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Potential protocol for earthworm monitoring under adverse soil conditions or in small plots

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ABSTRACT

Many methods for earthworm sampling have been developed with various sample sizes and soil volumes for physical, chemical, and electrical extraction. Each method has its benefits and shortcomings, as for example, in hard and dry soils aestivating earthworms are often unrecorded with chemical or electrical sampling. However, this study tested an alternative physical sampling method (Cylinder) for dry soils or small plots with a sample area of 78.54 cm^2 which was compared with a more universally-used Spade-method (400 cm^2). The Cylinder method used six soil augers per plot of 10 cm diameter and 20 cm length, driven into the soil by a heavy-duty electrical demolition hammer. Each Cylinder sample was hand-searched for earthworms for 15 min. The Spade-method comprised four soil monoliths per plot of 20 × 20 × 20 cm, with each hand-sorted for 45 min. Earthworm parameters such as abundance and biomass of total, adult, juvenile and ecological groups were compared for each technique. The study was part of an on-farm research project in Austria and eight sites were selected due to different soil types, climate and management systems (conventional vs. conservation). Conventional field management included soil tillage (depth 20–25 cm) without cover cropping whereas conservation practices comprised of no-till and the use of cover crops. The results for Cylinder were similar to Spade for Shannon index, abundance and biomass of most earthworm parameters, except for adult biomass (Spade was 7 % higher than Cylinder). In addition, power analysis showed that both methods are comparable and can detect a difference of 99 earthworms m^{-2} with 6 subsamples for Cylinder and 4 subsamples for Spade with $\beta = 80$ %. Nevertheless, due to the smaller sample volume, soil from Cylinder can be transported more easily than that from Spade and examined in the laboratory under more comfortable working conditions. In addition, Cylinder is less destructive to plots and can therefore be used for specific research purposes or in plots <2 × 2 m. The results of Shannon index, abundance and biomass of total, adult, anecic and endogeic earthworms were higher for conservation than conventional for both sampling methods. Results from this study may assist decision-making on choice of technique, depending on specific research objectives.

1. Introduction

To evaluate a habitat for its earthworm community, physical, chemical, and electrical methods have been developed over the years (Pelosi et al., 2009; Singh et al., 2016; Edwards and Arancon, 2022). Physical sampling, usually using a spade, needs to be done rapidly (before earthworms can try to escape) and with a standard soil area, anything from 20 × 20 cm to 100 × 100 cm (Butt and Grigoropoulou, 2010; Rutgers et al., 2016; ISO 23611-1, 2018). But sampling area, or the number of samples taken, may need to be limited if working in relatively small plots e.g., vegetable patches of 70 × 200 cm, experimental plots of 200 × 200 cm or e.g., by midden size of a species such as *Lumbricus terrestris* (Butt and Lowe, 2007; Euteneuer et al., 2024). In addition, finding an ideal time for sampling earthworms in large monitoring campaigns can be challenging, due to weather and soil conditions. Soils can become very hard when dried out and even more difficult to sample consistently. In particular, no-till soils, with their high soil resistance and bulk density, are more difficult to penetrate with a spade, but tend to have a higher earthworm abundance than ploughed fields (Dekemati et al., 2019; Liebhard et al., 2022). Under such solid soil conditions, some researchers use their full strength or stand on top of the spade and wiggle it into the soil, while others iteratively scrape the soil

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out of a slowly developing pit. Such attempts are very likely to disturb the earthworms and affect abundance records, as earthworms flee from vibrations (Mitra et al., 2009). Therefore, the quality of earthworm samples depends on the experience of the sampling team, plot size, number of samples and particularly soil conditions.

An obvious solution may be to use non-destructive, chemical (mustard suspension or formaldehyde solution), electrical extraction (e.g., the octet method) (Butt and Grigoropoulou, 2010) or a combination of physical and chemical extractions, but all methods are not ideal under particularly adverse soil conditions. Shortcomings of vermifuge extraction include poor infiltration of liquids into erodible soils (Weninger et al., 2019) or on slopes, so that earthworm extraction is not possible or biased. While electrical extraction may underestimate earthworm abundance and diversity (Rutgers et al., 2016) and underrepresent small earthworm species (Schmidt, 2001) and may only be effective at extracting earthworms living close to the soil surface or having burrows connected to the surface. These non-destructive methods will not extract aestivating earthworms from dry soils (Schmidt, 2001), so such earthworms normally go unrecorded.

Some areas where earthworms may be collected, such as the east of Austria, are diverse in soil types and weather conditions. For example, within 100 km of Vienna (Lower Austria), annual precipitation ranges from 425 to 1700 mm at an elevation of 146–900 m a.s.l., with the highest precipitation occurring mainly from May to September (68–91 mm month^{-1}; 14–20 °C; Supplementary Fig. S1) and mean annual temperatures are 6–12 °C (GeoSphere Austria, 2023). Since droughts have become more likely in the north-east of Austria due to climate change (Schönhart et al., 2014; Trnka et al., 2016), earthworm sampling methods need to be adapted accordingly. One possible solution for hard soils, and/or smaller plots is an electrically-driven soil auger. In this study, we tested the commonly used earthworm extraction method of digging (Spade) and an electrically-driven metal auger (Cylinder), in terms of sample volume taken, number of samples required and ease of handling.

To test the methods, an existing on-farm project with both conventional (ploughing) and conservation (reduced tillage) field management was used, because earthworms are sensitive to soil tillage. Earthworm abundance decreases with soil tillage intensity, e.g., in ploughed fields, a standard practice widely used in Austria, while conservation management with direct seeding or reduced tillage leads to higher earthworm abundance and species diversity (e.g., Briones and Schmidt, 2017; Dekemati et al., 2019, 2020).

