FunDivEUROPE

Functional significance of forest biodiversity in Europe

Project number: 265171

Earthworm sampling

FunDivEUROPE (FP7) field protocol

V1.0

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Content

1 Introduction

Earthworms are the main group of soil macrofauna in temperate forests. They are considered ecosystem engineers as they do not only contribute to litter decomposition but also to soil bioturbation, contributing to macro-aggregation, porosity and drainage of soils. It is known that tree species through their litter quality have a strong influence on the abundance and composition of earthworm communities. Earthworm species are often grouped in three functional categories: epigeics living in the litter layer, endogeics living in the topsoil, and anecics feeding in the litter but living in permanent vertical burrows in the soil.

Earthworms are typically sampled by handsorting of a soil monolith, combined with an ethological method, such as formalin or mustard extraction (Valckx et al. 2011; ISO 23611-1 standard, see Römbke et al. 2006; Jeffery et al. 2010). Basically, a litter sample is needed to find epigeic worms, a soil sample to find endogeic worms and a mustard extraction to find anecic worms. We developed two sampling procedures, one to catch epigeics (**procedure 1, litter sampling**), and another to catch epigeics, anecics and endogeics (**procedure 2, litter sampling, mustard extraction and soil sampling**). All plots in a site will follow the same procedure. In order to decide on the procedure for a given site, the tree plot with the highest expected earthworm activity will be sampled following procedure 2. This is the monoculture plot of the tree species with highest litter palatability (see tree species functional traits). Based on the functional categories of earthworms found in this pre-sampling, the procedure is selected to sample the rest of the plots.

2 Scope and application

The idea is to investigate the effect of tree species diversity on earthworm number, biomass, species diversity and functional diversity. All these features will be further related to microbial activity, litter decomposition and humus form.

3 Objectives

The objectives of this task are to determine earthworm densities (in Nb. per m^2) and biomass (in g per m^2) per species and per functional category (epigeic, endogeic, anecic) and to derive earthworm diversity indices.

4 Location of measurements and sampling

4.1 Field sampling design

4.1.1 Number of replicates

The (30 x 30) m^2 plots are divided in nine (10 x 10) m^2 subplots. 1 sample per (30 x 30) m^2 plot is taken in a randomly assigned subplot.

4.1.2 Sampling scheme

One sample is taken in a randomly assigned subplot, more specifically in the dedicated zone for soil biological sampling. In principle, within that dedicated zone, the sample is taken randomly, but samples should not be taken close to tree stems, and whenever possible, locations in between trees of different species should be preferred over crowns of single species. The tree IDs of, and the estimated distance to, the three nearest trees are noted on the Earthworm Sampling Datasheet (see below). Also the rest of the sampling sheet is filled before taking the sample.

4.2 Sampling equipment

The following equipment is needed in the field:

Procedure 1 (litter sampling):

- Wooden frame of (25 x 25) cm^2 inner size
- A 30 cm plastic ruler
- 1 transparent **polyethylene** bag with closing zipper of 10 litres with a label for writing sample code
- Garden shear
- Trowel
- Knife (long and sharp)
- Permanent marker
- Pencil and earthworm sampling datasheet

Procedure 2 (litter sampling, mustard extraction, soil sampling):

- Wooden frame of (25 x 25) cm^2 inner size
- Wooden frame of (100 x 50) cm^2 inner size
- A 30 cm plastic ruler
- 1 transparent **polyethylene** bag with closing zipper of 10 litres with a label for writing sample code
- 1 plastic stacking box of 30 litres with handgrips
- 1 plastic tag to label the soil samples and add to the boxes
- 2 plastic jerrycans of 10 L water with 30 g mustard powder in suspension
- 1 plastic jerrycan of 10 L water with 60 g mustard powder in suspension
- 1 plastic garden watering can of 10 L with sprinkler
- 1 labelled sample pot with 70% ethanol solution
- Garden shear

- Plastic tweezer
- Trowel
- Knife (long and sharp)
- Spade with a long straight blade of minimum 25 cm high
- Permanent marker
- Clinometer
- Pencil and earthworm sampling datasheet
- Watch

The following equipment is needed in the field station (central place near the plots where samples are temporarily stored and pre-treated).

- Storage space for one stacking box of 30 litres per plot.
- Place for hand sorting samples. This place needs very good light conditions. Possibly foresee extra light source above hand sorting table.
- Table for hand sorting of minimum 2 m^2, covered with **white** plastic protection.
- Polyethylene sample containers (urine cups with label zone for writing sample code)
- Tweezers
- Permanent marker
- Water source
- Two boxes with pre-weighed doses of mustard in labelled plastic bags, one box with single 30 g dose and one box with double 60 g dose

The following equipment is needed at the home base.

