TECHNICAL NOTE

Earthworm sampling

In brief

Earthworms are key organisms and indicators of soil quality, which also links to the productivity of an ecosystem. The higher the earthworm density and diversity, the better the earthworm's living conditions and the more fertile is the soil. They are relatively easy to detect and determine into three ecological groups.

The number and distribution of the three ecological categories and the numerous earthworm species depends on soil management, soil type, the vegetation and climate conditions.

In this technical note, various methods how to sample, measure and interpret earthworms are shown.

Three ecological categories

There are more than 4000 species of earthworms worldwide, around 400 in Europe and around 40 in our latitudes (Switzerland, Germany). Bouché (1971) in France and Lee (1959) in Australia defined the ecological categories of earthworms. Initially, they characterized the species according to morphological, demographic, ecological and anatomical criteria. Later, Lee and Forster (1991) included behavioural characteristics and over the years, three categories simplified the identification of ecological groups. Some species, however, may have mixed properties and even a certain behavioural plasticity.

Occurrence and ecological needs

Site-specific factors as soil pH, content of organic matter, soil texture, land use and farm practices influence the occurrence and abundance of earthworms. The different spatial way of life and preferences roughly determine the three ecological categories in the soil layer.

- **Epigeic species** (leaf litter dwellers, 2-6cm long) decompose fresh organic matter close to the soil surface. They are small and mostly dark red. Preferred habitats: grassland, forest and compost, rarely found in cropland due to lack of permanent litter layers. They have a vigorous reproduction rate but a short lifespan.
- **Endogeic species** (shallow burrowers, small up to 18cm long) decompose organic substances in the soil and live in horizontal unstable galleries in the root area of the upper soil layer. They are pale, not pigmented. Their reproduction rate is limited (8-12 cocoons/year) and lifespan is medium (3-5 years).
- **Anecic species** (deep burrowers, 15-45cm long) pull plant parts from the soil surface into their vertical stable burrows (diameter of 8-11mm), where they decompose partly and get ready for feeding. Because of their behaviour, they are particularly sensitive to soil tillage at times, when they are active. Reproduction rate is limited and lifespan long.
  Anecic *Nicodrilus* species are large and black-brown. Adults partially place their faeces at the soil surface (worm casts).
  Anecic *Lumbricus* species are large and red-brown. Adult animals deposit their faeces into the soil or above ground.

Diese Publikation wurde im Rahmen des von CORE Organic Plus geförderten FertilCrop-Projekts als Partner des FP7 ERA-Net-Projekts CORE Organic Plus erstellt.

Advantages and disadvantages of the techniques

Earthworms can be extracted from the soil by pouring an irritant solution on the soil surface as formalin, mustard or allyl isothiocyanate, which flows into the soil pores and earthworm burrows. Once it meets an earthworm, it irritates the skin and brings the earthworm to the soil surface. However, earthworms can hide in niches, where the irritant solution cannot go to, therefore excavate and screen the soil for the hidden individuals by hand is often necessary for a precise recording (Tab 1). Depending on the preferred location of earthworms and the type of burrows, irritant is aimed primarily towards anecic species, and no hand sorting method carries the risk of underestimating endogeic. The combination of the two methods is considered as the gold standard in detecting abundance and diversity of earthworms.

Table 1: Methods in comparison

	Extraction	Hand sorting
Efficiency	accurate	very accurate
Species coverage	anecic species (deep burrowers)	endogeic species (shallow burrowers)
Field material	high material requirements and logistic	low material requirements
Time requirement	Relatively fast, 25-30 minutes…(waiting time due to multiple infiltration)	time-consuming (1-2 h)
Ground disturbance	None/low (depending on solution)	low
Earthworm injury	Formalin: toxic Mustard and allyl isothiocyanate: high survival rate	injured worms by digging devices
Examination area	large, on steep hills no extraction is possible	small (especially for spade test)

Extraction with an irritant solution drives a large part of the worms to the surface, where the individuals can be collected easily. The use of mustard solution is less efficient as compared to formol or allyl isothiocyanate, abbreviated AITC (Pelosi et al., 2009), but in combination with hand sorting, nearly all earthworms can be found. Another difficulty is the standardization of the mustard concentration. The advantage of mustard is its availability and harmlessness to the users. The extraction efficiency of the irritating solutions differs.

Fründ and Jordan (2003) give the following ranking: table mustard < mustard-flour ≈ mustard seeds < formalin.