The major aim of this study was to compare earthworm-related results using the two techniques (Spade and Cylinder) in parallel, to remove any bias associated with environmental or sampling conditions. Our objectives were to compare results from each method for abundance and biomass of earthworm communities across a variety of soil types and contrasting management systems. In addition, specific earthworm subcategories relating to developmental stage and ecological grouping were investigated, as was species diversity. We hypothesised, that Cylinder and Spade would show similar results for the investigated earthworm parameters under different field managements.

2. Materials and methods

2.1. Experimental sites and set-up

The research was conducted in 2022 at eight field sites located in north-east Austria in a temperate, continental climate (Table 1). Sites were selected beforehand due to their management systems, not because of any adverse sampling conditions, such as high soil penetration resistance or small plot size. The aim of the original research project was to compare earthworm parameters under different field management systems. Each field site is comprised of two management systems i) regular soil tillage (plough 25 cm depth or cultivator 20 cm depth) and no cover cropping (conventional), and ii) advanced agro-ecosystem management with soil conservation practices such as no-till or minimum tillage (i.e., disking 5 cm depth) and multi-species cover cropping (conservation). Two of the field sites are long-term soil tillage trials (since 2006) with a completely randomised block design and three plots treatment^{-1} (replicates; plots size 6 m width, 12 m length) with treatments no-till, plough with or without cover cropping ($n = 24$). The field sites selected for on-farm research changed their agro-ecosystem management one to ten years ago ($n = 12$).

Soil penetration resistance was measured by an electronic penetrometer (Penetrologger, Royal Eijkelkamp, Giesbeck, Netherlands). The measurements were carried out at 10 points per field or plot. Soil penetration resistance values were measured by 1 cm, 1 N accuracy, with a penetration speed of 2 cm s^{-1}, with a 1 cm^2 cone.

2.2. Earthworms and sampling methods

Earthworms were sampled in March and October 2022 using two methods (Cylinder; Spade) in parallel on the same fields/plots. Earthworms were counted, weighed, categorised into ecological group according to Bottinelli et al. (2020) and adult earthworms were identified to species level (Christian and Zicsi, 1999). Unidentified juveniles of any species of *Lumbricus* were recorded as *Lumbricus* spp. Earthworms cut due to sampling were only counted as an individual if the head was present. The proportion of cut to unharmed earthworms is unknown.

For the Cylinder method, samples were taken with a soil auger of 10 cm diameter and 20 cm depth (1570 cm^3; Fig. 1). The auger was driven into the soil by an electrical, heavy-duty demolition hammer (TE 805; Hilti Austria GmbH) to ensure a rapid insertion of 3–6 s. The soil cores were pushed out of the cylinders by an extractor (Fig. 1), during which earthworms were neither damaged nor crushed, and total sample time was <2 min subsample^{-1}. Six soil subsamples per field/plot were collected, inserted into plastic bags and searched for earthworms for 15

Table 1
Field site specifics with time of sampling (March; October), mean soil penetration resistance (depth 0–20 cm), soil type (WRB, 2014) and long-term annual sum of precipitation and mean annual temperature from 1991 to 2021 (GeoSphere Austria, 2023). Two of the sites are long-term soil tillage trials with true replicates (replicates treatment^{-1}) and the remaining sites are on-farm sites with 1 replicate treatment^{-1}. All sites comprised of conventional management practices (plough or cultivator, without cover cropping) and no-till with cover cropping.

Sites	Site specifics	Replicates treatment^{-1}	Sampling time	Soil penetration resistance (Mpa)				Soil type	Precipitation	Temperature
				Conservation		Conventional				
				Mean	± SD	Mean	± SD		(mm)	(°C)
1	Long-term soil tillage trial	3	Mar	1.02	1.09	0.461	0.281	Chernozem	541	10.7
2	Long-term soil tillage trial	3	Oct	2.46	0.536	1.16	0.385	Stagnosol	798	10.3
3	Farmer	1	Mar & Oct	1.95	0.513	2.43	0.47	Chernozem	615	11.1
4	Farmer	1	Mar & Oct	2.51	0.663	1.98	0.633	Chernozem	573	10.9
5	Farmer	1	Mar & Oct	2.82	0.702	2.14	0.49	Cambisol	550	9.6
6	Farmer	1	Mar & Oct	2.48	0.478	1.97	0.692	Cambisol	563	9.8
7	Farmer	1	Mar & Oct	1.64	0.672	1.14	0.261	Luvisol	643	9.6
8	Farmer	1	Mar & Oct	1.69	0.662	0.801	0.723	Luvisol	643	9.6

Fig. 1. Cylinder extractor with six cylinders (10 cm diameter, 50 cm length), power generator, electric cable and electric heavy-duty demolition hammer.

min sample^{-1} after return to the laboratory. Spade samples were collected in four soil monoliths of 20 × 20 × 20 cm (8000 cm^3) per field/plot and searched for 45 min subsample^{-1} in the field. The 45 min searching time was selected due to the high proportion of relatively small juvenile earthworms (found in former studies under dry spring and autumn conditions in East Austria (Euteneuer et al., 2020)). Sampling locations were selected systematically and spread over the whole field/plot to represent the field topography and to reduce autocorrelation (Valckx et al., 2011). Spade and Cylinder samples were upscaled to square metre by multiplication factors of 25 and 127.38, respectively.

