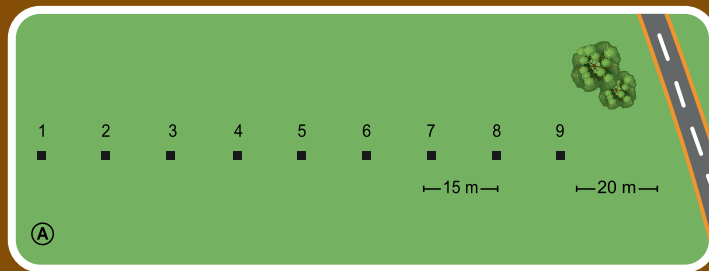
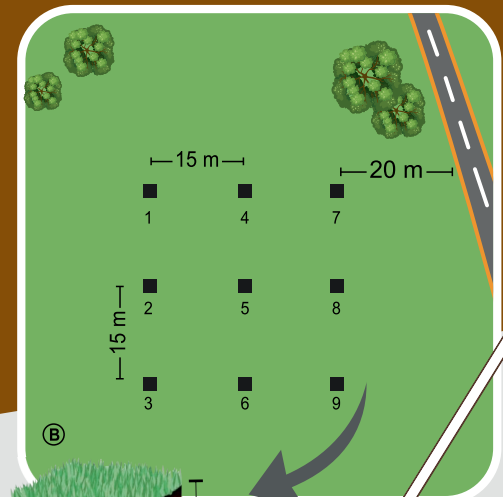


PROTOCOL FOR QUANTITATIVE EARTHWORM SAMPLING



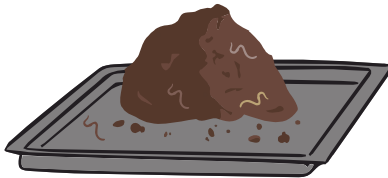
1

Starting 20 m or more from the edge (road, fence, forest or stream) of the field, delimit 9 sampling points in a straight line or in a grid, maintaining a distance of 15 m between each point.



2

Using a straight shovel, remove a 20 x 20 x 20 cm square monolith of soil and place it on a tarp. We recommend taking one sample at a time to avoid escape of the earthworms.



3

On a large plastic tray or small tarp place 2 to 3 handfuls of soil and look for any earthworms. It is important to sort a little bit of soil at a time to make it easier to see the earthworms.

4

Separate the earthworms from the remaining invertebrate fauna. Count and record the number of earthworms found in each hole. Be careful so the earthworms don't dry out. Even if an earthworm is cut during sampling, collect all pieces from the sample. If you find a piece in the hole, collect it also.



5

For each hole, use a screw-top bottle with 96% alcohol solution. Store the earthworms in the bottle, and add an internal label written on card paper, identifying the sampling point, date and collector. Fill the bottle up to the dotted line indicated in the figure to the left. If there are many earthworms or very large earthworms in the sample, use larger bottles and more alcohol. **Important:** always write the label using a pencil since alcohol removes ink from pens.



6

Fill in the form with all the necessary information.



7

For further details of the results obtained, contact those responsible (see "Information or questions" on the other page) and arrange shipment to the laboratory.



8

Species identification, counting and weighing of earthworms and the deposit in the Fritz Müller Oligochaetae Collection at Embrapa Forestry in Colombo - Brazil.



MATERIALS NEEDED:

- ✓ 1 straight cutting shovel
- ✓ 1 ruler or measuring tape
- ✓ 1 small tarp
- ✓ 1 large plastic tray (bigger than 30 cm x 20 cm)
- ✓ 9 plastic bottles with screw cap that seal well, with a capacity of 100 mL
- ✓ 1 liter of alcohol 96%
- ✓ Cardboard paper grammage minimum 120 for the labels
- ✓ Pencil (2b or 4b) to write the labels
- ✓ Clipboard and paper or cell phone to write down information about the area

INFORMATION THAT MUST BE CONSIDERED AND NOTED AT THE TIME OF THE SAMPLING

- ▶ - Date: ____/____/____
- ▶ - City, state, country: _____
- ▶ - Name of person responsible for the sampling: _____
- ▶ - Property owner name: _____
- ▶ - Property name: _____
- ▶ - Property Size (in hectares): _____
- ▶ - Sampled plot size (in hectares): _____
- ▶ - Geographical location of the sampled plot (central point). If you don't have a GPS, the location can be easily obtained using Google Maps or Google Earth. _____
- ▶ - Vegetation cover (what crop, what pasture, what cover crops/plants) at the time of sampling: _____
- ▶ - Briefly describe soil management practices adopted (for instance, tillage and which implement is used, no-tillage, no-tillage system, crop rotation or succession, use of cover crops, etc). If integrated systems are used, indicate the type: agropastoral, silvopastoral, agroforestry or agrosilvopastoral. _____
- ▶ - If you have information about the soil, indicate:
Soil type: _____
Soil texture: _____
Soil pH: _____
Soil organic matter content: _____
- ▶ - Record the number of earthworms collected in each hole:

1	2	3	4	5	6	7	8	9

To calculate abundance of earthworms in the area:

1. Count the number of earthworms per hole.
2. Average the abundance, that is, sum up all the earthworms in the samples and divide by nine.
3. The value obtained will be the average number of earthworms.
4. To obtain the number of individuals per square meter (ind.m^{-2}), multiply the average number of earthworms (item 3) by 25.
5. To obtain the number individuals per hectare (ind.ha^{-1}), multiply the number of earthworms per square meter (item 4) by 10.000

Classification of soil health in annual cropping under No-Tillage Systems using earthworm abundance (average number, no. ind.m^{-2} or no. ind.ha^{-1}) and total species richness (number of species) according to Bartz et al. 2024.

CLASSIFICATION CATEGORY	EARTHWORM ABUNDANCE			TOTAL RICHNESS
	Average number of individuals per sample	Individuals per square meter (ind.m^{-2})	Individuals per hectare (ind.ha^{-1})	Number of species
POOR	< 2	< 50	< 50.000	1
MODERATE	≥ 2 - < 4	≥ 50 - < 100	≥ 50.000 - < 100.000	2
GOOD	≥ 4 - < 6	≥ 100 - < 150	≥ 100.000 - < 150.000	3
VERY GOOD	≥ 6 - < 8	≥ 150 - < 200	≥ 150.000 - < 200.000	4
EXCELLENT	≥ 8	≥ 200	≥ 200.000	≥ 5

Note on number of samples: The proposed number of samples/holes is for areas of 1 ha or larger. This number of samples can be greater or the straight line/grid can be replicated in the area when in very large fields and, when in smaller fields (<1 ha), the number may be reduced to a minimum of 5 points.

Note on sampling season: Sampling should be carried out in the wet season (during or at the end of the rainy season or the higher rainfall period). Do not sample after more than 15 consecutive days without rain, or when the soil is very dry.

Information or questions: Marie Bartz: soil.soul.keeper@gmail.com or George Brown: minhocassu@gmail.com

Reference: Bartz, M.L.C., Dudas, R., Demetrio, W.C., Brown, G.G. Earthworms as health indicators in no-tillage agroecosystems, *European Journal of Soil Biology*, 2024.