

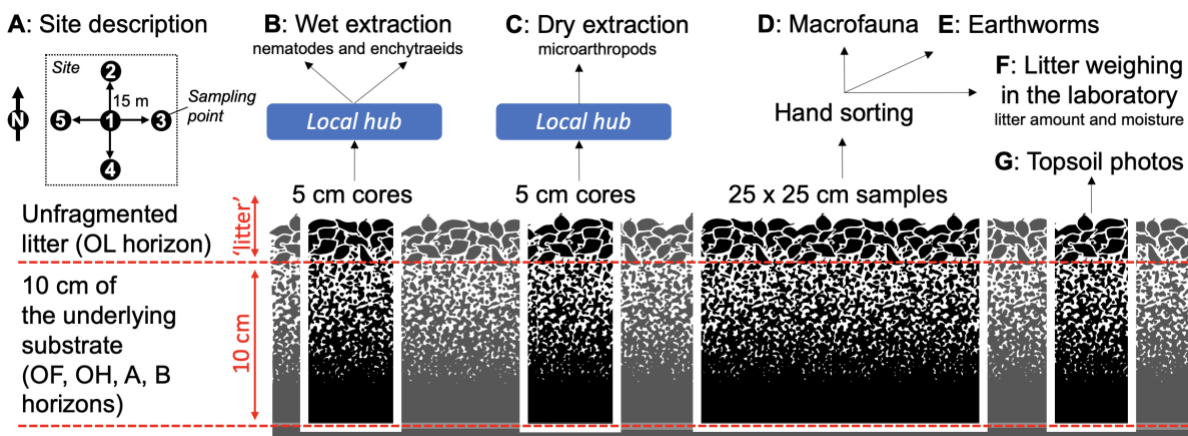
# Soil animal assessment - field protocol overview

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

VISIT THE WEB SITE: <https://soilbonfoodweb.org>

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 c. 3-4 hours.**



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## 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)

### LABORATORY EQUIPMENT

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

### 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 (15 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 water to wash earthworms (highly recommended)
- Gloves for field work (optional)

\*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.

# Fieldwork overview cheatsheet [to print]

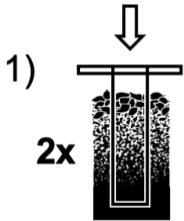
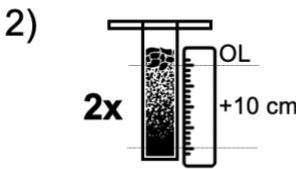
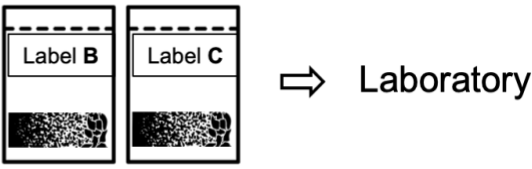
**DO ALL ACTIVITIES EXCEPT OF THE SAMPLING OUTSIDE THE SITE TO NOT DISTURB IT**

## A: Site description

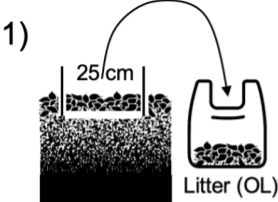
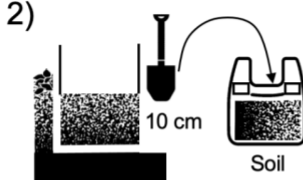
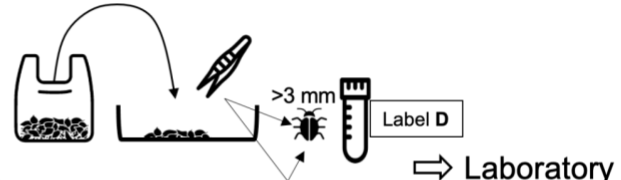
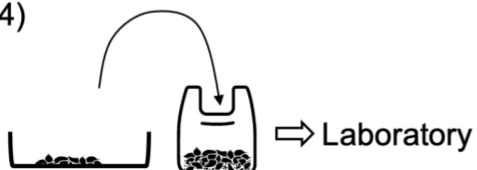
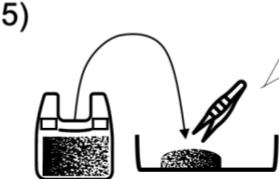
- 1) 
- 2) 
- 3) 

At each of the five sampling points:

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

- 1) 
- 2) 
- 3) 

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

- 1) 
- 2) 
- 3) 
- 4) 
- 5) 

## G: A photograph of topsoil profile

- 1) 
- 2) 

## A: Site and sampling event description [to print]

Make a photo of the entire sampling *site* overview with geolocation 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 in a table with exactly these columns, or upload this information via the submission system.