2.3. Data analysis

Earthworm parameters of abundance, biomass and Shannon diversity were analysed with a two-way linear mixed model (2-way LMM) with fixed factor method (Cylinder vs. Spade) and management system (conventional vs. conservation) without an interaction term. The model considered random effects month (March; October) as a repeated factor, and field site as a random factor (Piepho et al., 2003, 2004). Coefficient of variation was analysed similarly in a one-way LMM (1-way LMM) only with fixed effect method, but the same random effects including management systems. Mean soil penetration resistance (depth 0–20 cm) was analysed in 1-way LMM with fixed effect management system and the same random effects as earthworm parameters. Linear MM was performed with 'lmer' ('*lme4*' package) (Bates, 2015) in RStudio 6.1.524 (Posit team, 2023) using R 4.2.2 (R Development Core Team, 2023) using the residual maximum likelihood method. In addition, ANOVA was used with Wald-type *F*-tests and Satterthwaite's method to obtain denominator degrees of freedom (function 'anova' with type III hypotheses). The Shannon index was calculated with package '*vegan*' and function 'diversity' (Oksanen et al., 2020). In addition, normal distribution of residuals was inspected in QQ-plots and homogeneity of the variation was determined by plotting residuals against fitted values. To meet these assumptions, every dependent variable was square root transformed before analysis.

For power analysis, the required i) number of subsamples plot^{-1} (n_0) and ii) number of replicates (n_e) were calculated for each method. To increase the quality of these analyses, one data set of the long-term soil tillage trial at site 1 with three replicates ($n_e = 3$) and $n_0 = 4$ for Spade and $n_0 = 6$ for Cylinder was employed (Table 1). Number of n_0 for a given relevant difference of earthworm number m^{-2} or earthworm biomass g m^{-2} (δ) were calculated for a power of 80 % or 90 % by applying Eq. (1) (Piepho et al., 2022),

$$n_0 \approx \sigma_0^2 \left(\frac{n_e \delta^2}{2\left(z_{1-\alpha/2} + z_{1-\beta}\right)^2} - \sigma_e^2 \right)^{-1} \quad (1)$$

where σ_0 and σ_e are the variance of the subsamples and replicates and $z_{1-\alpha/2}$ is (1 − α/2) × 100 % and $z_{1-\beta}$ is the (1 − β) × 100 % quantile of the standard normal distribution. In addition, to determine the optimal number of n_e Eq. (2) was used,

$$n_e \approx \frac{2\sigma\left(z_{1-\alpha/2} + z_{1-\beta}\right)^2}{\delta^2} \quad (2)$$

where σ was calculated by Eq. (3).

$$\sigma = \sigma_e^2 + \frac{\sigma_0^2}{n_0} \quad (3)$$

3. Results

3.1. Earthworm monitoring

Results of earthworm parameters (Table 2) showed that the method

Table 2

ANOVA results of sampling data and coefficient of variation for total earthworm abundance (Total), total biomass of earthworms (Total_mass), abundance of adult earthworms (Adult), adult biomass (Adult_mass), abundance of juvenile earthworms (Juvenile), biomass of juvenile (Juvenile_mass), abundance of anecic earthworms (Anecic), anecic biomass (Anecic_mass), abundance of endogeic earthworms (Endogeic), endogeic biomass (Endogeic_mass) and Shannon index of a 2-way linear model with fixed factor management system (conventional vs. conservation) and method (Cylinder vs. Spade) with repeated measure month at eight sites in Lower Austria in two seasons. *F*-values; degrees of freedom = 1.

Parameter	Sampling data				Coefficient of variation			
	System		Method		System		Method	
Total	18.2	***	0.382		1.69		18.6	***
Total_mass	15.3	***	3.69		0.257		16.2	***
Adult	10.6	**	3.6		5.99	*	34.7	***
Adult_mass	7.63	**	8.09	**	3.14		36.9	***
Juvenile	3.39		0.479		2.08		8.99	**
Juvenile_mass	3.04		3.29		1.43		18.31	***
Anecic	7.53	**	3.49		1.35		13.1	**
Anecic_mass	16.4	***	0.145		0.526		10.8	**
Endogeic	9.99	**	0.164		0.566		14.8	**
Endogeic_mass	7.49	**	3.29		0.669		16.1	***
Shannon	15.4	***	3.77		1.17		5.04	

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

used had no effect on earthworm abundance or biomass (Fig. 2 A–C, E–J), except biomass of adults was 7 % higher for Spade than Cylinder (Fig. 2 D). Both methods showed similar results between management systems with higher number (178 ± 189; 117 ± 151 earthworms m^{-2}; respectively) and biomass of earthworms (44.2 ± 66.5; 27.1 ± 42.4 g m^{-2}; respectively) under conservation than conventional management (Fig. 2 A–D, G–J) with the exception of juvenile abundance (108 ± 145 earthworms m^{-2}) and biomass (14.1 ± 20.7 g m^{-2}) which were not different between management systems (Fig. 2 E, F).

The most abundant species was *Aporrectodea caliginosa* (Savigny, 1826) and *A. rosea* (Savgny, 1826) was the second most abundant with both methods (Table 3). Adult *Lumbricus terrestris* (Linnaeus, 1758) were found by both methods in the conservation system and at a slightly higher number for Cylinder than Spade, but could not be detected with Cylinder in conventional, while this species was found with Spade. *Aporrectodea longa* (Ude, 1885) was only present in conservation and recorded by both methods. A single epigeic earthworm species, *L. rubellus* (Hoffmeister, 1843), was only present in very small numbers and not considered for ecological group analyses. The Shannon index was similar for both methods and was higher for conservation than conventional within methods (Fig. 3). The coefficient of variation for Shannon index was similar in both methods (Supplementary Fig. S2), while for all other earthworm parameters the coefficient of variation was always higher for Cylinder than Spade. Management system had no effect, except for adult earthworms, when coefficient of variation for conventional was higher than conservation (Supplementary Fig. S3 A–J). In addition, mean soil penetration resistance (0–20 cm) was lower for conventional (1.58 ± 0.829 MPa) than for conservation (2.05 ± 0.794 MPa) ($F_{1,328} = 25.5$; $P < 0.001$; Table 1).