It is possible to combine the earthworm extraction with the spade test by hand sorting the excavated soil first and then use the mustard extraction in the hole. However, the tremor produced by the digging and taking the spade sample may chase some earthworms to remote areas, where they can no longer be reached. This approach may therefore lead to a lower or more variable numbers.

Research question and experimental conditions

Occurrence and activity of earthworms vary greatly depending on weather, season and cultivation. The following conditions need to be considered for earthworm sampling:

- The ideal time to do the earthworm counting is their time period of maximum activity (March-April and September-October; Central Europe).
- Since the site is disturbed, be sure not to sample again on the same place. Sampling before farm practices as soil tillage.
- In dependence of soil heterogeneity and due to a general high variability, 4 to 6 replicates are recommended per field.
- The sampling design should be adapted to the particular research question.

Under the following conditions, studies on earthworm population are more difficult or impossible (Fig. 1):

- Dry, wet or waterlogged soil
- Hot weather and temperatures below zero
- In heavy clay soils, the extraction is difficult. In sandy soils earthworms occur in low numbers.

Figure 1: During winter coldness and summer drought deep burrowers remain rolled in and inactive (hibernation, aestivation periods). Photo: K. Huber

Instructions: Spade test followed by earthworm extraction

The extraction of earthworms can be combined with a spade test, which is based on the approach of Capowiez (2012). Note potential negative side-effects of the combination on sampling efficiency. After the excavation of soil for the spade test from the 30 × 30 x 30 cm volume, the earthworm burrows and macro pores are identifiable at the bottom of the hole. They indicate the activity and density of deep burrowing soil organisms. The spade sample can be described according to the spade test protocol of the FertilCrop technical note "Spade diagnosis to assess soil structure in the field" and afterwards it is hand sorted for earthworms as described above. Then the irritating solution is poured into the hole following the instructions above to collect the earthworms.

In FertilCrop, hand sorting was done before extraction.

Material

- Instruction and determination/identification help (ecological categories)
- Measuring tape / yardstick
- Spade
- Plastic sheet
- Plastic gloves
- Glass jars
- Waterproof pen for marking the jars
- Paper, pen
- Camera
- Stopwatch
- Watering can
- Canister whit 9 litres of water (for three extractions)
- 2 small commercial mustard pots (150 g *2) (for 9 litres of water)
- Laboratory balance
- 4% Formalin solution
- Filter paper

Prepare mustard solution

- For each watering, dilute 2 small commercial mustard pots (150 g *2) in a watering can with 10 liters of water.
- For each hole sampled: applied in total around 10-20 liters of spraying diluted mustard.

Hand sorting

- Excavate the volume of 30 × 30 x 30 cm and put the soil on the plastic sheet. Examine the crumbs for remaining earthworms.
- Look at the roots in particular, since they often hide in the dense roots below the shoot.
- Combine the earthworms with the previous ones.

Counting earthworm burrows

- Clear and level the surface at the bottom of the hole with a sharp knife to make the earthworm burrows visible. Count the open macro-pores on the total 30 × 30 cm area – use a frame to limit the sampling area.

Measure infiltration

- Place a yardstick at a visible area of the whole.
- Pour 3 litres of mustard solution into the whole (Fig. 2).
- Start a stopwatch immediately and stop record the time needed for infiltration per cm.

Spread irritant solution in the hole

- Every 10 minutes apply one third of the irritant test solution in the test area.
- The last worms can come out of the ground up to 30 minutes after the last dose - so wait.

Pick up the earthworms

- Carefully pick up worms with a tweezer as soon as they have completely come out.
- Place the individuals in a labelled tray, filled with moist paper.
- Rinse them with water to remove the irritant.

In the laboratory

- Wash and count immediately.
- Place individuals on filter paper to dry them superficially.
- Differentiate between adult (with clitellum / ring) and juvenile (without clitellum) depending on the research question.
- Group them into ecological categories by consulting an expert or a classification guide.
- Experts can help with the identification of the species.
- Count individuals (density) and weigh them with a good balance (biomass).
- Calculate the density and biomass per square meter.

Earthworm conservation and transport

Earthworms can be conserved in:

- 4% formalin: DNA will be damaged (solution for earthworm transport by air).
- 70% ethanol: DNA will be preserved (however, the ethanol has to be changed in the first weeks and photos help to identify the colour that changes in ethanol).
- Transport in a solution of 4% formalin is possible.

