Materials and reagents

BIOSERVICES

Earthworms and macrofauna

a. Wire or wooden frame to mark the area (25 x 25 cm)
b. Garden scissors (secateurs)
c. Spade (flat blade) to open the pit
d. Large plastic sheets (e.g. large bin bags or trays)
e. Watering cans of 5-10 L volume (e.g. for garden use)
f. Umbrella for sunlight protection
g. Plastic containers (vol.: ca. 1 L) with cold tap water
h. Cooler with cool packs
i. Disposable protective gloves
j. Plastic tweezers/forceps
k. Tissue paper or paper towels
l. Earthworms: Allyl-isothiocyanate (AITC), synthetic grade (about 94% to 97% (volume fraction)). [Aldrich 37,743-0]
m. Earthworms: Isopropanol [2-propanol] 100 % (volume fraction)
n. Earthworms: Test tubes or vials (50 mL) for stock solution
o. Earthworms: Sealable glass containers with a 4% formalin + 96% ethanol mixture (ratio: 1:1)
p. Earthworms: Empty, chemical-resistant canister for the used formalin-ethanol mixture
q. Earthworms: Test tubes or vials (50 ml) (e.g. Falcon tubes) properly labelled and half filled with 4% Formalin
r. Macrofauna: Test tubes or vials (50 ml) (e.g. Falcon tubes) properly labelled and half filled with 70% Ethanol

Mesofauna

a. Working gloves
b. Auger (0-20 cm, 4 cm diameter) and hammer (e.g. impact-free soft-face mallet with plastic or rubber inserts)
c. Beakers that suit to the Kempson/MacFayden extractor, or plastic bags or sealable containers properly labelled)
d. Spatula for the division of the two depth levels (0-10 cm; 10-20 cm) and to help transfer the soil from the auger
e. Cool box with cool packs

Soil structure

a. Auger (0 – 30 cm) with hammer
b. Plastic liners properly labelled both for sample code and depth (write down on liner 0 and 30 cm in both extremes)
c. Parafilm/aluminium foil and paper to prevent soil from moving from the plastic liner if it is not fully filled with soil
d. Boxes to store the plastic liners

Physicochemical properties

a. Disponsable gloves to avoid contamination
b. Auger (0 – 20 cm) (to be disinfected with 70% ethanol after every sample)
c. Paper bags properly labelled (to avoid contamination with plastics)
d. Spatula to help transfer soil to the bag (to be disinfected with 70% ethanol after every use)
e. Rigid plastic containers (500 mL apprx.), properly labelled, to collect soil for aggregate analysis
f. Boxes to store the bags and the rigid plastic containers

Microbiota

a. Disposable gloves to avoid contamination
b. Sterile 2 mL Eppendorf tubes (6 tubes per sample; 8 tubes per sample in the Mediterranean region)
c. Spatula to collect soil from bag (to be disinfected with 70% ethanol after every use)
d. DNA/RNA shield for preservation: https://zymoresearch.eu/collections/dna-rna-shield/products/dna-rna-shield (1.5 mL per Eppendorf tube previously prepared in the laboratory)
e. 5 cm sieves to sieve soil (to be disinfected with 70% ethanol after every use). Useful to have 2 sieves in the field
f. Falcon tubes (viable cells)

Mineral nitrogen and microarthopods by metabarcoding

a. Falcon tubes (50 mL) or ziploc bags properly labelled
b. Cooler
c. Ice, ice plates or cool packs

Nematodes

a. Auger (0-20 cm) (to be cleaned after every sample)
b. Disposable gloves to avoid contamination
c. Plastic bags properly labelled (close well with twist tie (preferred) or piece of rope)
d. Cooler
e. Ice, ice plates or cool packs (preferred)
f. Styrofoam plate or similar else in cooler for compartmentalization to avoid the soil samples touching the ice or cool packs (samples should not get frozen)

In the field: pre-sampling

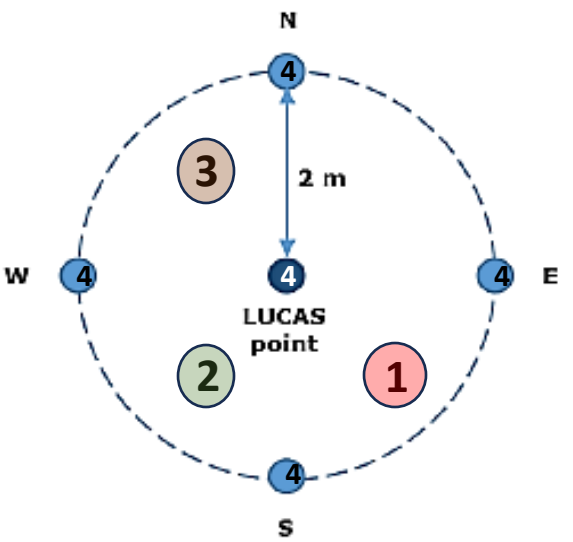


Figure 1. Location of sampling spots

Vegetation

a. Record the main vegetation species within the sampling area for each grid (circle of 4 m diameter, see Figure 1 above). If it is a cropland, record the crop/s present at sampling time.