- A laboratory fume hood
- Disposable gloves
- Safety glasses and gloves (to use for pouring formalin solution in the collection pots)
- Polyethylene sample containers (urine cups with label zone for writing sample code)
- 5% formalin (this solution should not be prepared locally, but in a laboratory with all needed protection of skin, eyes and respiratory system. It is made by adding 6 units of water to 1 unit of technical formaldehyde 35%. In practice 833 ml formaldehyde 35% is diluted with 5 l water).
- Tweezers
- Permanent marker

4.3 Frequency of sampling

Sampling is done only once in every plot, for the Experimental Platform in 2011, and for the Exploratory Platform in 2012. The period of sampling is spring and autumn, more specifically a period with humid soil conditions and positive temperatures (preferably no night frost). In spring sampling can start from the moment that all the snow is melted and no night frost occurs (probably March in Mediterranean, April in cold temperate and early June in boreal zone). In autumn, sampling can be done starting from full litterfall period onward (probably September in boreal, October in cold temperate and November in Mediterranean zone). At the same time the description of the humus form and the sampling of micro-organisms can be performed; preferably at the same time as soil sampling.

Attention! Avoid sampling of water saturated soils, as earthworm presence during that moment will most likely be biased. In addition, heavy rain makes the protocol hard or even impossible.

4.4 Sample collection, transport and storage – quality control in the field and between plots and sites.

Sample collection

Procedure 1

If ground vegetation is present, cut it away just above the ground with a garden shear on an estimated 30 by 30 cm square. Put the 25 x 25 cm wooden frame on the ground in the middle of the cleaned area. Carefully remove the ectorganic horizon from within the frame and put it in a labelled polyethylene bag. Use trowel or knife to cut the sides perpendicular to the surface and scrape the bottom of the sample. Sample OL, OF and OH, but not the mineral soil (more details in Futmon report on soil and water sampling: Cools & De Vos, 2010). Close the bag. It is ready for transport to the field station, where it will be hand sorted.

Procedure 2

If ground vegetation is present, cut it away just above the ground with a garden shear on an estimated 120 by 70 cm rectangle. Put the 100 x 50 cm wooden frame on the ground in the middle of the cleaned area. If the slope of the terrain at the sampling location is more than 5%, measure the slope with a clinometer, note it in the datasheet for later correction of the area sampled. Put the 25 x 25 cm wooden frame on the ground in the middle of the larger frame. Remove the litter from within the small frame and put it in a labelled polyethylene bag. Use trowel or knife to cut the sides perpendicular to the surface and scrape the bottom of the sample. Sample OL and OF, but not OH and the mineral soil (more details in Futmon report on soil and water sampling: Cools & De Vos, 2010). Close the bag, it is ready for transport to the field station, where it will be hand sorted. Take away the small wooden frame and remove now also the organic layer to exactly the same depth from within the large wooden frame and a narrow zone around it. We are ready for mustard extraction.

A jerrycan of single dose mustard suspension (30 g/10 L) is stirred and poured into the watering can. The suspension is sprinkled over the area making sure that it is well distributed including the narrow zone around the frame. Earthworms appearing at the soil surface inside the frame are captured with tweezers and put into a labelled collection pot with a bit of ethanol 70% solution. After 5 minutes a second dose single mustard suspension (30 g/10 L) is stirred and poured into the watering can. The suspension is sprinkled over the area and captured earthworms are added to the same pot. After another 5 minutes a jerrycan with double dose mustard suspension (60 g/10 L) is stirred and poured into the watering can.

The suspension is again sprinkled over the area and captured earthworms are added to the same pot until 10 minutes have past. The pot is carefully closed.

Finally a soil sample of 25 x 25 cm square and 20 cm deep is taken in the middle of the area, where the litter sample was taken before. Use the spade to cut out the soil monolith. Make sure the walls of the pit are at right angles to the surface slope (Fig. 1). Use the ruler to check the correct dimensions. It can be useful to mark a 20 cm depth line in the steel of the spade. For the depth the thickness of the ectorganic horizon should not be included, so measure 20 cm from the transition between OH and mineral soil; more details in Futmon report on soil and water sampling: Cools & De Vos, 2010). Put the soil sample in a box. It is not important to have an intact monolith. Even to take out the sample from the soil it can be easier to cut a cross in the sample with the spade and take it out in four smaller blocks. Add a labelled tag to the sample in the box. Add the bag with the litter sample and the collection pot with the worms from the mustard extraction to the box. The sample is ready for transport to the field station.