ProjectID	ID of the project, fill "SBF" for the Soil BON Foodweb project
Researcher	Full name (first+second) of the PI, e.g. "Anton Potapov"
ContactEmail	Email of the person to contact regarding the data use
SiteID	ID of the <i>site</i> , should be unique to the project (avoid using "Site1" etc)
Country	Country name, e.g. "Nauru"
Locality	Nearest named geographical location, e.g. village or mountain name
DecimalLongitude	WGS84 coordinates in decimals, max 30 m uncertainty, e.g. 51.5377
DecimalLatitude	WGS84 coordinates in decimals, max 30 m uncertainty, e.g. 9.9371
SamplingDate	Date of the sampling in YYYYMMDD format, e.g. 20220710
SamplingMethods	"ABCDEFGH"=core methods, add "H", "I", "J" for auxiliary methods
Habitat	One from the list: "forest", "grassland", "shrub", "agriculture", ...
ProtectedArea	Fill "YES" for officially protected and "NO" for unprotected
CurrentVegetation	Free sentence to describe the age and type of the dominant vegetation
LanduseHistory	Known info on the site history in the last ~50 years; ecosystem age
Comments	Deviations from the protocol, if any. Any further relevant information

### FIELD CHECKLIST

- A:** A photo of the *site* and *site* information
- B:** 5 soil cores for water extraction
- C:** 5 soil cores for Berlese 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
- Site* and sampling event descriptions were submitted
- Photographs were named (see labels) and submitted
- Fresh and dry weights of litter were submitted



**Fig. A1** | Examples of the sampling *site* overview photographs



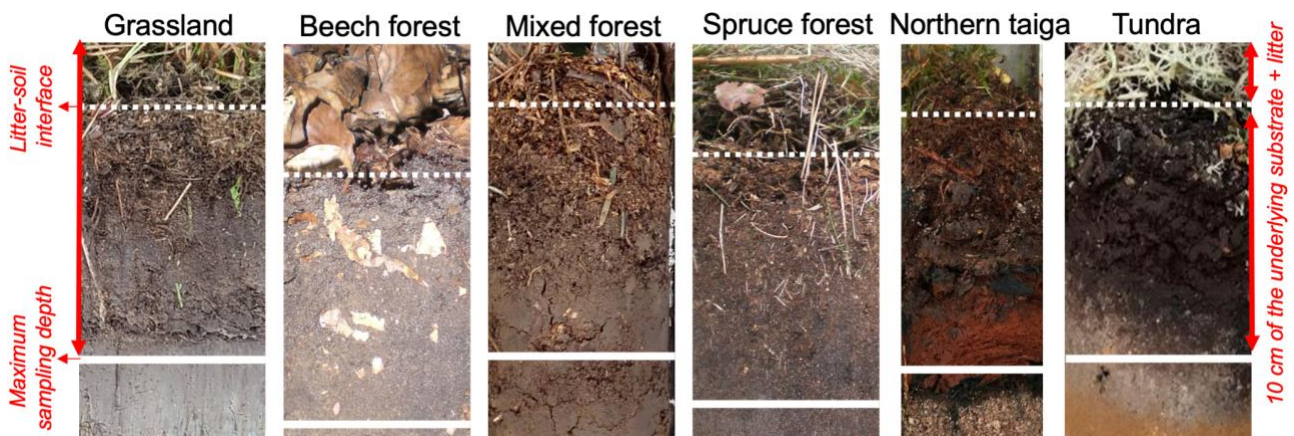
## B and C: Samples for wet and Berlese extraction

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 cm<sup>2</sup> area). The sample includes **litter** (green vegetation remains, mosses, lichens and unfragmented dead leaves 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 (~60 mins/point/pers)

One sample 25 x 25 cm (625 cm<sup>2</sup>) 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 ( $\geq 3$  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  $\geq 3$  mm in body length) are ignored (Table 1). Hand sorting takes **a minimum time of 30 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 can be done in the laboratory/field station if litter and soil can be safely transported there within a day.

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  $\geq 3$  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 fauna from the collected underlying soil** by placing small amounts from the bag into a sorting tray and breaking soil aggregates. Collect macrofauna  $\geq 3$  mm in body length and earthworms in separate vials. Wash earthworms in a tank with water to avoid dirty samples. 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. Air-dry all 5 litter samples separately in well-ventilated room until they are completely dry (>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.

## 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. 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 the storage of extracted animals. **Wet hubs** receive central support from the initiative and are closely enrolled in coordination, add-on project planning, and lead regional-scale synthesis studies. Field sampling is can be done by collaborators from this area. 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 is expected to be done NOT by the hub coordinators, but by the *Central team*. The protocols and manuals regarding the equipment and extraction process are provided to the registered hubs and will be available from [www.soilbonfoodweb.org](http://www.soilbonfoodweb.org)

## Dry extraction hubs (dry hubs)

**Dry hubs** are responsible for a standardised microarthropods extraction and are established in each country. Each hub does extraction and animal imaging from up to 30 *sites* in the sampling year, i.e. up to 50 soil cores (one extraction per core). The hubs are also responsible for the storage of extracted animals. **Dry hubs** receive central support from the initiative and are closely enrolled in coordination, add-on project planning, and lead regional-scale synthesis studies. Field sampling is can be done by collaborators from this area. 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 is done NOT by the hub coordinators, but by the *Central team*. The protocols and manuals regarding the equipment and extraction process are provided to the registered hubs and will be available from [www.soilbonfoodweb.org](http://www.soilbonfoodweb.org)