **P < 0.0001**
M. phosphoreus	χ^2 (4) = 2.25 P = 0.6899	n.a.	χ^2 (6) = 9.64 P = 0.1405	n.a.

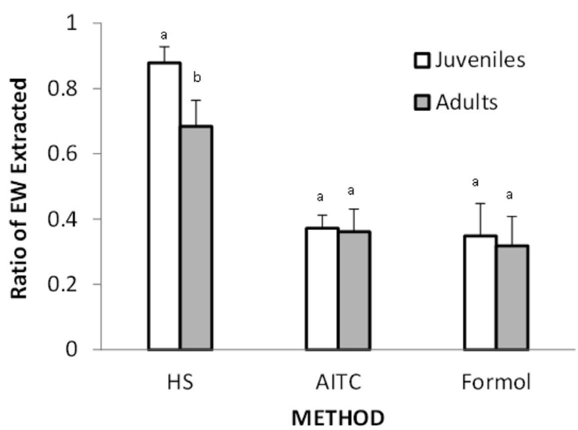

Fig. 1. Proportion (±S.E.) of earthworms (EW) (all species pooled) extracted with different methods in relation to the developmental stage in the small-scale survey. Percentages were calculated with respect to the total number of earthworms extracted with the combined method (chemical expellant + subsequent hand-sorting). For significant differences among adults and juveniles for each species, refer to Table 5. HS: hand-sorting.

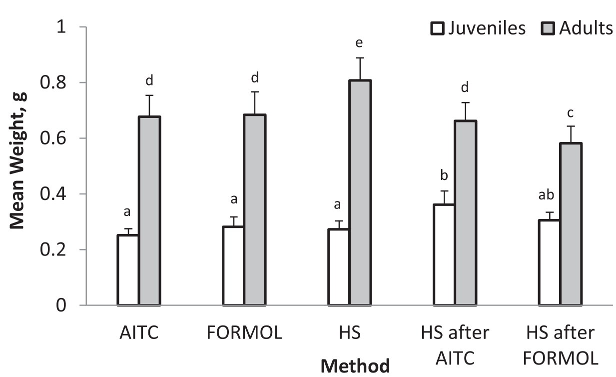

Fig. 2. Mean size (weight ± S.E.) of earthworms (all species averaged) extracted with different methods as a function of the developmental stage in the small-scale survey. Different letters denote significant differences at 0.05 level. HS: hand-sorting.

and 33% of individuals expelled with AITC and FORMOL, respectively. The low efficiencies of the two chemicals in the studied soils and land uses can be explained by a combination of two factors: the low hydraulic conductance of the soils and the composition of the earthworm community in the study region.

Table 6
Results of GLM model comparing mean values of earthworm weight (g per individual) taking into account method of extraction in the small-scale sampling, species (3 species: *Z. ibericus, A. rosea, A. trapezoides*) and maturity class (adult and juvenile) and the respective combination of these factors. D.f. means degree of freedom for the factor and error and F refers to Snedecor statistic.

Factors	D.f.	F	P_{value}
Methods (MT)	4, 234	0.584	0.674
Species (SP)	2, 234	133.4	**<0.001**
Maturity Class (MC)	1, 234	106.0	**<0.001**
MT* SP	8, 234	0.698	0.694
MT* MC	4, 234	0.735	0.569
SP* MC	2, 234	30.20	**<0.001**
MT* SP* MC	8, 234	0.411	0.914

4.1. *The importance of soil properties*

Although some authors did not find a significant relationship between the effectiveness of the extraction method and soil attributes [19], other authors indicated that the efficiency of the methods using chemical expellants would probably depend on temperature, soil moisture content and soil tillage [33]. The efficiency of expellants is expected to be reduced by dry soil conditions because chemical infiltration depends on soil moisture, but in saturated or near-saturated soils, in which many of the pores are already full of liquid, expellant penetration may also be poor. The portion of the chemical solution that does enter the soil will be diluted by soil moisture and thus be less effective. In non-saturated soils, greater penetration and lower dilution will increase efficiency as long as the earthworms are active. In our study, the two sampling campaigns were conducted in the spring when soil moisture and temperature favour earthworm activity, and individuals move to the upper soil layers. Indeed, when conducting exploratory digging during that period, we did not capture any earthworms below a depth of 20 cm. The low organic matter content of Mediterranean soils and the shallowness of the organic horizons [48,49] probably favour the concentration of earthworms in the uppermost few cm of the soil during peaks of maximum activity, as was described for the endogeic *Hormogaster elisae* [50]. Even under these favourable conditions, chemical expellants exhibited low efficiency, regardless of their composition (AITC or formalin).

