Soil animal assessment - field protocol overview

WATCH THE FIELD TUTORIAL VIDEO: https://www.youtube.com/watch?v=PkZuW0rJtZI

This protocol has been developed by the Soil BON Foodweb Team to sample soil animals from Soil BON sites (it is not the main Soil BON protocol). Each site represents a habitat. For the Soil BON site selection, please contact National coordinators. This protocol can also be applied beyond Soil BON to produce comparable data across studies. On each site, five sampling points are assessed: one at the georeferenced center of the site, and four at sampling points 15 m in each of the four directions, N, S, W, E from the center. All samples at each sampling point are taken randomly within one square meter area. In total, the following materials and information are collected from each site: (A) Site and sampling event description; (B) 5 soil cores for wet extraction; (C) 5 soil cores for dry extraction; (D) 5 vials with hand-sorted large macrofauna; (E) 5 vials with hand-sorted earthworms; (F) 5 plastic bags with litter samples for weighing; (G) 5 photographs of topsoil profiles.

Assessment of one site by two persons takes at least 3-4 hours.

CONTENTS

Soil animal assessment - field protocol overview 1
- Materials for the field sampling [to print] 2
- Fieldwork overview cheatsheet [to print] 3
- A: Site and sampling event description [to print] 4
- B and C: Samples for wet and Berlese extraction 5
- D, E and F: Hand-sorting of macrofauna and litter collection 6
- G: Topsoil photographs 7
- H: Pitfall traps (auxiliary) 8
- I and J: Deep soil macrofauna and earthworms (auxiliary) 8

Wet extraction hubs (wet hubs) 9
Dry extraction hubs (dry hubs) 9
Materials for the field sampling [to print]

**GENERAL FIELD EQUIPMENT**
- Tape-measure, 15 m
- 5 cm diameter soil corer or a PVC/metal tube
- A sharp knife to cut soil
- A ruler to measure sampling depth
- Flat-shape spade to dig soil samples
- 25 by 25 cm frame to measure area
- Secateurs to cut ground vegetation
- Plastic tray(s) to sort macrofauna
- Tweezers and brushes to catch fauna
- A phone with camera/digital camera
- Background laminated template with a printed ruler for photographs
- A pencil or ethanol-resistant marker
- A hammer to hammer cores (optional)
- A saw to cut large roots (optional)
- Headtorch (optional)
- Stander for vials (optional)

**FIELD EQUIPMENT PER SITE**
- 10 containers (~500-1000 ml) with lids or bags for transportation of soil cores
- 5 vials (15 ml+) with ~96% ethanol for macrofauna
- 5 vials (30 ml+) with ~96% ethanol for earthworms
- 2-3 backup vials for large animals with ~96% ethanol
- 96% ethanol (~150-200 ml)
- 5 plastic bags (~20-40 l) for litter
- 5 strong plastic bags (~40-60 l) for soil
- Labels* for vials and containers (6/sampling point, 30/site)
- Laminated label with the site code (if the site is not labelled by Soil BON and labelling of the site is allowed)
- Any bucket with water to wash earthworms (highly recommended)
- Gloves for field work (optional)

**LABORATORY EQUIPMENT**
- Scales to weigh the litter (min 0.1 g precision)

*Labels should be robust, ethanol/waterproof and go inside and outside of vials. Use a laser printer, or a pencil. Minimum information: Project ID (e.g. SBF = SBF Team), PI Surname, Site ID, Sample type (B / C / D / E / F / G) and sampling point number (1-5), Date (YYYYMMDD). Example, plot code, sample type and sampling point are emphasised in bold:

SBF_Potapov_BF3_B2_20220710

Fig. 1 | Field sampling equipment. Example solutions.
**DO AS MUCH AS POSSIBLE OUTSIDE THE SITE TO NOT DISTURB IT**

**A: Site description**

1) ![Camera](image1)
2) ![Site 1](image2)
3) ![Notebook](image3)

At each of the five sampling points:

**B and C: Soil cores for wet and dry extractions**

1) ![Soil core](image4)
2) ![Soil core with label](image5)
3) ![Laboratory](image6)

**D, E and F: Hand sorting of large macrofauna and litter collection**

1) ![Litter collection](image7)
2) ![Soil collection](image8)
3) ![Labeling](image9)
4) ![Laboratory](image10)
5) ![Laboratory](image11)

**G: A photograph of topsoil profile**

1) ![Litter collection](image12)
2) ![Photograph](image13)
A: Site and sampling event description [to print]

Make a photo of the entire sampling site overview with geolocalisation turned on (Fig. A1). Firmly fix the laminated label with the site code at the centre of the site (if it is allowed). Record the coordinates. Ensure to record complete site-level data (listed below). This and additional information must be provided in an Excel template available from the web page.

