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BENCHMARKS soil sampling protocol

Guidelines for urban systems

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Abstract

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This document presents a comprehensive sampling protocol for a general assessment of soil health indicators (SHI) in urban systems. In addition, the protocol suggests a sampling design to address the impact of land fragmentation in an urban context. Geo-referenced sampling points are identified by random and informed sampling based on fragmentation maps generated using Sentinel 2 imagery and Landscapemetrics.

To evaluate soil health, a diverse set of biological, chemical, and physical SHIs is proposed. To optimize soil samples for subsequent analyses, SHI-compliant sampling protocols are implemented, aligning with the BENCHMARKS sampling scheme.

For baseline site characterisation, soil samples will be collected using BENCHMARKS protocols for bulk soil, bulk density, earthworms, and mesofauna. Depending on site-specific challenges, additional samples may be collected using protocols tailored to plastic sampling or hydraulic property sampling.

The document also provides detailed guidelines on sample processing, shipping, and storage tailored to each soil sampling protocol. Lastly, it outlines the SHIs recommended for assessment both in the laboratory and directly in the field.

Keywords: BENCHMARKS soil sampling protocols, soil sample processing, sample storage, sample shipment, soil health indicators, laboratory analysis, urban fragmentation

Contents

1. BENCHMARKS soil sampling design

The protocol suggests a sampling design to address the impact of land fragmentation in an urban context. For each city, fragmentation was calculated as an edge density of non-sealed patches identified based on 10-m Sentinel 2 images (ESA WorldCover v2) and Landscapemetrics R tool (Hesselbarth et al., 2019). The resulting raster maps were classified in four fragmentation categories (low <1, moderate 1-5, high 5-10 and very high > 10), and points representing each category were selected by random sampling or based on the previous research, giving the total of 20 locations per city. To evaluate soil health, a diverse set of biological, chemical, and physical SHIs is proposed. To optimize soil samples for subsequent analyses, SHI-compliant sampling protocols are implemented, aligning with the BENCHMARKS sampling scheme.

2. BENCHMARKS sampling protocols

To assess soil health, a range of biological, chemical and physical SHIs are proposed. To ensure that soil samples are optimized for subsequent analysis, we use SHIs-compliant sampling protocols from the BENCHMARKS sampling scheme (Figure 1). At each sampling site, samples are collected following the BENCHMARKS bulk soil, bulk density, earthworm and mesofauna sampling protocols. Where specific soil functions or site challenges dictate, additional samples are taken following the plastic or hydraulic properties sampling protocols.

Sampling is recommended before any major management operations (e.g., fertilisation or tillage), generally at the start of the growing season when baseline SHI values are most representative. However, timing may be adjusted based on climatic zone. Weather and soil-moisture conditions should be monitored to ensure the soil is neither excessively wet (to prevent compaction) nor overly dry (to prevent sampling bias and disturbance). To minimize disturbance and avoid cross-contamination between different sample types, the following overall sampling order should be followed, and the designated sampling locations for each type must be respected (no trampling). 1. earthworm sampling, 2. mesofauna sampling, 3. plastic sampling and then the remaining samplings. A list of soil sampling materials and equipment is provided in Appendix 1.1.

For subsequent years, the sampling design will remain consistent, but sampling points will be slightly adjusted to avoid exact overlap with prior locations. The sampling timeline should also be maintained, allowing a flexibility of ±1 week to accommodate local weather conditions.

