STANDARD OPERATING PROTOCOL · EnGeL-FAUNA-MAR-MORPH

Sampling, MacFadyen extraction and sorting of soil microarthropods

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Area	Fauna sample processing (FAUNA)
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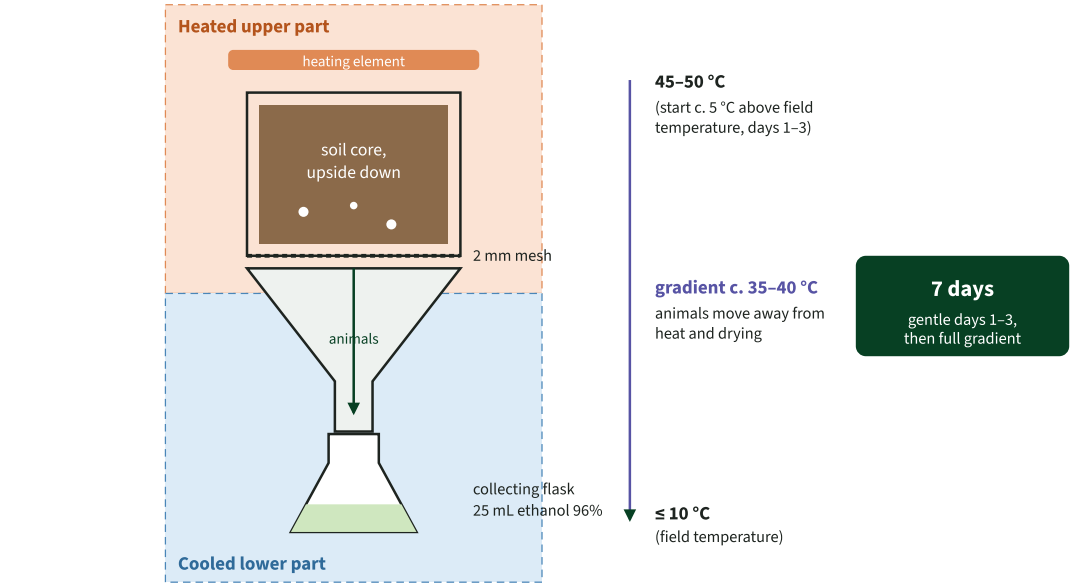
Licence	Text: CC BY 4.0 · Code: MIT
Main hazards	 Ethanol 96%: highly flammable, serious eye irritation (H225, H319); keep away from the extractor heating elements; Hot extractor parts (45–50 °C)

1 Purpose and scope

This protocol covers the field sampling of soil microarthropods from the litter/organic layer and the first 5 cm of mineral soil, their extraction with a MacFadyen high-gradient extractor (7 days), and their sorting into major groups and morphotypes. Sorted animals are preserved in 96% ethanol for identification and for DNA-based community analysis (EnGeL-MOLB-DNA-MAG, EnGeL-MOLB-DNA-COL, EnGeL-MOLB-MB). Results are densities, morphotype richness and diversity, and indices of soil quality (QBS-ar) and Collembola life-forms.

2 Principle

Soil cores are taken with a split corer (litter layer) and a PVC tube (undisturbed mineral soil). In the laboratory the cores are placed upside down in a MacFadyen extractor: the upper part is heated gradually while the lower part is cooled, so the animals move down, away from the heat and drying, and fall into flasks with 96% ethanol. The extracted animals are sorted under a stereomicroscope into major groups and morphotypes based on life-form traits (Macfadyen, 1962; Dindal, 1990).



Schematic, not to scale. Values from EnGeL-FAUNA-MAR-MORPH, Procedure.

Figure 1: MacFadyen high-gradient extraction. The core sits upside down on a 2 mm mesh; the upper part is heated (45–50 °C after a gentle start) and the lower part cooled (≤ 10 °C), so the animals move down into the flask with 25 mL of ethanol 96% over 7 days. Schematic, not to scale. Figure: EnGeL-UC, CC BY 4.0.