<table>
<thead>
<tr>
<th>projectID</th>
<th>ID of the project, fill “SBF” for the Soil BON Foodweb project</th>
</tr>
</thead>
<tbody>
<tr>
<td>researcher</td>
<td>Last (family) mane of the main contact person, e.g. “Potapov”</td>
</tr>
<tr>
<td>contact</td>
<td>Email of the person to contact regarding the data use</td>
</tr>
<tr>
<td>locationID</td>
<td>ID of the site, should be unique to the project (avoid using “Site1” etc)</td>
</tr>
<tr>
<td>country</td>
<td>Country name, e.g. “Nauru”</td>
</tr>
<tr>
<td>decimalLatitude</td>
<td>WGS84 latitude in decimals, max 30 m uncertainty, e.g. 9.9371</td>
</tr>
<tr>
<td>decimalLongitude</td>
<td>WGS84 longitude in decimals, max 30 m uncertainty, e.g. 51.5377</td>
</tr>
<tr>
<td>eventDate</td>
<td>Date of the sampling in YYYYMMDD format, e.g. 20220710</td>
</tr>
<tr>
<td>samplingMethods</td>
<td>“ABCDEFG”=core methods, add “H”, “I”, “J” for auxiliary methods</td>
</tr>
<tr>
<td>habitat</td>
<td>One from the list: “forest”, “grassland”, “shrub”, “agriculture”, ...</td>
</tr>
<tr>
<td>protectedArea</td>
<td>Fill “YES” for officially protected and “NO” for unprotected</td>
</tr>
<tr>
<td>vegetation</td>
<td>Free sentence to describe the age and type of the dominant vegetation</td>
</tr>
<tr>
<td>ecosystemHistory</td>
<td>Known info on the site history in the last 100- years; ecosystem age</td>
</tr>
<tr>
<td>eventRemarks</td>
<td>Deviations from the protocol, if any. Any further relevant information</td>
</tr>
</tbody>
</table>

FIELD CHECKLIST
- A: A photo of the site and site information
- B: 5 soil cores for wet extraction
- C: 5 soil cores for dry extraction
- D: 5 vials with hand-sorted large macrofauna
- E: 5 vials with earthworms
- F: 5 plastic bags with litter samples for weighing
- G: 5 photographs of topsoil profiles

POST-FIELD CHECKLIST
- Cores+vials transported to Local hubs within 1 week
- Sample list prepared in an Excel template
- Fresh and dry weights of litter recorded in the list
- Photographs were named (see labels) and submitted

Fig. A1 | Examples of the sampling site overview photographs
B and C: Samples for wet and Berlese extraction

Target groups: nematodes, enchytraeids, microarthropods, small macrofauna (~10 mins/point)

One core for wet extraction and one for dry extraction are taken at each of 5 sampling points (10 cores in total per site). Each core has 5 cm inner diameter and is taken with a corer/PVC/metal pipe or a 5 x 4 cm rectangle is cut (~20 cm² area). The sample includes litter (green vegetation remains, mosses, lichens and unfragmented dead leaves/needles and wood = OL horizon, if present; Figs. 1 and B1) and the underlying soil to a depth of 10 cm from the litter-soil interface (OF+OH+A+B horizons). In shallow soil, cores are taken down to the maximum possible depth. If bulk cores are not feasible to sample (e.g. rocky soils), litter can be collected by hand from a 5 x 4 cm area and the underlying soil (excluding rocks) can be extracted with a knife to a depth of 10 cm to fill 200 ml volume.