Figure 2: Mustard solution is poured into the hole and the infiltration is measured. Photo: D. Antichi

Classification of the results

The following factors contribute to a high earthworm density:

- Little disturbances in soil (soil tillage, plant protection)
- Vegetation (grassland, green manure)
- Dead plant material (compost, mulch)

Information on density and biomass differs widely in the literature. Main reasons for the different presence are the specific conditions at each location. In addition, there is small-scale inhomogeneity in the soil, as well as seasonal differences in the distribution of earthworms. The population densities in soil depends on the intensity of farm practices (soil tillage, input of pesticides, diversity of crop rotation, use of heavy machinery), food supply (below and above ground) and soil moisture (rainfall, irrigation, …).

Regarding total density and biomass, in some countries, there are national or regional thresholds that can indicate if the amounts of earthworms found are at low, medium or high level. For instance in France, the threshold is at 150 earthworms per hectare (Cluzeau, 2015). However, this threshold does not take into account soil and climate conditions.

It is recommended to take repeated measurements over time (years) to monitor the change and confirm differences.

Note: The earthworm biomass better represents the ecological effect.

Interpretation of the findings

Observations	Possible conclusions and recommendations
Earthworms abundance and biomass	▪ The colonisation of the soil volume is depending on anthropogenic factors as farming intensities, crop rotation, pesticides etc., and site factors as soil type, food and moisture (Pfiffner, 2014). ▪ The higher the earthworm biomass and abundance, the better the related ecosystem services, such as decomposition of organic residues are carried out and the porosity is improved for infiltration. ▪ When glyphosate-based herbicides are used, the activity of anecic species on the soil surface stops a few weeks after application. While endogeic species in the soil are not affected in their activity, their reproduction is reduced by 56% within three months after the application (Gaupp-Berghausen et al., 2015). ▪ Use of herbicides reduces earthworm population by reducing the availability of residues (Pfiffner, 2014).
Ecological categories	▪ The ecological categories of earthworms provide different soil services. The grouping of earthworms into the categories indicates soil biodiversity and, which of the associated services they carry out. ▪ Best practice to increase anecic earthworms is to stop ploughing and cover the soil permanently with crops and green manure (Cuendet, 1997) ▪ Endogeic species are less susceptible to ploughing but as soon as crop residues are exported too, they decrease. Endogeic species seem to adapt better to disturbances caused by ploughing and may benefit from soil inversion because of the incorporation of organic matter (Pelosi et al., 2009). ▪ In arable soils, epigeic species occur in low numbers, depending on the amount of organic residues at the soil surface, since they are living in the mulch layer. ▪ While anecic earthworms are most susceptible to the superficial application of pesticides due to their behaviour, endogeic species react more sensitive to toxic pesticides being incorporated into the soil (Pfiffner, 2014).
Age of earthworms	▪ Adults and juveniles can indicate if soil has been disturbed: more juveniles indicate soil disturbance. ▪ A high number of juveniles may also show a high reproduction rates and therefore good living conditions. ▪ The total earthworm density increases significantly with reduced tillage compared to ploughing, mainly due to the increased number of juveniles, while cocoons have been shown to be five times higher with reduced tillage (Kuntz et al., 2013).
Key measures for promotion of earthworms	▪ Main reasons for the decline of earthworms are monoculture, crop residues removed, to long periods of bare soil, heavy machinery and intensive soil tillage as frequent ploughing, rotary cultivator and pesticides (e.g. herbicides). →What could be done: Sufficient food (plant material), no/less use of harmful pesticide, reduced tillage, no-till, avoidance of soil compaction, promotion of well-structured and aerated soils, appropriate fertilization, balances humus management within crop rotation (Pelosi et al., 2014)

More information

For more information about earthworms sampling and determination, see the website on the French participatory observatory of earthworm https://ecobiosoil.univ-rennes1.fr/OPVT_accueil.php

For more information about earthworm diversity in Europe, see the atlas of soil biodiversity http://eusoils.jrc.ec.europa.eu/content/latlas-europ%C3%A9en-de-la-biodiversit%C3%A9-des-sols

In the FiBL Shop you will find further technical guides about the earthworms with the following information: determination according to ecological categories, significance and measures to increase earthworm populations in agricultural soils https://shop.fibl.org/

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Imprint

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About FertilCrop
Fertility Building Management Measures in Organic Cropping Systems – FertilCrop is a project funded by the CORE Organic Plus funding bodies, being partners of the FP7 ERA-Net project CORE Organic Plus. The overall aim of FertilCrop is to develop efficient and sustainable management techniques aimed at increasing crop productivity in organic farming systems. More information about FertilCrop is available at www.fertilcrop.net.