3 Before you start

Item	Details
Hands-on time	[TO CONFIRM]
Total time	Field sampling + 7 days of extraction + sorting [TO CONFIRM: sorting time per sample]
Sample	Litter/organic layer (split corer) and mineral soil core 5 cm × 5 cm (c. 100 cm^3) per sampling point
Sample condition	Time between sampling and extraction ≤ 5 days; keep samples in an aerated space at room temperature
Prepare in advance	Sampling design; labelled boxes, bags and tubes; extractor set up; flasks with 25 mL ethanol 96%
Key safety points	Ethanol near heating elements: see Safety and waste

4 Materials

4.1 Equipment

- MacFadyen high-gradient extraction apparatus (canisters with 2 mm mesh, funnels, collecting flasks, ceramic heating elements, cooled lower part)
- 5 cm diameter plastic split corers (litter / organic layer)
- PVC (or stainless-steel) tubes, 5 cm diameter × 5 cm height (c. 100 cm^3), with plastic caps (mineral soil)
- Wooden or rubber block, mallet, spade and knife
- Measuring tape
- Stereomicroscope
- Drying oven
- Forceps (including soft entomological forceps), pipette, fine painting brush, fine needles
- Wash bottle

4.2 Consumables

- Collecting flasks
- Petri dishes
- Plastic vials and glass vials
- Plastic bags, boxes or flasks to store soil samples; paper tape
- Styrofoam boxes for shipping
- Pencil, notebook, permanent and water-resistant markers, labels

4.3 Reagents and kits

- Ethanol 96% (25 mL per collecting flask, plus storage vials)

5 Reagent preparation

Not applicable: 96 % ethanol is used as supplied.

6 Procedure

6.1 Before starting

- Define sampling points according to the sampling design of the study.
- Register the samples in the sample master data file to obtain EnGeL labels (type of sampling `Macf`).

6.2 1. Collecting the soil samples

The aim is to sample animals in the litter/organic layer and the first 5 cm of soil (mineral layer). The litter layer is collected to extract epigeic microarthropods, which are important in forests (Potapov et al., 2016, 2018; Pollierer and Scheu, 2017). Agricultural sites often have no litter layer; in that case only the mineral core is taken.

1. At each sampling point, use a split corer to collect a soil sample.
2. Open the corer. Optionally, take a photo of the soil core profile to complement the site characterisation.
3. Separate the sample inside the corer into the litter/organic layer (including the humus horizon; variable thickness is expected) and the mineral soil.
4. Discard the mineral soil in the split corer (mineral soil is collected with an additional PVC tube).
5. Record the depth of the litter layer.
6. Place the litter sample in a labelled plastic box (Annex 1).
7. For the undisturbed mineral soil, remove the litter layer to expose the mineral soil and take a core to a depth of 5 cm with a PVC tube (5 cm diameter × 5 cm height, c. 100 cm^3).
8. Drive the PVC tube gently into the soil using a wooden or rubber block and a mallet, to avoid compacting the soil.
9. Remove the PVC tube from the soil with a spade placed underneath it. Remove excess soil around the tube with a knife.
10. Close both ends with plastic caps, seal them with paper tape to preserve the soil structure, and place the tube in a labelled plastic bag.

> **CRITICAL** Avoid disturbing the core structure and compacting the soil, which could kill the animals. The time between sampling and extraction should not exceed 5 days, to minimise effects of confinement and shifts in the micro-populations. Keep samples in an aerated space at room temperature until shipping.

11. For shipping, pack the samples in a styrofoam box.

6.3 2. Extracting microarthropods

12. In the laboratory, set up the MacFadyen extraction system.
13. Place each sample core upside down in a canister with a plastic or metal net (2 mm mesh) at the bottom.
14. Connect the canister to a funnel attached to a collecting flask containing 25 mL of 96% ethanol.