b. Record the number of different plant species (vegetation richness). If you cannot identify all species names, at least record the number of the different species present in the sampling area for each grid (circle of 4 m diameter, see Figure 1 above).

c. If possible, estimate the vegetation cover (%): percentage of the 4 m diameter area covered by vegetation.

d. Important to request the owner of the field: crop yield or timber yield for sampling year, when available.

Weather conditions

a. Record the weather conditions on the sampling day (sunny, cloudy, rainy, windy, foggy, etc).

Images and videos (use the guidelines created by the Communication Team)

a. Please, take photos and videos of the general study site.

b. Please, take photos and videos of each sampling spot.

c. Please, take photos and videos of the different sampling types.

Sampling

a. Avoid edge effect with roads, pathways, etc

b. Disinfect the augers and sampling materials with 70% ethanol (flaming optional) for the next soil sample.

c. Samples should be collected with disposable gloves to avoid any cross contamination.

d. USE OFFICIAL CODES TO LABEL ALL BAGS, TUBES AND RECIPIENTS.

e. Record the geographical coordinates (decimal) of the central point of the sampling scheme in each area (see Figure 1). For the LUCAS sampling with the auger, you can sample additional points within the LUCAS circle to get 3 kg.

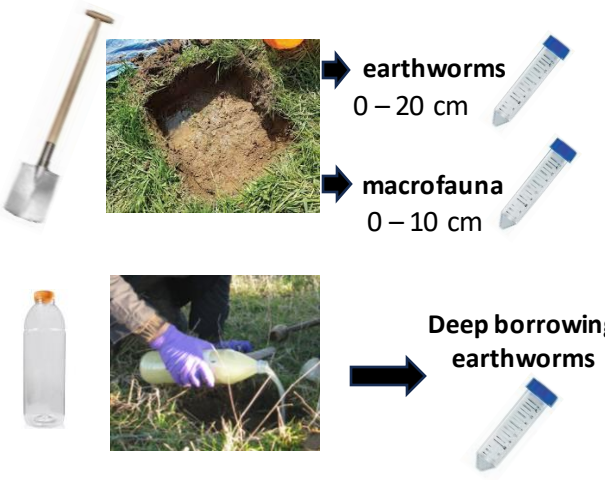
In the field

BIOSERVICES

1

1. Earthworms and macrofauna

- Excavate a square pit with an area of 25x25 cm and a depth of 20 cm
- Separate 0-10 cm (earthworms and macrofauna) and 10-20 cm (earthworms only) and place the 2 soil samples on 2 different plastic sheets
- Collect earthworms and macrofauna out of the excavated soil, roots, litter etc. by hand-sorting and picking up with plastic tweezers/forceps
- Earthworms are rinsed in tap water, put on tissue paper for carefully drying, then fixed in a 1:1 solution of 4% formalin and 96% ethanol until stop moving and collected in labelled test tubes with 4% formalin
- Other macrofauna are collected in labelled test tubes with 70% ethanol
- Add 2.5 L AITC solution into the pit to expel deep burrowing earthworms up to the surface. Pick them up with plastic tweezers/forceps, put them in tap water and treat them as described above



2

2. Mesofauna

- Collect a soil core of 4 cm diameter and 0-20 cm depth with a soil core sampler
- Separate the two depth levels 0-10 and 10-20 cm using a spatula
- Place the soil cores directly into the beakers that suit to the Kempson/MacFayden extractor or in plastic bags or containers properly labelled
- Transport the samples to the laboratory in cool boxes with cool packs
- In the lab, keep at 4ºC and extract the animals as quickly as possible. (Do not store for longer than 4 weeks)

3

3. Soil structure, bulk density and SOC: Collect at 0-30 cm with plastic liner auger.

- During soil fauna sampling, try to avoid impact on this sampling area
- Plastic liners properly labelled both for sample number and depth (0 and 30 cm in both extremes)
- If the plastic liner is not filled with soil, please seal it with parafilm/aluminum foil and paper to prevent it from moving during storage and transport
- Storage: Keep plastic liners at room temperature

4

4. Physicochemical properties, microbiota and nematodes

- Record the coordinates of the central point
- Collect ± 3 kg composite sample from 5 LUCAS points (2 m forming a cross from de central point) at 0-20 cm with an auger. Homogenize all subsamples.
- Note for microbiota: Homogenize using a 5-mm sieve.