The low efficiency of the chemical expellants compared with hand-sorting could have been caused by poor liquid diffusion into the soil. Farm soils are frequently compacted/degraded by either repeated cultivation of the soil (olive farms) or by continuous grazing (dehesa farms), as confirmed by Pulido-Fernández et al. [51] for soils in the same study region, and soil water repellency is a common feature of Mediterranean rangelands [52]. In previous studies of the area, mean soil bulk density values of 1.49 g/cm^{-3} for the upper horizon were measured on dehesa farms [53], and soil organic carbon averaged 1.4% [54] and 1.6% [51] in the uppermost soil layer (0–10 cm), which contributes to low soil porosity. Alternatively, many soils were saturated or near saturation during the sampling campaign, and liquids probably moved more horizontally than vertically through subsurface Hortonian runoff. Corcobado et al. [55] showed that the dehesa soils in the study region are frequently saturated in the early spring, but they dry very quickly, making the selection of an optimal time for earthworm sampling with a chemical expellant difficult. The usually high spatio-temporal heterogeneity of soil moisture in undulating Mediterranean landscapes [11] could also complicate the selection of sampling methods, whose efficiencies could depend on a highly variable condition, soil moisture.

Moreover, our results reveal the importance of soil properties and habitat type for chemical expellant efficiency; AITC efficacy was influenced by factors such as soil depth, texture, and soil water regime. AITC was more efficient in crops and pastures with shallow and mesic soils, and the strong dependence of chemical expellant (AITC) efficiency on site characteristics suggests that earthworm behaviour and distribution could vary with soil characteristics and status (e.g., depth and moisture). Moreover, our results indicate that the response to the site/soil characteristics depends on the species; some species are much more sensitive to site heterogeneity.

4.2. Low efficiency of chemical expellants in endogeic-dominated communities

Many authors have shown that different earthworm species respond to chemicals differently, concluding that their extractive use is suitable for only certain species [18–20,25]. Chemical expulsion has typically been found to be more efficient for sampling deep-burrowing anecic species, which may not be captured by hand-sorting. Alternatively, chemicals inefficiently extracted endogeic earthworms, which are better extracted by hand-sorting. Bartlett et al. [24] explained these results in terms of the pore structure of the soil. Anecic earthworms create permanent vertical burrows that are well connected to the surface, which enable the rapid percolation of chemical solutions, so these species are the first to be exposed to expellant solutions. Endogeic species create horizontal or irregular burrows that may slow the movement of these earthworms to the surface, so their response would be to migrate horizontally through the soil instead.

In our study, we found a strong predominance of endogeic earthworms, which are the most abundant ecological group in the Iberian Peninsula [56], and they react more poorly to chemical expellants than epigeic and anecic species [18,21]. However, epigeic earthworms, which live in the litter layer and feed on leaves on the soil surface, were not observed. This could be explained by the lack of a litter layer in Mediterranean soils and the high rates of litter decomposition in these soils [48].

As expected, the efficiencies of chemical earthworm expellants varied significantly among ecological groups but less so among the species within each group. For endogeic species, such as *Z. chitae*, *Z. ibericus*, and *A. rosea*, and hygrophilous species, such as *A. molleri*, extraction efficiency was very low with AITC (4–20% of the specimens were extracted with AITC; Table 2). Only *A. trapezoides*, which is mainly considered to be anecic but is very variable in its behaviour, and *M. dubius* and *M. phosphoreus*, which are endogeic soil surface-dwellers, were highly efficiently with AITC (45–65% of specimens).

4.3. Developmental state or body size?

Our results also show that the extraction efficiencies of chemical expellants also depend on the development stage of the earthworm. Lawrence and Bowers [19] and Bartlett et al. [24] observed that a mustard solution was slightly more effective at extracting adults than juvenile earthworms, whereas Chan and Munro [18] observed the opposite result. Our study did not confirm a dependence of chemical expulsion on earthworm maturity; the percentage of earthworms extracted with chemicals (AITC or FORMOL) with respect to the whole community (AITC + HS or FORMOL + HS) did not differ among developmental stages (on average, 34 and 36% of adult and juvenile earthworms, respectively, were sampled with the application of chemicals). Juveniles usually inhabit more superficial soil layers while adults inhabit deeper ones, but this behaviour did not seem to affect their response to chemical expellants. However, hand-sorting alone was better at extracting juveniles than adults, so it seems that chemical extraction is more important to the reliable sampling of adults than juveniles because adults, which live more deeply in the soil, could escape more easily due to soil tremor from digging. This conclusion seems to be more valid for certain species, such as *A. rosea*.

Different authors, such as Bartlett et al. [24] and Pelosi et al. [33], have observed that large earthworms can be more effectively sampled by applying an irritant solution to the soil, and differences between adults and juveniles have been interpreted as being due to size-dependent effects. For instance, Azevedo et al. [57] found formalin to be more efficient at collecting adult earthworms and larger species, whereas hand-sorting extracted a higher number of juvenile and small specimens. However, our study did not confirm a dependence of chemical expellant efficiency on earthworm size (weight), and differences among developmental stages seem to be due to behavioural or physiological responses than to size-dependent responses. Although size could be an important factor in the response of certain earthworm species to chemical expellants (e.g., large earthworms could more easily reach the soil surface from deep burrows than small earthworms), the lack of a significant effect of earthworm size on chemical efficiency was independent of species in our study.