15. Build up a temperature difference of about 35–40 °C between the upper and lower parts: heat the upper part to 45–50 °C while cooling the lower part to a maximum of 10 °C (field temperature). Circulate heated air in the canister area and cooled air in the collecting area.
16. Increase the temperature of the upper part gradually, starting at about 5 °C above field temperature during the first 2–3 days, to prevent sample desiccation and animal death.
17. Then intensify the gradient (upper part 45–50 °C) for the remaining days. The total extraction time is 7 days.

6.4 3. Sorting and preserving

18. After extraction, label the animal samples for identification and prepare them for storage until sorting.
19. Place each sample in a Petri dish under the stereomicroscope.
20. Sort the animals into major groups and morphotypes according to life-form traits (pigmentation, antenna length, presence of ocelli, furca and scales/long hairs). Handle animals with a pipette or a fine brush.
21. Transfer each group/morphotype to a plastic vial with 96% ethanol and store at room temperature (ideally in the fridge if there is space) for molecular analyses.

> **PAUSE POINT** Sorted animals in 96% ethanol can be stored at room temperature (ideally in the fridge if there is space) until identification or DNA extraction.

6.5 After finishing

- Clean the bench with 70% ethanol.
- Empty the small bench bin into the right waste bin.
- Wash your hands.
- Turn off equipment and lights.
- Lock doors, fridge and freezer.

7 Calculations

Density

$$D = \frac{N}{A}$$

- D: density (individuals m^{-2})
- N: number of individuals in the sample (litter and mineral core together)
- A: core cross-section (m^2); for a 5 cm diameter core, A = 0.00196 m^2, so $D \approx N \times 509$

Worked example (illustrative value): 120 individuals in a 5 cm core → 120 / 0.00196 ≈ 61 100 individuals m^{-2}.

Diversity: morphotype richness S (number of morphotypes) and Shannon diversity $H' = -\sum_i p_i \ln p_i$, where p_i is the proportion of individuals in morphotype i.

QBS-ar $= \sum \mathrm{EMI}$: the sum, over the microarthropod groups present, of each group's highest eco-morphological index score (EMI, 1–20) (Parisi et al., 2005).

Collembola life-form index: each collembolan morphotype is scored on its life-form traits (ocelli, antenna length, furca, pigmentation, scales/hairs) following Martins da Silva et al. (2016); the sample value is the abundance-weighted mean of the morphotype scores.

8 Quality control

Control	Purpose	Acceptance criterion
Time from sampling to extraction	Avoid population shifts	≤ 5 days
Extractor temperature gradient	Efficient extraction without killing animals	Upper part 45–50 °C, lower part ≤ 10 °C after the gradual start [TO CONFIRM: how temperatures are logged]

Control	Purpose	Acceptance criterion
Sorting check	Consistent morphotyping	[TO CONFIRM: e.g. second sorter on a subset]

9 Data and metadata

- **Outputs:** counts per major group and morphotype per sample; litter depth; densities, richness, Shannon diversity, QBS-ar and Collembola life-form index (dimensionless indices).
- **Sample labels:** EnGeL labels from the sample master data file (type of sampling `Macf`); sorted vials keep the sample label.
- **Where:** [TO CONFIRM: project sheet / Lab Pipeline *Sample_Tracking* (Total_abundance column)] and the lab book.

Minimum to record

- EnGeL sample label; site, coordinates, sampling date, litter depth, photo (if taken)
- Date the extraction started and ended; extractor position
- Counts per group/morphotype; sorter and date
- Storage of the sorted vials (room temperature or fridge; location)

10 Safety and waste

Follow the manufacturer's instructions and safety data sheets for each reagent. Biological samples are potentially hazardous and are handled following the facility's procedures. EnGeL lab rules:

- Before you enter: wear your lab coat, use appropriate gloves, closed shoes only, tie back long hair.
- Not allowed: eating or drinking; touching phones or laptops with contaminated gloves; bags or coats on benches.
- Label all reagents (name, concentration, date) and treat all chemicals as hazardous.
- Ethanol is highly flammable and causes serious eye irritation (H225, H319). Keep it away from the extractor heating elements and other heat sources, store it in a flammables cabinet, and wear gloves and safety glasses when filling flasks and vials.

