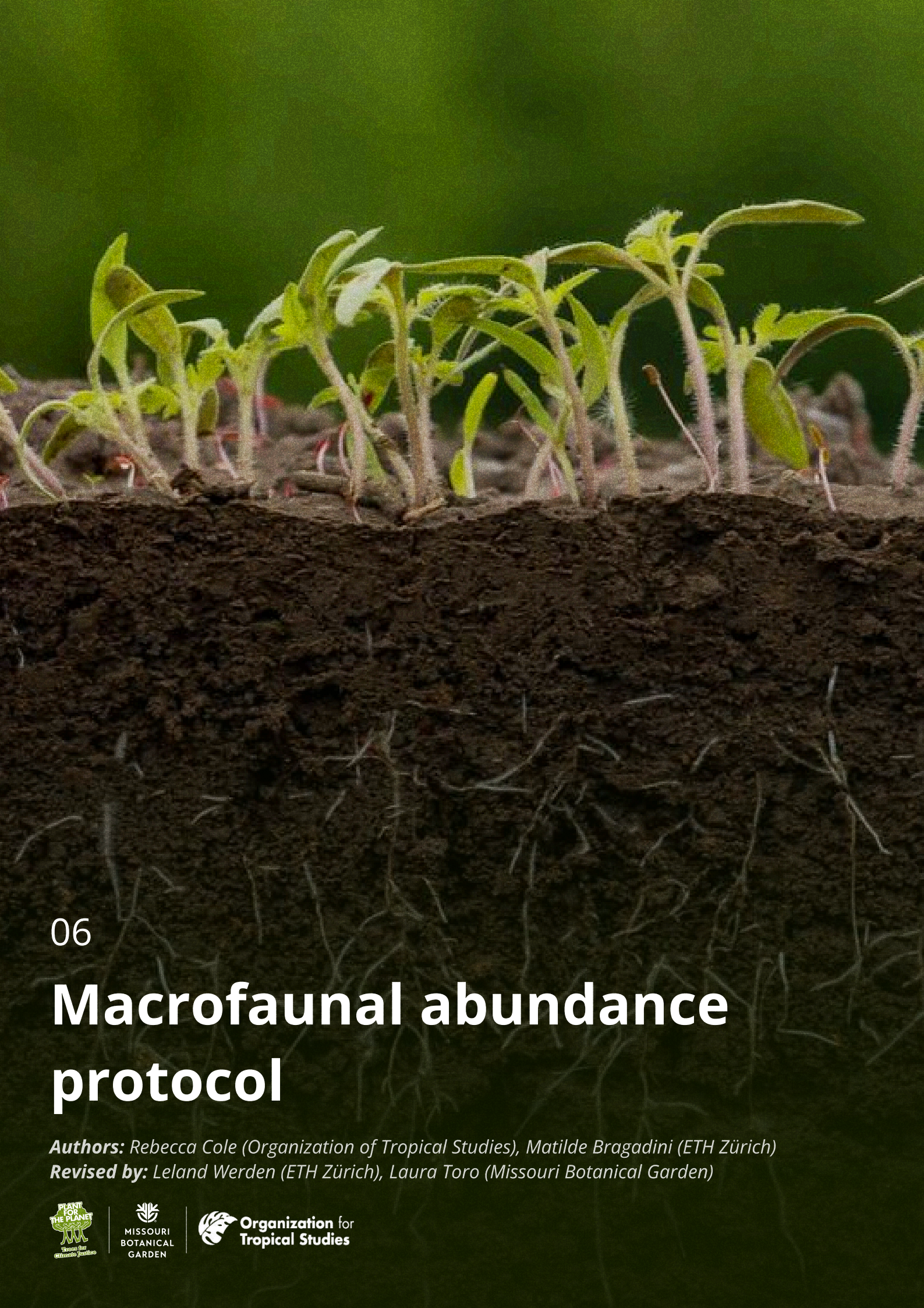




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Macrofaunal abundance protocol

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**Organization for
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Why measure this?