4.4. Practical recommendations: on the selection of the best method

Throughout most of the Iberian Peninsula, where endogeic earthworms are abundant, any behavioural collection method will probably yield poor results. The findings of this study, which are in agreement with previous results [24,33,58], confirm that earthworm populations are greatly under-estimated when sampled with chemical expellants without hand-sorting. Researchers must try to adapt their sampling methods to the type of habitat, the soil conditions and the composition of the earthworm community and employ techniques that provide a consistent estimation of relative abundance. In our study, the low efficiency of the use of chemicals alone for the extraction of earthworms, regardless of the chemical, discourages their recommendation for broad surveys of earthworm abundance in Mediterranean agricultural soils. As indicated by Zaborski [20], combination methods may yield the most complete and accurate characterization of the size and structure of the earthworm community.

In contrast, the chemical method effectively characterized the species richness of the study region. Although different species responded differently to chemical expellants, all the species recorded at every site during the large-scale survey were sampled with AITC (the same is true of formalin in the small-scale sampling campaign). That is, when species richness is the only variable of interest, the application of a chemical expellant can be a time-efficient method (if water is easily available to dilute the chemicals). This is especially important for Mediterranean regions where earthworm populations are commonly small and fragmented [10]. Given the high site-dependence of earthworm species assemblages, the rapid chemical method is preferred over time-consuming hand-sorting for the estimation of farm- or landscape-level species richness, for which a high number of samples are needed. However, the efficiency of chemical expellants decreases in terms of determining abundance, with the consequent risk of losing species, so this method is not applicable in small-scale studies with a low number of samples. Although hand-sorting method is time-consuming, labour-intensive and physically destructive, it appears to be necessary for an accurate sampling of local earthworm communities, especially endogeics and small anecics [33]. The scarcity of anecic earthworms reduces the concern over not capturing the species that react quickly to soil tremor during digging. Removing a block of soil and bringing it to the lab could be more effective, but it can be employed over only small areas near a laboratory and could underestimate rare species. Therefore, there must be a compromise between the efficiency of the extraction method in each medium, the viability of the sampling and the size of the area sampled (an important factor for less abundant species).

Formalin is lethal to earthworms, plants and other organisms. It is also carcinogenic to humans, and its use is forbidden due to national health and safety regulations [59]. Valckx et al. [17] found AITC to be even more successful at extracting endogeics and subadults than formalin, although other studies comparing AITC and formalin efficiency [20,31,33] found no significant differences between the two chemical expellants. Our study confirms the similarity of the two compounds in terms of efficiency; both of the combined methods (FORMOL + HS and AITC + HS) yielded similar results. Both of the chemical methods (FORMOL and AITC) also presented the same efficiencies, although they were much lower than the combined methods. The use of AITC has some clear advantages over formalin: it is inexpensive and may be less toxic to earthworms and much safer to use. Moreover, AITC has lower persistence because it degrades faster in the soil, so it is more environmentally friendly than formalin [20,31]. Nevertheless, as Valckx et al. [17] note, AITC should also be used with care because it is very toxic to aquatic organisms; its preparation can cause irritation, and it can cause epidermal loss in earthworms.

5. Conclusion

In the early spring, direct hand-sorting without prior chemical application, yields results similar to the combined method, i.e., chemical expellant application and further digging, in terms of earthworm richness, but the estimation of earthworm abundance is poorer as a certain proportion of the adults can be lost. Using only a chemical expellant (preferably AITC) is a more time-efficient and cost-effective method to assess earthworm richness at a large scale in Mediterranean regions, where populations are small and fragmented. If both species richness and abundance are of interest, the combination of chemical expellant plus hand-sorting is preferred. Therefore, we conclude that method selection depends on the scale of the work and the targeted parameters, such as species richness alone or more elaborate diversity indexes that include the relative abundances of species.

Chemical expellant efficiency was much better for anecic and epi-endogeic earthworms than endogeic species, which explains the low efficiency of chemical expellants in the studied systems, which are endogeic-dominant communities. We found low differences among species within the same ecological group, and although efficiency was independent of earthworm size, the use of chemical expulsion was more effective for adult (but not for larger) individuals, which may react more strongly to soil tremor during digging than juveniles.

Finally, the results confirm our expectation of high differences among land uses/habitats and soil types in terms of abundance and species composition, which further complicates the selection of a single best method for the study of earthworm communities in the farmed landscapes of the Mediterranean region.

Acknowledgements

We thank the farmers for access on their land and facilities provided during field work. This study was funded by the European Union through FP7 project BioBio (Indicators for biodiversity in organic and low-input farming systems; grant agreement nº 613520; www.biobio-indicators.org).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejsobi.2016.01.005.

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