3.2. Sample size

The sample size analyses of site 1 showed that both methods can detect a significant difference for total earthworms of 114 earthworms m^{-2} between both management systems with β = 90 % (Fig. 4 A). For β = 80 %, these differences were reduced to 99 earthworms m^{-2} (Fig. 4 A). Conversely, to find a difference of 75 total earthworms m^{-2} the required number of replicates would need to be 7 or 5 (β = 90 % or β = 80 %, respectively) for both methods (Fig. 4 A). In addition, in an experiment with only 3 replicates, 9 or 4 subsamples $plot^{-1}$ for Spade and 8 or 6 subsamples $plot^{-1}$ for Cylinder (β = 90 % or β = 80 %, respectively) would be needed to detect a difference of 100 earthworms m^{-2} (Fig. 4 B). But Spade would need 5 times more subsamples $plot^{-1}$ (β = 90 %) than Cylinder for a delta of 90 earthworms m^{-2} between the management system.

Power analyses (β = 90 %) for abundance of adult, juvenile, anecic, endogeic earthworms showed that with 3 replicates $treatment^{-1}$ Spade and Cylinder detected similar differences between conventional and conservation and the delta was only 5–9 earthworms m^{-2} (Fig. 4 C, G, H). For endogeic earthworms, Cylinder needed 25 more earthworms m^{-2} than Spade to find significant differences between the management systems (Fig. 4 I). Similarly, for 10 subsamples $plot^{-1}$, results for Spade and Cylinder differed by only 3–14 earthworms m^{-2} including endogeic earthworms (Fig. 4 D, F, H, J). But for juvenile abundance, Spade would need 6 times more subsamples $plot^{-1}$ than Cylinder to find a significant difference for 72 earthworms m^{-2}.

Outcomes for biomass (β = 90 %) of total, adult, juvenile, anecic and endogeic earthworms were close to abundance. By using 3 replicates $treatment^{-1}$, the differences between the methods were 1–7 g m^{-2} and for 10 subsamples $plot^{-1}$ the differences ranged from 0 to 10 g m^{-2} (Fig. 5 A–J). Only results for anecic earthworms showed that Cylinder would need 17 replicates $treatment^{-1}$ to find a delta of 5 g anecic earthworms m^{-2}, while Spade would need only 6 replicates $treatment^{-1}$.

4. Discussion

Although Cylinder and Spade differed in total sampling volume of hand-searched soil, their outcome was similar for 10 of 11 investigated earthworm parameters within management systems. The exception was for adult biomass where Spade had 7 % higher biomass than Cylinder. A possible explanation can be the smaller sampling volume of Cylinder and the damage to earthworm bodies during sampling. Such damage is more likely to happen to adult than juvenile earthworms, related to their body size, but was not recorded in the current study. This could have led to a lower biomass for adult earthworms. Although vibration caused by the heavy-duty demolition hammer could have resulted in earthworms fleeing, we expect these vibrations to have limited effect. Driving a cylinder into the soil takes only 3–6 s, depending on soil conditions, and due to the speed of sampling. Mitra et al. (2009) investigated earthworms escaping to the soil surface, due to low level vibrations (<500 Hz), a technique used to catch earthworms for fishing bait. However, Mitra et al. (2009) reported that *Diplocardia* spp. came to the surface within 54–131 s and were rather large exemplars of 7–30 cm. It is possible that earthworms flee in any direction from the heavy-duty demolition hammer and adults possibly faster than juveniles, but we observed no earthworms on the soil surface and numbers of earthworms were similar for both methods.