4.a. Physicochemical properties: Separate ± 2 kg in paper bags and keep at ambient T (avoid plasu bags for plastic analysis)

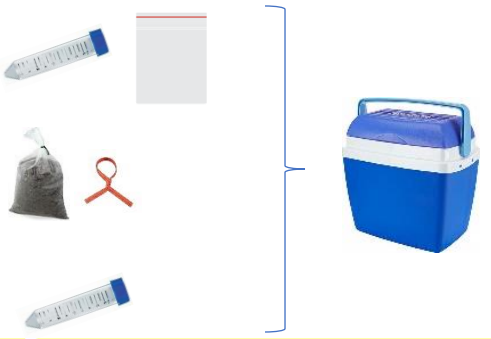
4.b. Aggregates: Separate 300 g in rigid container and keep at ambient T

4.c. Microbiota: Separate, with gloves, soil in 6 (8 in the Mediterranean) sterile 2 mL Eppendorf tubes. Previously in the lab, add 1.5 mL of DNA/RNA Shield in each 2 mL Eppendorf tube with a pipette. In the field, add ~0.5 g of soil in each tube. Shake the tube to get a slurry and so, efficient suspension, and keep at ambient T.

4.d. Mineral N and microarthopods by metabarcoding: Separate ± 40 g in 50 mL Falcon tubes or Ziploc bags properly labelled and keep in cooler with ice

4.e. Nematodes: Separate ± 600 g in plastic bags with gloves and keep in cooler with ice or cool packs. Plastic bags should be properly labelled (close well with twist tie (preferred) or piece of rope. Use a Styrofoam plate or something else to avoid the soil samples touching the ice or cool packs

4.f. Viable bacteria: Separate, with gloves, ± 10 g in a Falcon tubes and keep in cooler with ice



NOTES:

- Avoid edge effect with roads, pathways, etc
- Disinfect augur with 70% ethanol (flaming optional) for the next soil sample.
- Samples should be collected with disposable gloves to avoid any cross contamination.
- To avoid plastic contamination, if possible, try to wear non-synthetic clothes (cotton, linen)
- Record coordinates of central point and sampling date.

USE PROPER CODES TO LABEL ALL BAGS, TUBES, AND RECIPIENTS

In the lab

BIOSERVICES

1

1.a. Earthworms:
Fixing solution: 1:1; 4% formalin : 96% ethanol (for at least 2 minutes until not moving anymore).
Storage solution: 4% formalin .

1.b. Macrofauna i.e. Isopods, Myriapods (Millipedes and Centipedes), Insects and Spiders :
Preservation and storage in 70% ethanol.

→ Ship in in boxes at ambient T

2

2. Soil samples for mesofauna: keep at 4ºC in lab no longer than max. 4 weeks. Extract the animals as quickly as possible.

- Beakers for mesofauna: Extraction via Kempson/MacFaden extractor.
- Preservation and storage in plastic vials with screw cap and storage solution: 96% ethanol.

2a. Unextracted soil samples: Ship immediately after sampling in boxes with cool packs

2b. Extracted and preserved mesofauna: Ship in boxes at ambient T

3

3. Plastic liners for soil structure, bulk density and SOC: keep at room T → Ship by road at ambient T to UVigo

4

4.a. Paper bags for physicochemical properties (± 2 kg): air dry → Ship by road at ambient T to UPCT in paper bags

4.b. Rigid container for aggregates (300 g): air dry and return to rigid containers. Keep at room T → Ship by road at ambient T to UPCT in rigid containers

4.c. Eppendorf tubes for microbiota with DNA/RNA shield: keep at room T for a maximum of 30 days. After 30 days, they must be stored at -20 ºC.
IMPORTANT: If samples are not shipped within 30 days, they can be stored at -20ºC. However, it must be shipped later in dry ice. → Ship only 2 tubes (4 tubes for Mediterranean) to TUM at ambient T within 30 days. Keep 4 tubes as backup at -20ºC

4.d. Falcon tubes or Ziploc bags for mineral N and microarthorpods by metabarcoding (40 g): keep at -20ºC (if more than 1 week at -80ºC) → Ship to UPCT in polystyrene box with dry ice (24h delivery)

4.e. Plastic bags for nematodes (600 g): keep at 4ºC in lab (never frozen) no longer than max. 2 weeks. → Ship to ILVO in polystyrene box with ice plates (24h delivery)

4.f. Falcon tubes for viable bacteria (10 g): keep at -20ºC and send before 1 week time. → Ship to CREA in polystyrene box with dry ice (24h delivery)

Back-up samples
- Please, kept 500 g of soil (air-dried but NOT sieved) at room temperature in bags or rigid containers.
- Please, keep two 4 mL Eppendorf tubes with soil+DNA/RNA shield at -20ºC.