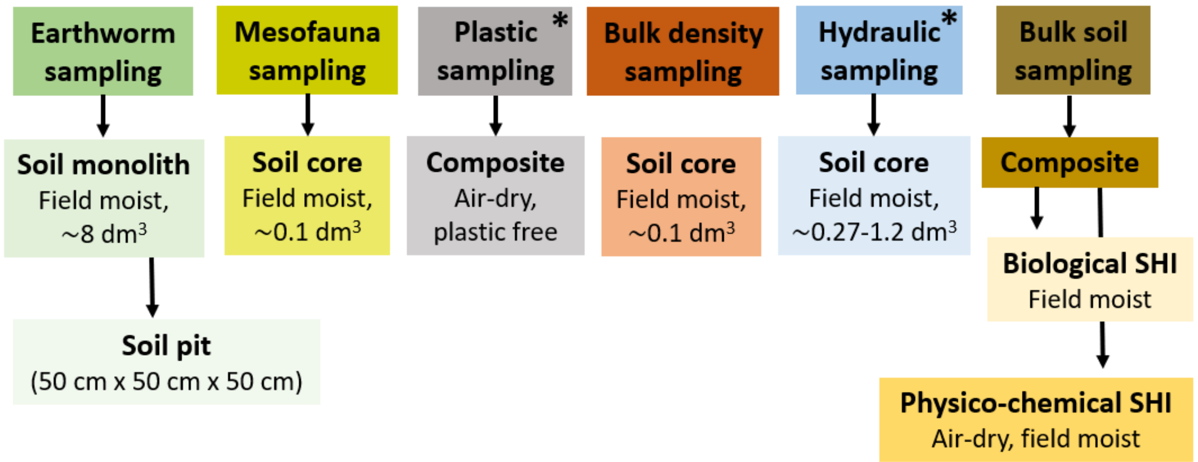

Figure 1. BENCHMARKS sampling scheme. Overview of the sampling protocols suggested for soil health indicator (SHI) assessments. The upper box indicates the name of the sampling protocol and the box below specifies the type of soil sample to be collected, required sample condition, and the sample volume. * Sampling protocols chosen for selected sites to address specific challenges or study specific soil functions.

2.1. Define the sampling area

At the sampling location, a homogeneous and representative sampling area is selected and a geo-reference point recorded with a GPS or GIS-enabled device, then the point is marked with a stake. To define the sampling area, attach one end of a tape measure to the geo-referenced point and use it to draw a circle with a radius of 2 m around the center point (Figure 2a). If sampling green spaces along streets, urban gardens, or similar, where no circle of 1 m fits (Figure 2b), calculate the length and width of your sampling area to reach an area of at least 3 m^2 excluding the area around tree trunks (if possible, matching the diameter of the tree crown). Describe the exact sampling area in the BENCHMARKS field observation protocol (Appendix 1.2). With a compass, define the three cardinal directions (north, west, east) with individual sticks to facilitate the specific sampling protocols: north for the mesofauna core, west for the hydraulic sampling, and east for the earthworm, bulk density and the soil pit. To ensure consistency, prevent overlap, and minimize trampling of adjacent subplots.