The management systems had a higher effect on numbers of

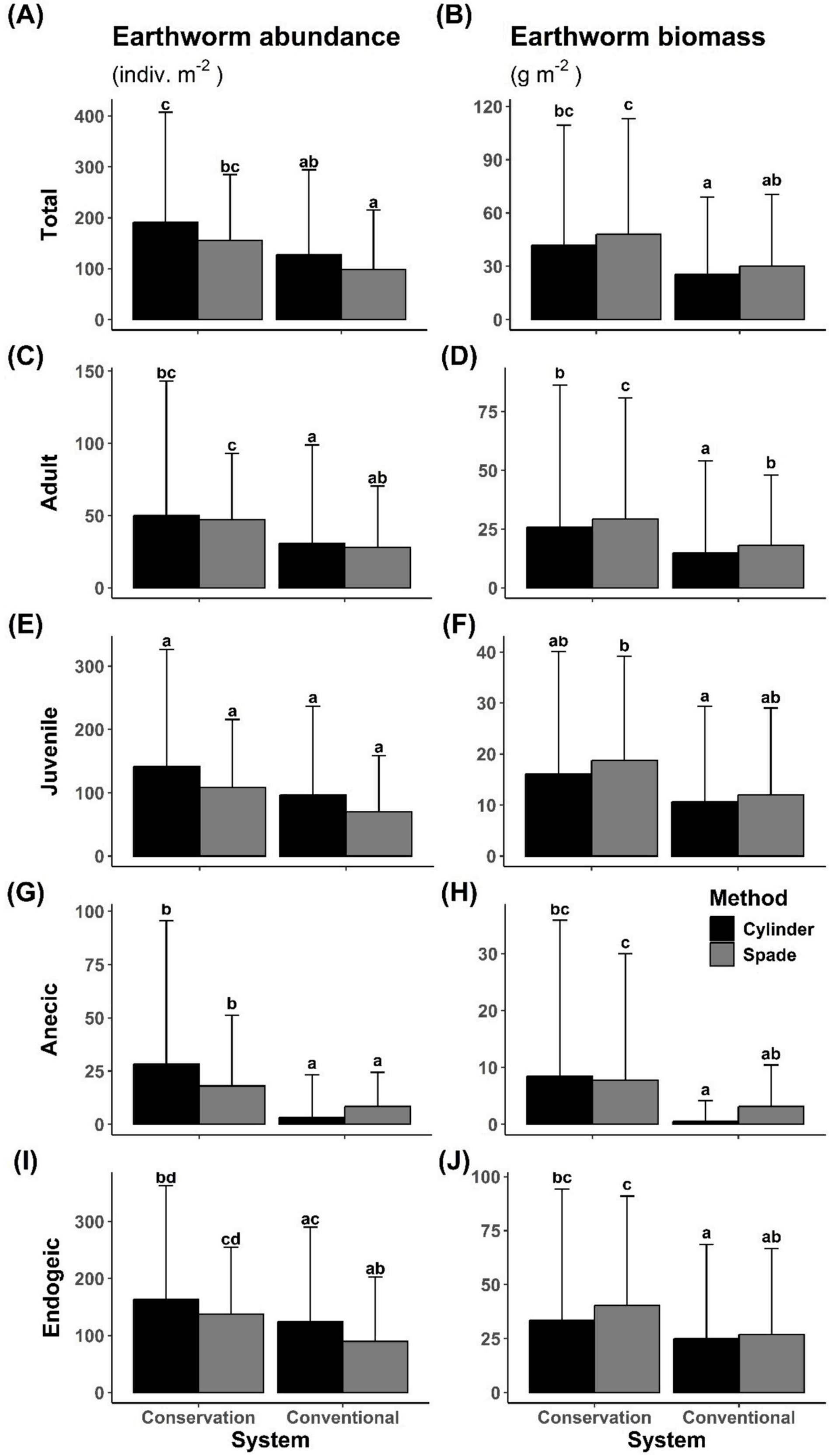

Fig. 2. Comparison of methods Cylinder vs. Spade with total earthworm abundance m^{-2} (A; Total), total biomass of earthworms g m^{-2} (B), adult earthworm abundance m^{-2} (C; Adult), adult biomass of earthworms g m^{-2} (D), juvenile earthworm abundance m^{-2} (E; Juvenile), juvenile biomass of earthworms g m^{-2} (F), anecic earthworm abundance m^{-2} (G; Anecic), anecic biomass of earthworms g m^{-2} (H), endogeic earthworm abundance m^{-2} (I; Endogeic), and endogeic biomass of earthworms g m^{-2} (J) at eight sites, two management treatments in one or two seasons. Methods per management system methods having no letter in common are significantly different by pairwise comparison (two-way linear mixed model, Tukey; $P < 0.05$). Values are mean + Standard deviation.

Table 3
Proportion of earthworm species found for each method (Cylinder or Spade) in conventional or conservation management systems in spring and autumn 2022 at eight sites in Lower Austria (Austria).

Earthworm species	Relative proportion			
	Conservation		Conventional	
	Cylinder	Spade	Cylinder	Spade
Allolobophora chlorotica	0.014	0.019	0.045	0.044
Aporrectodea caliginosa	0.397	0.161	0.401	0.183
A. rosea	0.137	0.099	0.200	0.061
Octolasion cyaneum	0.041	0.043	0.022	0.013
L. terrestris	0.041	0.011	0.000	0.017
L. rubellus	0.014	0.005	0.000	0.013
A. longa	0.014	0.005	0.000	0.000

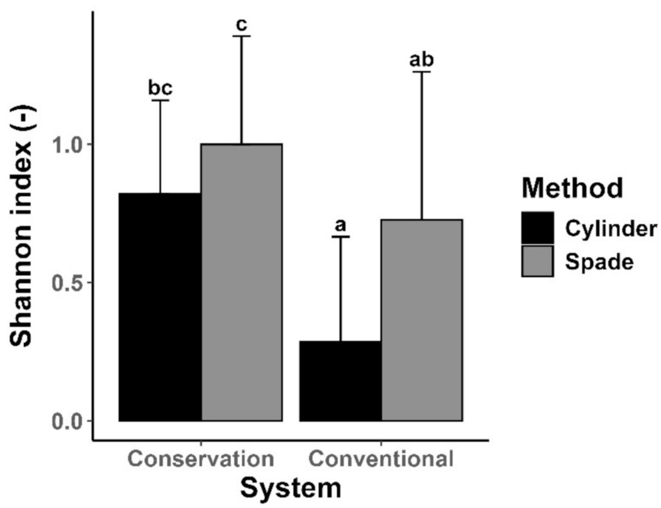

Fig. 3. Shannon index for comparison of methods Cylinder vs. Spade at eight sites, two management treatments in one or two seasons. Methods per management system having no letter in common are significantly different by pairwise comparison (two-way linear mixed model, Tukey; $P < 0.05$). Values are mean + Standard deviation.