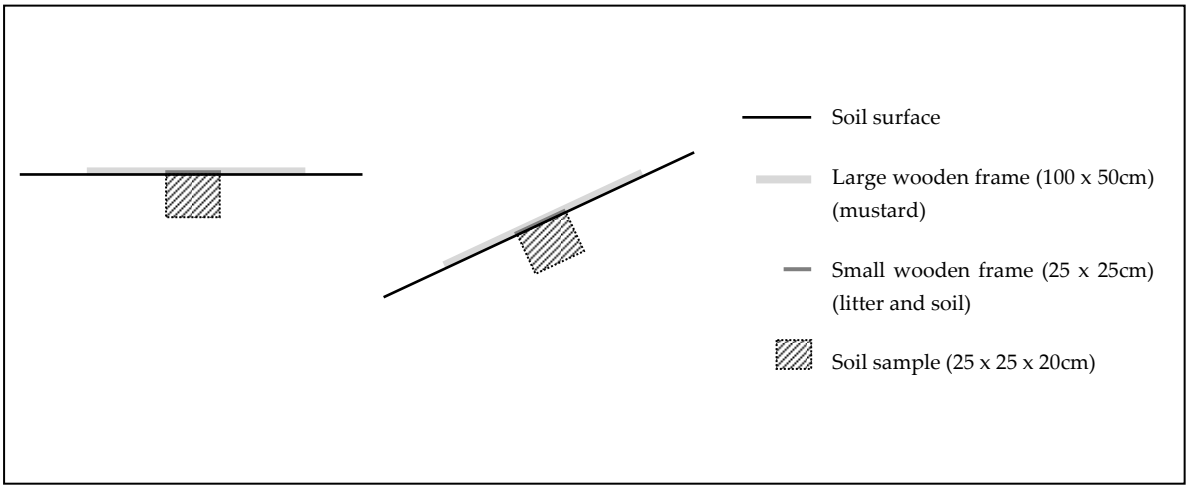

Figure 1: Schematic representation of earthworm sampling on horizontal and sloped terrain.

Sample storage

Boxes are stacked in the store. Each box contains a soil sample of 25 x 25 x 20 cm^3 and a plastic bag with the corresponding litter sample of 25 x 25 cm^2. Storage conditions: 5-15° C. Under permanent light if boxes are not hermetically closed to avoid escape of earthworms. If they are hermetically closed, they must be daily aerated during minimum 15 minutes. Storage time: max 4 days, then they have to be hand sorted locally.

Hand sorting

The sample (soil monolith or litter sample) is spread on one side of the table. In the middle of the table the material is crumbled between thumb and fingers of both hands and spread over the white table cloth in a thin layer, in order to detect earthworms, even very small ones.

Then the crumbled material is pushed on a pile towards the other side of the table. Once the sample is completely sorted and moved towards the other side, the whole procedure is repeated a second time until the material is brought back to the original position. For litter samples, it is important to separate intact tree leaves from each other and to inspect both sides of the leaves. Small epigeic worms are hard to discern, but thanks to the good light conditions and the white table cloth, their skin will appear more glossy than the more dull litter and soil material. Repeating the whole sorting procedure a third time, for litter samples, is therefore recommended. Found earthworms are taken by hand or with tweezers and added to a labelled collection pot with some 70% ethanol solution. Worms of litter samples and soil samples are kept in separate sample cups. These cups are carefully closed and stored in airtight plastic boxes.

Some recommendations for hand sorting

- Concentration and patience are important. Sorters may find it helpful to hide in the sample a few plastic worms or insects in order to keep the attention. This is helpful on condition that these items are about the same size and colour of real earthworms, otherwise they might distract the attention.

- Rhythm is also important. Not more than 40 minutes per sample should be spent by one sorter, or 20 minutes per sample by two sorters.

Transport

At the end of the field campaign when all plots are sampled, the collection pots are carefully packed in strong airtight boxes Inform to check if there are no special health/safety regulations to transport ethanol samples.

Fixation of earthworms

After arrival of the collection pots at the KU Leuven. The earthworms preserved on ethanol are transferred to pots with 5% formaldehyde solution. Formaldehyde makes earthworms hard and therefore easier to handle when determining them to species level. Working with formaldehyde always takes place under controlled lab conditions by means of a laboratory fume hood. After 3 weeks of fixation in formaldehyde, samples can be transferred again on 70% ethanol for further preservation.

Avoid contact of skin and eyes with formaldehyde, it is an irritating and carcinogenic substance. If contact rinse with plenty of cold water, and if necessary, see a doctor.

5 Datasheet Earthworm sampling

should contain:

- Date of sampling:
- Name(s) of sampler(s):
- Weather conditions (general weather description at the day of sampling: temperature, precipitation, cloudiness):
- Soil condition (water saturation%)
- Plot number:
- Diversity level:
- Tree ID's of and distances to the three nearest trees to sample point:
- Observations made: (e.g. slope percentage for every sample where slope > 5%)
- Problems encountered (indicate for which plot):

6 References

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