1. Cut and remove the ground vegetation at the ground surface at the sampling spot; keep mosses and lichens.
2. Take a soil core down to a sufficient depth. If using a short corer/pipe, two or three layers are sampled sequentially and combined.
3. Measure 10 cm from the litter-soil interface (Fig. B1) in the sampling device. Remove and discard the deeper horizons.
4. Put the remaining litter and underlying soil in a plastic container/bag. Try to preserve the soil structure as much as possible.
5. Put a label inside, write the sample code outside (e.g. BF3_B2).
6. Close the container/bag and prepare it for transportation (a cool box is preferred).

All cores must be transported to the laboratories for extraction as soon as possible (one week maximum). Five cores are transported to the local Wet extraction hubs for wet extraction, five cores to the local Dry extraction hubs for dry extraction. Personal delivery is preferred; postal deliveries should be tested beforehand and materials packed appropriately. During the storage and transportation soil samples should not be pressed, strongly shaken, overheated or overdried; avoid direct sunlight.

Fig. B1 | Examples of topsoil profiles with sampling depths marked. Each sample includes the entire litter layer + 10 cm of the underlying substrate below the litter-soil interface (i.e. ‘soil’). Dotted white lines show litter-soil interfaces; solid white lines show maximum sampling depths.
**D, E and F: Hand-sorting of macrofauna and litter collection**

**Target groups:** Large macrofauna, earthworms, social insects, litter weight (~90 mins/point/pers)

One sample 25 x 25 cm (625 cm²) is taken with a spade at each of 5 sampling points (5 samples in total per site). The sample includes litter and the underlying soil to a depth of 10 cm (see Fig. B1). All macroinvertebrates (≥ 3 mm in body length) are hand-sorted using tweezers or paint-brushes and put in a vial with ~96% ethanol. Earthworms are washed in water and placed in a separate vial with ~96% ethanol. Mesofauna taxa except very large specimens (e.g. springtails ≥ 3 mm in body length) are ignored; enchytraeids are ignored. Hand sorting takes a **minimum time of 45 minutes per sampling point (two persons) and until the entire sample is checked.** Use a headtorch in limited light conditions. The animal collection must be done **outside of the site** to avoid disturbance and is highly recommended to be done at a laboratory within 2 days after the sampling.

1. Put the **25 x 25 cm frame** on the ground and press it down to fix.
2. Cut and **remove the ground vegetation** within the frame; keep mosses+lichens.
3. **Collect litter (+fauna)** inside the frame with your hands and place it in a plastic bag. Wear gloves if dangerous animals are present in the area.
4. **Excavate the underlying soil** (10 cm) with a spade and put it in another bag.
5. **Sort fauna from the collected litter** by placing small amounts from the bag into a sorting tray. Collect macrofauna ≥ 3 mm in body length and earthworms in the vials.
6. **Place the litter back** in the plastic bag after all animals are captured. Close the bag, put the corresponding label inside and write the sample code on the bag.
7. **Sort the collected underlying soil** by placing small amounts from the bag into a sorting tray and breaking soil aggregates. Collect macrofauna ≥ 3 mm in body length and earthworms in separate vials. Earthworms should be washed before fixation in ethanol to minimise the amount of dirt. Animals from litter and soil are bulked together. Put the checked soil back and **leave the site minimally disturbed**.
8. Put a **label** inside both vials (E for earthworms and D for other macrofauna).
9. Transport and submit the vials with animals to the local **Dry extraction hub**.
10. Transport the litter in plastic bags (5 samples) to your laboratory.
11. **Back at the laboratory:**
    a. Weigh each litter sample with at least 0.1 g precision (=fresh weight).
    b. Completely air-dry 5 litter samples separately in ventilated room (>48 hours)
    c. Weigh each litter sample again (=dry weight).
    d. Submit both fresh and dry weight to the **Central team**

---

**Fig. D1** | Hand-sorting of macrofauna: Litter removal within the 25 x 25 cm frame (a); Excavation of the soil monolith (b); Checking of substrates and animal collection (c).
G: Topsoil photographs

Target variables: Diagnostic horizons, soil type and morphology (~5 mins/point)

5 photographs of topsoil profiles (1 at each sampling point) are taken using a phone camera with a flashlight on (Fig. G1). The photo is taken in the pit excavated for macrofauna collection and should include the full litter layer and the underlying soil to a depth of 20 cm, or down to the maximum possible depth if the soil is shallow). Please, turn on geolocalisation on your phone to keep tracking the sample location.