earthworms found than the applied sampling method. Overall, we observed greater numbers (178 ± 189; 117 ± 151 earthworms m^{-2}; respectively), biomass (44.2 ± 66.5; 27.1 ± 42.4 g m^{-2}; respectively) and Shannon index (0.904 ± 0.368; 0.497 ± 0.502; respectively) in conservation than conventional tillage. This agrees with many previous studies (e.g., Crittenden et al., 2014; Dekemati et al., 2019). Earthworms are sensitive to soil disturbance and as seen in this study, especially anecic earthworms *L. terrestris* and *A. longa* decrease with increasing soil tillage intensity in conventional management systems (Crittenden et al., 2014; Stroud, 2016). The number of earthworms retrieved by Cylinder or Spade are similar to previous studies from north-east Austria and Hungary (Dekemati et al., 2019; Euteneuer et al., 2019, 2020). For example, in north-east Austria Euteneuer et al. (2019, 2020) found a range of 78–358 earthworms m^{-2} in March and 81–296 earthworms m^{-2} in October under various cover crop treatments and reduced soil tillage by using the Spade method and hand-searching in the laboratory. Additionally, in Hungary, Dekemati et al. (2019) found 117–154 earthworms m^{-2} in no-till compared to <50 earthworms m^{-2} under plough in April and October 2016 by using ISO standard (ISO 23611-1, 2018) with 25 × 25 × 30 cm, four subsamples $plot^{-1}$, and 30–40 min searching time in situ. Like Dekemati et al. (2019), we used no chemical expellant, as our observations show that chemical methods also depend on soil moisture under dry conditions and cannot expel aestivating earthworms. In addition, soils with high silt content or managed by ploughing are highly erosive, silt up when pouring liquids on to the soil surface or into soil pits and can prevent infiltration for many hours (Weninger et al., 2019). In a study of Čoja et al. (2008) it was seen that hand-searching and the electrical octet method produced 3–3.5 times less earthworms than the Kempson apparatus (mean 440 earthworms m^{-2}) (Meyer, 1996). Briefly, the Kempson apparatus (Meyer, 1996) uses soil samples of 50 × 25 × 25 cm and extraction of earthworms with heat within 12 days in the laboratory, while the octet apparatus sends pulses of electricity from eight electrodes inserted deeply into the soil (Thielemann, 1986). In addition, Čoja et al. (2008) compared i) hand-searched soil monoliths (50 × 25 × 25 cm) and then added 10 l of 0.4 % formalin following the ISO standard (ISO23611-1, 2007) and ii) used chemical extraction only via formalin or allyl isothiocyanate (AITC) in different concentrations (50 × 50 cm). The study showed that the Kempson method found highest abundance of earthworms followed by the octet method, hand-searching and chemical extraction. Čoja et al. (2008) suggested that the Kempson method and hand-searching produced similar numbers of adult earthworms (29 %), but hardly any adults were found using the octet method (<10 %). They stated that the use of the octet method is limited in low soil moisture and declared formalin and AITC as non-destructive alternatives. But Chan and Munro (2001) reported that mustard extraction revealed only 58 % of endogeic *Aporrectodea trapezoids* (Dugés, 1828) compared with hand-searching. Conversely, hand-searching underestimated the number of anecic *Anisochaetae* spp. (Wiecek and Messenger, 1972) by 21 % and biomass by 67 % compared to mustard extraction. In the current study, we found more *Lumbricus* spp., *L. terrestris* and *A. longa* with Cylinder than Spade, but overall numbers were very low and could not be statistically analysed. Further research could usefully investigate the effect of sampling method Cylinder and Spade on earthworm species retrieved.

Coefficients of variation were per se lower in Spade than in Cylinder and similar was seen in a method comparison by Andriuzzi et al. (2017), when hand-searching a greater sampling area (35 × 35 × 20 cm) showed less variation than a smaller sampling area (25 × 25 × 25 cm). Andriuzzi et al. (2017) indicated that the higher coefficient of variation can also be affected by spatial heterogeneity and dependence of biological variables. Hence, considering the experimental set-up and applied factors, soil tillage variables often show huge differences, as seen by Dekemati et al. (2019) particularly when comparing no-till to ploughed fields. Due to the higher variation in Cylinder than Spade, it seems possible that using Cylinders for weaker factors may not be sensitive enough, but the analysis of the sample size showed that 6 subsamples $plot^{-1}$ of Cylinder are comparable to 4 subsamples $plot^{-1}$ of Spade to find a difference of 99 earthworms m^{-2} when 3 replicates are used. Replicates for both methods need to be increased to 7 or 5 (β = 90 % or β = 80 %, respectively) to find differences of 75 earthworms m^{-2}. The delta range for total, adult, juvenile, anecic and endogeic earthworms for both methods was only 0–25 earthworms m^{-2} or 1–10 g m^{-2}. In addition, we believe a delta of 25 juvenile earthworms or 10 g adult earthworms m^{-2} between treatments is rather low, but an alternative for a raised number of replicates or subsamples are repeated measurements, for example in spring and autumn, according to Piepho et al. (2022).

Regarding the sampling area, 6 Cylinder samples take an area of 471 cm^2 while 4 Spade samples still use 1600 cm^2. This smaller demand of area for Cylinder can be an advantage for small plots such as experimental vegetable patches. In addition, due to the smaller sampling volume, and therefore lower destructive nature of Cylinder than Spade, Cylinder can be used for specific research purposes such as targeting the burrows of *Lumbricus terrestris* (Butt and Lowe, 2007; Euteneuer et al., 2024), on-farm research in market gardening with plot width of <1 m, mesocosms of 0.3 × 0.4 m (Andriuzzi et al., 2015) and generally plots smaller than 2 × 2 m. The smaller sample size also enables their transportation to the laboratory, while the larger and heavier Spade samples are usually directly searched in the field. Hand-searching Cylinder samples in the laboratory can be considered as more precise due to better working conditions such as sorting at a table in a comfortable sitting, working position, at room temperature, with an artificial light

