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Comparison of two widely used sampling methods in assessing earthworm community responses to agricultural intensification

Walter S. Andriuzzi[a,*], Mirjam M. Pulleman[b,c], Daniel Cluzeau[d], Guénola Pérès[e,f]

[a] Department of Biology, Colorado State University, Fort Collins, CO, 80523, USA
[b] International Center for Tropical Agriculture, AA6713, 763537, Cali, Colombia
[c] Department of Soil Quality, Wageningen University, P.O. Box 47, 6700AA, Wageningen, The Netherlands
[d] University of Rennes, CNRS OSUR-EcoBio, France
[e] UMR1069 SAS (Sol Agro et Hydrosystème Spatialisation), INRA, AGROCAMPUS − F-35042 Rennes, France
[f] Forensic and Applied Sciences, University of Central Lancashire, PR1 2HE, Preston, UK

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ABSTRACT

To assess whether different sampling protocols provide similar results on earthworm community responses to land use, comparisons across different environments are required. Using an ongoing experiment in France, we assessed whether two protocols, widely used in international projects and global databases, provide similar estimates of earthworm abundance, and detect the same community responses to agricultural intensification. Method A consisted of hand-sorting composite samples of three soil monoliths 35 × 35 × 20 cm each, and applying formalin in the resulting holes. Method B consisted of applying formalin over a 1 m^2 contiguous area and subsequently hand-sorting a 25 × 25 × 25 cm soil monolith within it. Higher abundance was obtained from Method A than from Method B, but the two methods led to the same ecological conclusions. Firstly, they both showed that earthworm biomass and density decreased with agricultural intensification. Secondly, they showed similar land use effects on earthworm ecological group proportions, age structure, and body size distribution, pointing to a relative loss of large-bodied earthworms with agricultural intensification. These findings suggest that data from the two methods are both suitable to investigate the community response of earthworms, whereas assessments of earthworm abundance per se are more sensitive to the sampling protocol. Merits and drawbacks of the methods in terms of time and labour needed and of statistical variation are discussed.

1. Introduction

Earthworms are an important component of soil biodiversity in terms of biomass and function, with strong effects on soil structure and processes (Blouin et al., 2013; Brown et al., 2000; Darwin, 1892; Pulleman et al., 2012) and on plants and other soil biota (Andriuzzi et al., 2015, 2016; Newington et al., 2004; Nuzzo et al., 2015). It is therefore unsurprising that they are the focus of many studies. What may surprise is the lack of conformity in sampling protocols, even though standardized methods exist (Anderson and Ingram, 1993; ISO/DIS, 2006): field studies with similar aims often differ in the technique used to extract earthworms, the amount of soil sampled, the size and number of sampling units. This complicates comparisons between studies, as has been emphasized recently in the earthworm distribution map for Europe by Rutgers et al. (2016). Rigorous comparisons of sampling techniques across multiple land use types are few, and it is not clear how the spatial extent (e.g. sampling area, simple or composite samples) may affect the results. In this study we sought to compare two sampling methods that are widely used in international research and that contribute to global databases. Specifically, we evaluated whether they provide similar results in terms of earthworm community response to agricultural intensification, and derived recommendations in terms of data quality and practicality for future studies as well as for the integration of data obtained by the different methods.

Both methods are based on a combination of hand-sorting and chemical extraction (Anderson and Ingram, 1993; et al., 2006; Van Vliet and de Goede, 2006; Bartlett et al., 2010). Hand-sorting consists of excavating a soil monolith and searching for earthworms manually; chemical extraction consists of applying an irritant, non-lethal solution that infiltrates into the soil and induces earthworms to come to the surface. Other methods are available (reviewed in Bartlett et al., 2010), but hand sorting and chemical extraction techniques have the advantage of not requiring specialised equipment, and used together they ensure reliable sampling (Bartlett et al., 2010). Therefore, the current

* Corresponding author.
E-mail address: ws.andriuzzi@gmail.com (W.S. Andriuzzi).

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ISO protocol for earthworm sampling in temperate soils recommends a combination of hand-sorting and chemical extraction (ISO (International Organization for Standardization), 2004; Roembke et al., 2006).

Apart from the sampling *technique*, there are important variations in sampling *protocols* that may affect the results. Firstly, whether the chemical is applied on the undisturbed soil surface (e.g. Bouché, 1972), or after excavation of the soil below the sample (e.g. Van Vliet and de Goede, 2006); secondly, how many samples are collected and the size (area, depth) of each sample. There is scarce experimental evidence to assist researchers in making choices on trade-offs between sampling area and number of replicates or sub-replicates – for instance, is extracting earthworms from a composite soil sample of several small sub-replicates equivalent to extracting from fewer sub-replicates but larger size?

The two sampling protocols that we compare in this study differ in operational details, sampling area, and the number of spatially distinct sub-samples. Using an ongoing experiment in a European experimental field site, we assessed (1) whether the two methods provided similar results in terms of earthworm abundance (density, biomass) and community characteristics (diversity, ecological groups, development stage, and body size distribution); and (2) whether they provided similar results for earthworm response to agricultural management practices of different intensity. We also discuss merits and drawbacks of the two methods in terms of feasibility (time and labour needed) and statistical variation. Lastly we discuss whether harmonization of the results obtained by different methods is feasible. Our aim was not to identify the better of two competing approaches, not only because we lack reference data provided by an infallible sampling method, but especially because our interest was to evaluate the potential for linking data obtained through different protocols. Assessing whether soil biological data obtained with distinct sampling methods are comparable is a necessary first step before integration of single studies into larger databases.