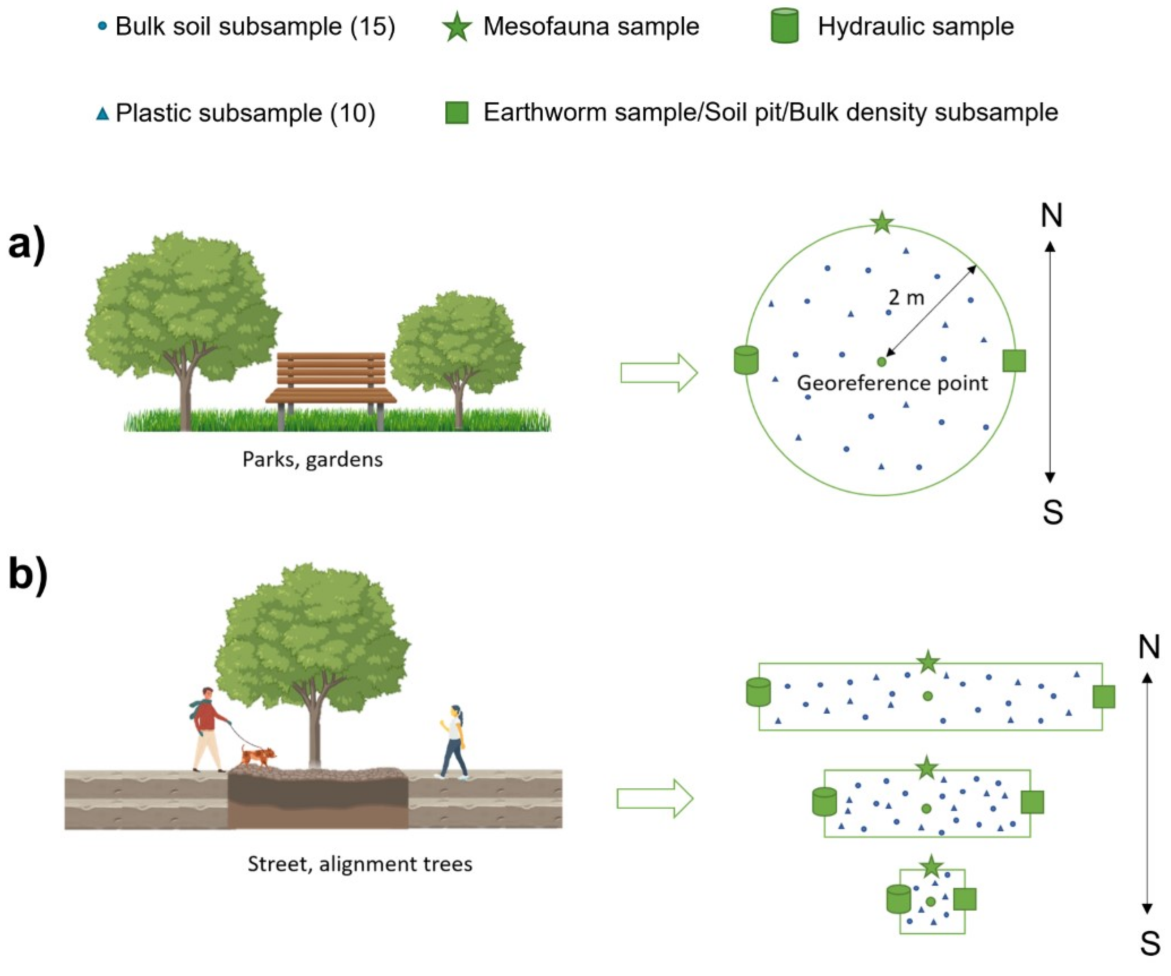

Figure 2. BENCHMARKS sampling approach. Define the sampling area in parks/recreational areas, green spaces, urban forests or similar (a) or along streets or small allotment gardens (b). The bulk soil sample consisting of 15 subsamples is randomly sampled within the sampling area. The plastic sample consisting of 10 subsamples is randomly sampled in the sampling area (same as the bulk soil), but only at a depth of 0–10 cm. The mesofauna sample is taken on the north side, the earthworm/bulk density sample and soil pit on the east side, and the hydraulic property sample on the west side.

2.2. BENCHMARKS earthworm sampling protocol

On the east side of the sampling area, excavate a soil monolith of 20 cm x 20 cm x 20 cm with a spade and transfer it onto a large black plastic bag or tray. Earthworms present in the soil monoliths are hand-sorted and transferred to a 50 ml reaction tube (or similar air-tight container) filled with >90% ethanol for fixation.

Before returning the soil, the earthworm pit will be further expanded for soil characterisation (see section BENCHMARKS soil observation protocol) and for bulk density sampling (see section BENCHMARKS bulk density sampling protocol).

2.3. BENCHMARKS mesofauna sampling protocol

With the BENCHMARKS mesofauna sampling protocol, collect an undisturbed soil core at the north side of the sampling area using a PVC cylinder (5 cm diameter x 5 cm height, ~100 cm^3 volume) from a depth of 5 cm. Remove the vegetation (upper 1–2 cm) or organic (O) layer (if present in forest systems). Extract the soil core by gently driving the PVC tube into the soil using a wooden block and a mallet. This avoids the compaction of soil. Remove the PVC cylinder from the soil with the help of a spade/spatula placed underneath the cylinder. Remove the excess soil around the PVC cylinder with a knife. Wrap the cylinder with plastic film and seal it with paper tape to preserve the soil structure inside the cylinders (make a few tiny holes with a needle on the top cover of each cylinder). Transfer samples into a labeled plastic bag and double pack each sample (put the labeled bag inside another plastic bag to prevent losing the label in case it detaches from the bag). Transport samples in a cooling box. To prevent compaction, either use separate cooling boxes for different samples or place buffer material between layers of samples.

In urban forests, also collect a mesofauna sample from the O layer, if present. Use a split corer (5 cm diameter) to collect one core from the entire O layer. Take a photo of the core profile to complement the site characterisation using a ruler as a scale. Report the thickness of the O layer in the field observation protocol. Discard the mineral soil layer and transfer the O layer into a labelled plastic bag and double pack each sample (put the labeled bag inside another plastic bag to prevent losing the label in case it detaches from the bag).

2.4. BENCHMARKS bulk soil sampling protocol

With the BENCHMARKS bulk soil sampling protocol, take a composite bulk soil sample of approximately 2 kg at each sampling site. The composite sample consists of at least 15 subsamples taken with a soil corer (3-5 cm diameter) randomly within the sampling area from a depth of 0–20 cm.