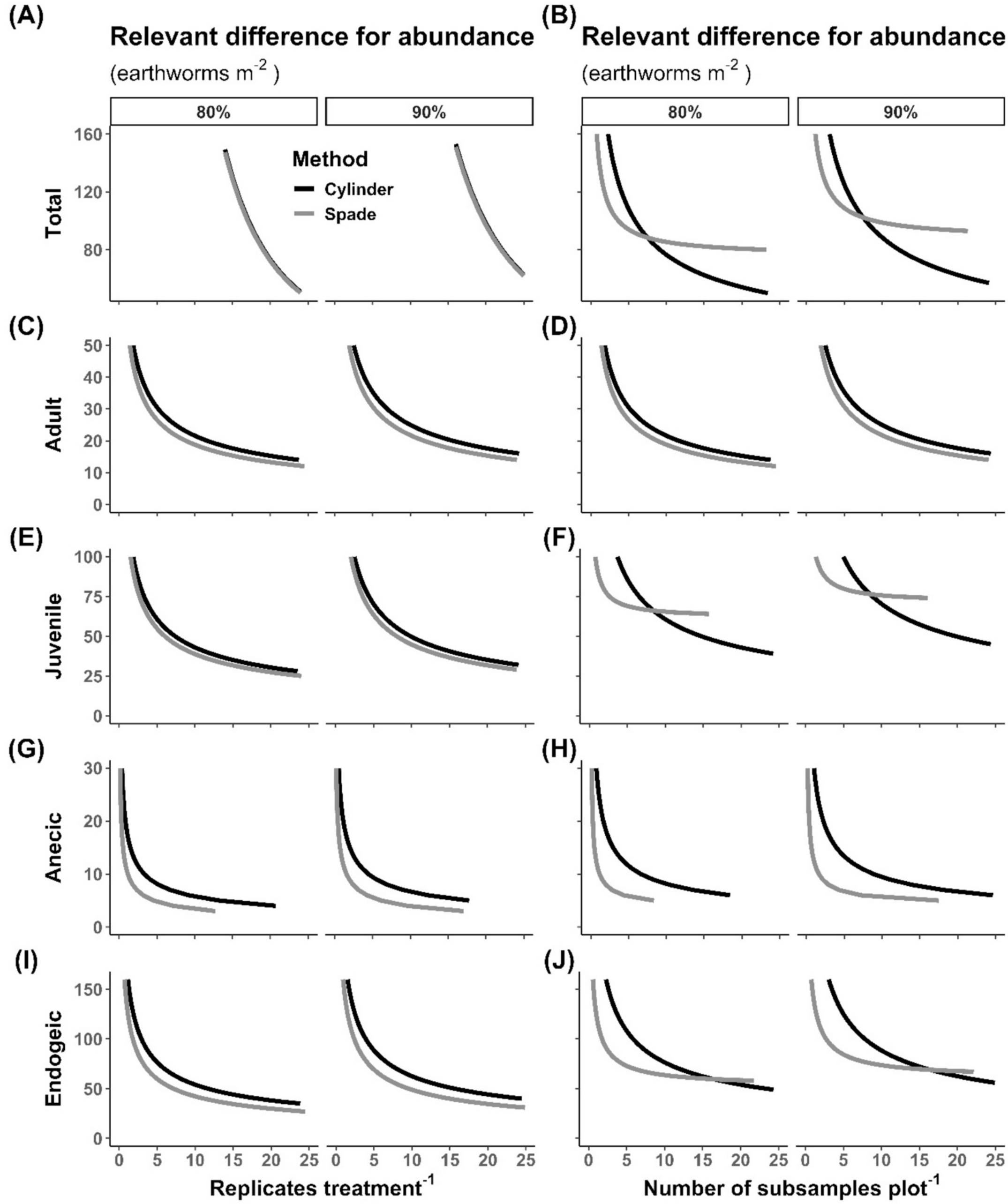

Fig. 4. Samples size analyses of optimal numbers of replicates treatment^{-1} (A, C, E, G, H) or subsamples plot^{-1} (B, D, F, H, J) for Spade and Cylinder based on Site 1, a complete randomised block design with a given number of replicates = 3 and subsamples plot^{-1} = 4 or 6 for Spade or Cylinder, respectively to detect a relevant difference of total, adult, juvenile, anecic and endogeic earthworm abundance (indiv. m^{-2}) with a power of 80 % or 90 %.

source of adjustable light intensity, rather than working at soil surface level in changing weather and light conditions. In addition, sampling with the electrical-driven Cylinder is more rapid (3–6 s), takes less time for hand-searching (90 min for 6 subsamples plot^{-1}) than Spade (180 min for 4 subsamples plot^{-1}) and is less dependent on the physical strength of the operator when driving the cylinder into the soil, especially in no-till plots, grassland, dry soils or in any other harder soil conditions. By contrast, the usage of a spade can be restricted by soil, weather, land use or management conditions such as compaction, low soil moisture, grassland or no-till. Cylinder is less affected by abiotic or working conditions and can be considered similar in terms of data quality to Spade. However, the current trial was performed during March and October, when the highest earthworm activity could be expected in East Austria, but the precipitation range of 146–1700 mm year^{-1} and the high temperature fluctuations from 14 to 20 °C makes it difficult to meet the ideal sampling time for Lower Austria. When main precipitation period began in May 2022 (31–171 mm), maximum temperatures also rose to 27–33 °C, which is not optimal for earthworm sampling. Thus, especially in the drier eastern part of Lower Austria, the sampling time is in constant conflict with the amount of precipitation and high temperatures. This is also reflected in the soil penetration resistance, which is mainly affected by soil moisture and soil tillage and increases in drier soils or under conservational management (Dekemati et al., 2019). Soil penetration resistance of conservation was 1.29-times higher than conventional and one site reached 2.8 MPa. Using a spade was not difficult at that time, but Dekemati et al. (2019) showed that during dry conditions, soil penetration resistance for no-till can exceed 6 MPa. It can be argued that earthworm samples should not be taken in such soil conditions, as earthworms are probably not active, but that depends on the research question, as seen with Dekemati et al. (2019)

