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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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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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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