Before taking a soil core, remove vegetation, litter, stones, etc. from the soil surface. Collect the soil cores, separate the cores with a knife according to the required sampling depths and place each layer in the appropriate labelled plastic bag, which are placed in clearly labelled buckets. Repeat this procedure until all subsamples have been collected. Remove bigger stones (> 6 cm). Double pack each sample for transportation (put the labeled bag inside another plastic bag to prevent losing the label in case it detaches from the bag). Transport samples in a cooling box cooled with freezer packs but avoid samples lying directly on the ice packs (add a layer of isolation material).

In urban forests, also collect bulk samples from the O layer. Extract a soil core from the entire O layer using a soil corer (3–5 cm diameter). Measure the depth of the O layer with a ruler (record it in the field observation protocol as well as the diameter of the soil corer used). Take a picture. Transfer the entire volume of the O layer into a labelled plastic bag. Repeat this procedure until all subsamples have been collected. Pack and transport samples as described for bulk soil samples.

To avoid cross-contamination of samples, always wear laboratory gloves when touching the soil and equipment and try to touch the soil as little as possible. Sampling equipment needs to be cleaned with water and dried with tissue paper after each composite sample. In addition, the first soil core taken at a new sampling site will be discarded.

2.5. BENCHMARKS soil profile observation protocol

Expand the earthworm pit to a min. of 50 cm x 50 cm x 50 cm, then fix a measuring tape vertically along the exposed soil profile. Describe the soil profile following the FAO guidelines (FAO 2006). Take a clear photo of the soil pit with the measuring tape fully visible, avoiding partial shadows and ensure the area is either in full sunlight or completely shaded.

2.6. BENCHMARKS bulk density sampling protocol

In the soil profile observation pit, use a vertical measuring rod to identify the midpoint of each sampling depth perpendicular to the soil profile.

Coat the outside of a metal cylinder (5 cm diameter x 5 cm height, 100 cm^3 volume) with a very thin layer of Vaseline or grease (only if necessary for easier soil penetration). Insert the cylinder horizontally into the soil at the targeted depth using a wooden block and mallet (Figure 3). Once the cylinder is fully inserted, remove the surrounding soil gently and carefully extract the cylinder using a spade or shovel placed underneath if needed. Trimm any excess soil extending beyond each end of the cylinder with a straight-edged knife. Transfer the cylinders into a labeled plastic bag and double pack each sample (put the labeled bag inside another plastic bag to prevent losing the label in case it detaches from the bag). Repeat the procedure for all sampling depths, positioning the cylinder at the midpoint of each layer.

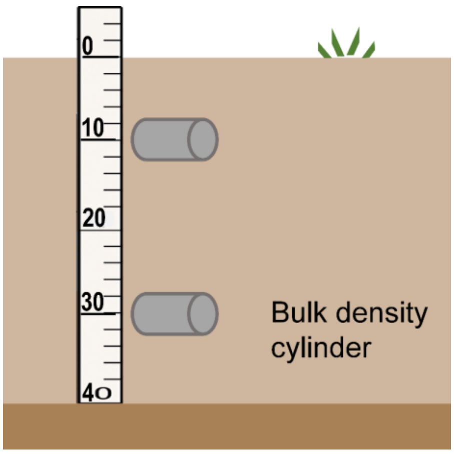

Figure 3. BENCHMARKS bulk density sampling protocol. The bulk density cylinder is inserted horizontally at the midpoint of each sampling depth in the expanded earthworm pit.

2.7. BENCHMARKS soil hydraulic properties sampling protocol

With the BENCHMARKS hydraulic properties sampling protocol, collect undisturbed soil samples at the south side of the sampling area using a metal cylinder of varying size depending on the subsequent analysis:

- Cylinders of 7 cm diameter × 7 cm height, 270 cm^3 volume (or 5 cm diameter × 5 cm height, 100 cm^3 volume for HYPROP2) to determine water retention curves (equilibrium method)
- Cylinders of 8 cm diameter × 15 cm height, 670 cm^3 volume (or 7.2 cm diameter × 6.2 cm height, 250 cm^3 volume) unsaturated hydraulic conductivity and water retention curves simultaneously (evaporation/wind's method).