2. Materials and methods

2.1. Sampling methods and study sites

Two earthworm sampling protocols were compared. Method A consists of excavating and hand-sorting a soil monolith (35 × 35 cm, 20 cm deep), and then applying 0.5 L of a 0.2% formaldehyde solution in the resulting pit to extract deep-dwelling earthworms. This method is widely used (Bartlett et al., 2010; Crittenden et al., 2014; Roembke et al., 2006; Van Vliet and de Goede, 2006) and technically based on ISO protocol 23611-1. Method B consists of first applying formalin to a 100 × 100 cm square (three applications of 10 L every 15 min, with 0.25% formaldehyde in the first two applications and 0.4% in the last one); afterwards, a 25 × 25 × 25 cm soil monolith in the square is hand-sorted to collect remaining earthworms, and these data are extrapolated to the rest of the square to complement the results of formalin application. This method has been used in extensive earthworm surveys in France (Cluzeau et al., 2012; Pèrès et al., 2011). Details for both methods are summarized in Table 1.

Table 1
Characteristics of the two earthworm sampling methods. HS = hand-sorting, F = formalin application. Area f refer to each statistical sample (which for Method A was a composite of 3 monoliths per plot).

	Method A	Method B
Soil monolith	35 × 35 × 20 cm, n = 3 per sample	25 × 25 × 25 cm
HS sample area	0.367 m^2	0.062 m^2
HS sample volume	0.0735 m^3	0.016 m^3
F application	Below monolith	On soil surface
Statistical samples per treatment	N = 3	N = 3

The study site was the Long Term Observatory "SOERE-ACBB" of Lusignan, France (46°25′12.91″N, 0°07′ 29.35″E), managed by INRA, where different crop rotation systems are compared in a randomized block design since establishment in 2005. The soil is a Cambisol with a silty-loam texture (14% sand, 63% silt and 23% clay), a pH 6.4, an organic carbon and total N content of 14.0 g C kg^{-1} and 1.6 g N kg^{-1} (Chabbi et al., 2009; Moni et al., 2010). Sampling was performed in the following selection of agricultural treatments, each replicated in three blocks: "Cr", a conventional arable annual crop rotation, with maize (2011), wheat (2012) and barley (2013); "CrGr", a six-year rotation system with three consecutive years of grassland (mowed annually, 2008–2010) followed by three annual crops (maize in 2011, wheat in 2012 and barley in 2013, as in Cr); and "Gr", permanent grassland since 2005. Cr and CrGr were sampled in autumn 2012 and 2013, Gr was sampled only in autumn 2013. At each plot (block × treatment combination), three 35 × 35 × 20 cm soil monoliths were sampled for Method A, at least 2 m apart and 2 m from Method B. For Method B one 100 × 100 cm^2 was sampled per plot. Therefore, Method A employed composite samples made of spatially discrete sub-samples, all of which were hand-sorted, whereas the sampling unit for Method B was a contiguous meter square plot that was treated with formalin, a small area of which was hand-sorted (Table 1; method A sub-samples were averaged before the analyses, see 'Statistical analyses' below).

2.2. Earthworm identification

Earthworm samples were stored at 4 °C on the same day of extraction and processed within the next two days. The fresh weight of each earthworm (gut content included) and total biomass were measured, and the number of individuals counted. Specimens were identified to species level according to Sims and Gerard (1999), Bouchè (1972) and Stöp-Bowitz (1969); juveniles that could not be ascribed to a species were identified to genus level. Earthworms were divided into ecological groups according to Bouchè (1977), i.e. anecic, endogeic and epigeic. *Lumbricus rubellus* was considered as an epigeic for simplicity, but we are aware that it may also be classified as an epi-anecic or an epi-endogeic.

2.3. Statistical analyses

To avoid pseudoreplication and have an equal sample size for the two methods, density data from Method A consisting of 3 subsamples per plot were averaged and scaled up to a m^2 basis; for Method B, data for the hand-sorted subset were also scaled up to one m^2, and added to the amounts obtained via formalin from the entire 100 × 100 cm square. Total sample size was thus n = 12 in 2012 (3 blocks × 2 treatments × 2 methods) and n = 18 in 2013 (3 blocks × 3 treatments × 2 methods).

All analyses were performed in the R statistical environment (R Development Core and Team, 2014). Sampling method and treatment effects on earthworm density, biomass, Shannon diversity, proportion of ecological groups (done both based on biomass and density data), and the ratio of adult to juvenile earthworms were analysed in mixed-effect models using the "nlme" package (Pinheiro et al., 2013), with treatment, sampling protocol and their interaction as fixed effects, and block as random effect. Methods were also compared in terms of variability of their results by calculating the coefficients of variation for earthworm abundance (CV = standard deviation/mean, expressed as %). To investigate the body size distribution in 2013, the relationship between abundance and body size in the four most abundant species (*Lumbricus centralis*, *Aporrectodea longa*, *Aporrectodea caliginosa* and *Allolobophora chlorotica*) was analysed with linear regressions of average body size against density (Local Size Density Relationship *sensu* White et al. (2007)), on a log–log scale as recommended by Turnbull et al. (2014). The more negative the slope, the greater the contribution of small-bodied earthworms to the community; vice versa, the more