Waste

EnGeL waste streams (choose the right bin):

- **Biological solid waste** (black bin, white bag): contaminated gloves, contaminated disposable materials, solid biological laboratory waste, contaminated PPE. No liquids or sharps.
- **Specific hazardous waste** (red bin, red bag): chemicals and pharmaceuticals designated for this waste stream, specific hazardous laboratory waste, anatomical material; sharps only in a rigid sharps container. No loose sharps.
- **Biological liquid waste** (jerrican, UN 3291): liquid biological waste and contaminated biological liquids only. Use a funnel; rinse the original container and the funnel with dH_2O and discard the rinse water in the same jerrican.

For this protocol: extracted soil [TO CONFIRM: route] used ethanol from the collecting flasks [TO CONFIRM: route].

11 Troubleshooting

Problem	Likely cause	Solution
Few animals extracted	Sample dried too fast, or temperature raised too quickly	Start about 5 °C above field temperature for the first 2–3 days, then intensify.

Problem	Likely cause	Solution
Animals dead in the core	Core compacted or disturbed during sampling or transport	Drive the tube in gently with a block and mallet; keep cores sealed and upright [TO CONFIRM].
Long delay before extraction	Shipping	Keep samples aerated at room temperature; extract within 5 days.

12 Remarks

Sampling device. A stainless-steel or aluminium corer, 40 cm long and e.g. 5 cm in diameter, can be used for the litter sample (length and diameter should not differ much from these values to keep conditions comparable). It can be made of two parts that fit together along the main axis, or of one tube, with a handle on top and a cutting edge at the bottom. For flooded soils, use the same corer with an auger tip to retain the soil.

Project note. For BENCHMARKS samples, the published sampling guidelines recommend keeping samples at 4–10 °C with bags open, instead of room temperature.

13 References

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14 Version history and citation

Version	Date	Change	By
1.0.0	2026-11-16	First EnGeL-UC version, from the UC BENCHMARKS protocol “Sampling, Sorting and Identification of Soil Microarthropods” (BM-BIO-MAR-MORPH v2.0, 2026-10-05; Luís Cunha, Pedro Martins, Filipa Reis); project-specific notes moved to Remarks.	Luís Cunha
1.0.1	2026-11-16	Schematic of the MacFadyen extraction set-up	Luís Cunha

Version	Date	Change	By
		(figure); ORCIDs of Filipa Reis and Pedro Martins.	
1.0.2	2026-11-17	GHS pictograms in the hazards row (flame, exclamation mark; ethanol 96%, H225, H319).	Luís Cunha
1.0.3	2026-11-17	Authorship: all who contributed are authors, with their roles; lead author first where identified, reviewer (Luís Cunha) last.	Luís Cunha
1.1.0	2026-11-17	Sorted animals in 96% ethanol stored at room temperature (ideally in the fridge if there is space) instead of −20 °C.	Luís Cunha
1.1.1	2026-11-20	Author order confirmed by Luís Cunha: Filipa Reis, Pedro Martins, Luís Cunha.	Luís Cunha

How to cite

Reis F, Martins P, Cunha L (2026). Sampling, MacFadyen extraction and sorting of soil microarthropods. EnGeL-UC protocol EnGeL-FAUNA-MAR-MORPH, version 1.1.1. Zenodo. DOI [TO CONFIRM].

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15 Open questions

1. DOI.
2. Annex photo: the source caption says “PVC tube with caps”, but the photo shows a round plastic container with litter. Which caption is right?
3. Hands-on time; sorting time per sample.
4. How extractor temperatures are monitored; sorting quality check.
5. Where counts are recorded (project sheet, Lab Pipeline *Total_abundance*?).
6. Waste route for extracted soil and used ethanol.
7. The UC source title says “Identification”, but the steps cover sorting into groups and morphotypes only. Is an identification step (keys, levels) to be added?

Annex 1 – Sample container



Figure 2: Sample container [TO CONFIRM: caption; the source reads “PVC tube with caps for keeping the soil mineral layer intact”].