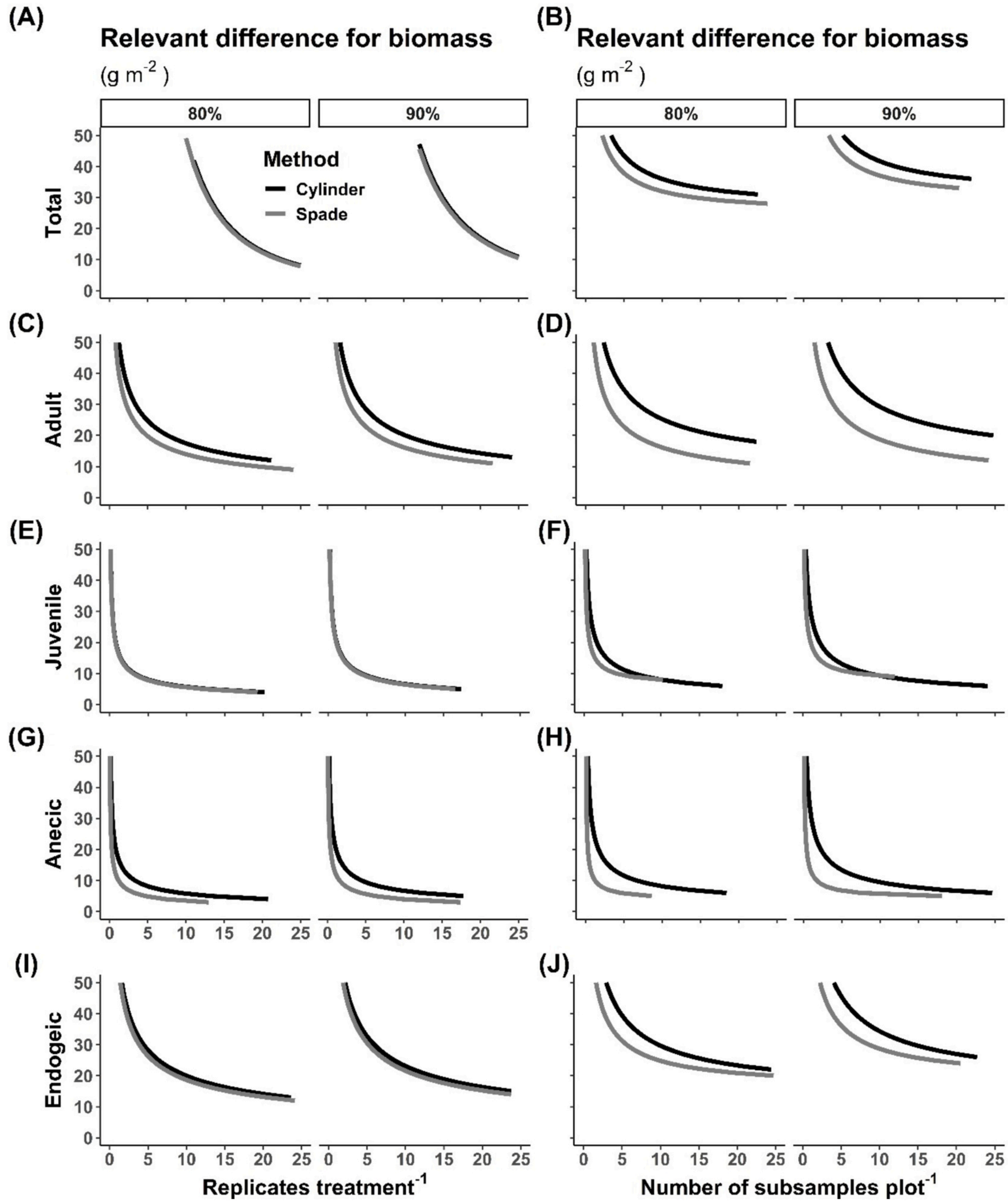

Fig. 5. Samples size analyses of optimal numbers of replicates treatment^{-1} (A, C, E, G, H) or subsamples plot^{-1} (B, D, F, H, J) for Spade and Cylinder based on Site 1, a complete randomised block design with a given number of replicates = 3 and subsamples plot^{-1} = 4 or 6 for Spade or Cylinder, respectively to detect a relevant difference of total, adult, juvenile, anecic and endogeic earthworm biomass (g m^{-2}) with a power of 80 % or 90 %.

who monitored earthworms from April to October. Dekemati et al. (2019) still found 41.7 ± 17.1 earthworms m^{-2} for no-till under dry conditions in June and a soil penetration resistance (0–20 cm) of 6.67 MPa. Comparing field management treatments and undertaking on-farm research can lead to a deeper insight of earthworms and real-world data, and to effects of adverse sampling conditions. We found that Cylinder is a comparable sample method to Spade which allows large sampling campaigns even across an area with various soils and challenging weather conditions.

5. Conclusion

Depending on the research objectives, Cylinder can be used to evaluate ecological effects e.g., on field management system and land use, if an appropriate subsample size is applied. This comparison of Cylinder vs. Spade can facilitate a decision on the applied method chosen, depending on objectives, location, space, time and human resources.

CRediT authorship contribution statement

P. Euteneuer: Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **K.R. Butt:** Writing – original draft, Validation, Supervision, Methodology, Formal analysis, Conceptualization.

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Declaration of competing interest

Pia Euteneuer reports financial support was provided by Boden. Leben. Pia Euteneuer reports a relationship with Boden.Leben that includes: board membership. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2024.105828.

Data availability

Data will be made available on request.

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