Soil macrofauna – earthworms, beetles, ants, termites – are the engineers of healthy soil. Their presence and abundance reflect the capacity of your soil to decompose organic matter, cycle nutrients, and maintain structure. Tracking macrofauna tells you whether the biological engine of your soil is recovering (Toro et al. 2025).

What it captures

Captures the diversity of macrofauna (more than 2 mm), such as earthworms and arthropods, their potential role in processes such as decomposition, and nutrient cycling activity. Soils with a high diversity and abundance of macrofauna tend to have high soil structure and nutrient availability.

Common tropical macrofauna groups to look for: earthworms, beetles (Coleoptera), ants (Formicidae), termites (Isoptera), woodlice (Isopoda), millipedes (Diplopoda), and centipedes (Chilopoda).

Sampling

- For a 1-hectare area, randomly assign five quadrat locations.
- Collect at least 3 samples per plot to capture macrofauna distribution.
- Sample during the growing or wet season, when biological activity is highest.
- Refer to the Sampling Protocol (00) for general plot layout and randomization.

Equipment needed

- Ruler or small quadrat (25 × 25 cm)
- Flags or markers
- Flat edge spade or shovel
- Plastic sack
- Trays (minimum three)
- Tweezers
- Plastic cups
- 4% formaldehyde solution (standard preservative for soil macrofauna)
- 70% ethanol (field alternative if formaldehyde is unavailable)
- Tape for labeling
- Paintbrush
- Notebook
- Pen or pencil
- Kitchen scale or balance (grams)
- Gloves

Procedure

1. Use a ruler or a small quadrat to mark out an area of 25 × 25 cm using flags or a similar marker.
2. Remove the litter from the quadrat (25 × 25 cm) and retain it for sorting.
3. Use a spade or a shovel to cut down a few centimeters outside the quadrat, then dig a 20 × 30 cm deep trench around it. This facilitates cutting the sample into horizontal layers and collecting animals escaping the block. Excavate the pit in as few shovel loads as possible to avoid damaging the macrofauna.
4. Find a comfortable place to work with adequate light.
5. Divide the quadrat into three layers (0–10, 10–20, and 20–30 cm) to capture variation in vertical distribution.
6. Begin sorting within 2 hours of excavation. If sorting is delayed, seal soil in plastic bags and store in shade. Sort within 24 hours.
7. Hand-sort soil and litter material in trays. Scoop handfuls of 500 ml and use tweezers to gently pick out any organisms that are > 2 mm in size. After removal of the animals from the tray, empty it and process another sample.
8. Preserve invertebrates in 4% formaldehyde, which is the standard preservative for soil macrofauna (Anderson and Ingram 1994). If formaldehyde is unavailable, 70% ethanol is an acceptable field alternative. Keep earthworms separate from other groups and store in a refrigerator.
9. Record abundance and fresh (preserved) weight of invertebrates in the litter and each of the three strata.



Calculating results

Report results as individuals per square meter (individuals/m²):

For a 25 × 25 cm quadrant (area = 0.0625 m²), multiply the raw count by 16: Individuals/m² = count × (1 / 0.0625) = count × 16

Important considerations

- It can take between 20–40 minutes for 3–4 people to sort out a bag.
- If there are lots of small ants or termites, use a small paintbrush dipped in ethanol to collect the rapidly moving macrofauna.
- Earthworms should be weighed as soon as they are collected and 24 hours later to get their actual weight.

References

Toro, L., et al. (2025). Integrating belowground recovery into tropical forest restoration design and monitoring. BioScience. <https://doi.org/10.1093/biosci/biaf097>

See also

Sampling Protocol (00), Decomposition Rate Protocol (05). Macrofauna drive decomposition; their abundance is linked to litter turnover rates.

Useful resources

Anderson, J.M. and Ingram, J.S.I. (1994). Tropical Soil Biology and Fertility: A Handbook of Methods. CAB International - The standard reference for tropical soil macrofauna sampling methods

Questions or Feedback?

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