1. **Excavate soil** from the macrofauna pit (D,E,F samples) down to a depth of ~25 cm.
2. **Slice one of the walls** in the excavated pit to make it vertical and flat.
3. **Place a ruler** (cm scale) on the cleaned wall, zero is the litter-soil interface.
4. **Place the corresponding label** on the cleaned wall near the ruler.
5. **Make a photograph** with a flashlight on.
6. **Upload all photographs** via the submission system.

---

*Fig. G1* | Example photograph of a topsoil profile with a template.
**H: Pitfall traps (auxiliary)**

**Target groups:** Mobile macrofauna, social insects

**Non-obligatory task, communicate with the National coordinator prior to implementation.** Five pitfall traps (1 at each sampling point) are installed. Each trap is a jar with a diameter of 7.5 cm and a height of ca. 9 cm (400 ml). Jars are dug into the soil so that the top edge of the glass is exactly level with the **ground surface** to avoid creating an obstacle for surface-active invertebrates. The traps are protected from rain and other disturbances by a roof (any waterproof material) and filled with 100 - 150 ml 75% propylene glycol. If there is high pressure from grazing livestock, the poles supporting the roof can be fitted with metal plates to prevent the roof from being trodden into the ground (Fig. H1). Pitfall traps are left in the field for 14 days. When collecting, the glass jars are closed with a lid and transported to the lab, where the invertebrates are rinsed with tap water and transferred to vials with ~96% ethanol for preservation. The five vials with collected fauna are submitted to the local **Dry hub** for imaging and storage.

![Fig. H1 | Installed Pitfall trap.](image)

**I and J: Deep soil macrofauna and earthworms (auxiliary)**

**Target groups:** Soil-living earthworms and other invertebrates

**Non-obligatory task, communicate with the National coordinator prior to implementation.** J and I samplings are incorporated in the core macrofauna sampling (D+E). After the top 10 cm of soil is removed, another underlying 10 cm are excavated and put in another plastic bag. The excavated soil is hand-sorted; macrofauna and earthworms are captured and preserved in ~96% ethanol in separate vials following the same approach as described above. This sampling results in two additional vials per **sampling point** and 10 additional vials per **site.** J and I samples are stored and processed separately from D and E samples. The 10 vials with collected fauna and earthworms are transported and submitted to the local **Dry hub** for imaging and storage.
Wet extraction hubs (wet hubs)

Wet hubs are responsible for a standardised wet extraction of nematodes and enchytraeids and are established in each country/area. Each hub is expected to do extraction and animal imaging from up to 10 sites in the sampling year, i.e. up to 50 soil cores (one extraction for nematodes and one for enchytraeids per core). The hubs are also responsible for imaging and storage of the extracted animals in a freezer at ca. -20°C (in case no freezer is available, individual solutions should be found). Each collaborator team that does field sampling is affiliated to a Wet hub. Soil cores must safely and swiftly be transported to the hub (within one week; see B and C). Logistics should be clarified with National coordinators prior to the sampling. Animal sorting and identification from images is expected to be done NOT by the hub coordinators, but by the Central team. All protocols and manuals regarding the equipment and extraction process are available from https://soilbonfoodweb.org/protocols-and-manuals/

Dry extraction hubs (dry hubs)

Dry hubs are responsible for standardised dry extraction of microarthropods and are established in each country/area. Each hub does extraction and animal imaging from up to 10 sites in the sampling year, i.e. up to 50 soil cores (one extraction per core). The hubs are also responsible for the imaging and storage of the extracted animals in a freezer at ca. -20°C (in case no freezer is available, individual solutions should be found). Each collaborator team that does field sampling is affiliated to a Dry hub. Soil cores must safely and swiftly be transported to the hub (within one week; see B and C). Logistics should be clarified with National coordinators prior to the sampling. Identification from images is done NOT by the hub coordinators, but by the Central team. All protocols and manuals regarding the equipment and extraction process are available from https://soilbonfoodweb.org/protocols-and-manuals/