Remove the vegetation or O layer (if present in forest systems) and level the soil using a spatula, scraping off 1–2 cm of the uppermost layer. This ensures that the inserted cylinders sample the 0–10 cm soil layer effectively. Coat the outside of the metal cylinder with a thin layer of Vaseline or grease to reduce friction during insertion. Position the cylinder on the soil surface, optionally stacking a second cylinder or a 1–2 cm extension of equal diameter and thickness on top. Gently insert the cylinder into the soil using a wooden block and a mallet. Ideally, a specific sample ring insertion tool is available fitting the selected ring size. It is critical that the insertion proceeds vertically and slowly to minimize soil compaction. Once the cylinder has been inserted 3–4 cm, check that the inner soil surface aligns with the outer soil surface. If not, compaction has likely occurred, and the procedure must be repeated at a new location. Then, remove surrounding soil using a spatula to release lateral pressure on the cylinder. Continue to gently insert the remaining part of the cylinder into the soil. Extract the cylinder carefully using a spade or large spatula placed underneath. Trim excess soil from both ends of the cylinder using a sharp spatula or straight-edged knife. Wrap the sample tightly in plastic film, sealing both ends with paper tape to preserve structure and prevent soil loss. If available, use metal or plastics lids to close both ends before wrapping. If an empty space (1–2 cm) remains at the top, fill it with soft material (e.g. leaves, paper, etc.) to stabilize the soil inside. Transfer the cylinders into a labeled plastic bag and double pack each sample (put the labeled bag inside another plastic bag to prevent losing the label in case it detaches from the bag) and pad with cushioning material to protect the cylinders from vibration.

2.8. BENCHMARKS plastic sampling protocol

The BENCHMARKS plastic sampling protocol follows the same procedure than the BENCHMARKS bulk soil sampling protocol with some adaptations: i) sampling depth is restricted to 0–10 cm and ii) do not use plastic tools and do not wear synthetic clothing, instead use metal, glass or wooden tools and wear cotton or other natural fibres or wear a cotton lab coat over your clothes to avoid contamination. For sample transport and storage, use e.g. aluminium containers. Sampling equipment needs to be cleaned with water and dried with tissue paper after each composite sample.

3. Sample processing, storage and shipping

Processing of samples from the BENCHMARKS bulk soil sampling should be done latest the day after sampling (better the same day). Gently break big soil aggregates and mix the soil to take a homogeneous subsample of 150 g for aggregate stability analyses and of 500 g for chemical analyses (store at 4 °C until shipping by regular post), a subsample of 350 g from the most upper soil layer for nematode analyses (store at 4 °C, keep bags open until express shipping max. one week after sampling, see below) and a backup sample of 200 g (store locally at 4 °C). Sieve a subsample of 500 g at 2 mm, or at 5 mm for clay-rich and peat soils, and take a subsample of 50 g for nitrogen mineralization analyses, 200 g for pollutant (persistent organic pollutants (POPs), pesticides and metals) analyses and 200 g for microbiological analyses. Ship sieved fresh samples immediately by express in a styropor/thermo box filled with cooling packs by express courier (e.g. DHL or FedEx and provide the tracking number to the recipient and inform beforehand to arrange shipping to preserve the characteristics of the samples. Take a subsample 10–20 g for molecular analyses in a 15 ml or larger reaction tube. Do not compact the soil in the tube. Either fix the label additionally with transparent tape or write the sample ID by hand onto the tube, since labels like to detach when frozen (store at -20 °C, express shipping dry ice). If available, freeze-dry the samples and ship by regular post. And take a backup sample of 20–30 g and store it locally at -20 °C. Air dry the remaining soil at max. 30 °C for 48 h or longer if needed. Sieve at 2 mm and take a subsample of 50 g for active carbon analysis and keep the remaining soil as backup (store locally at room temperature).

From urban forests, process samples from the O layer in the same way, but omit sieving and subsampling for specific analyses such as aggregates, microbial biomass, nitrogen mineralization, and active carbon, depending on the requirements.

Figure 4 gives an overview of bulk soil sample processing steps and shipping conditions. Before and after sieving, store samples in a cooling box. Ship samples in plastic bags (i.e. zip lock bags except those of the BENCHMARKS plastic sampling and those for molecular analyses) and double pack each sample (put the labeled bag inside another plastic bag to prevent losing the label in case it detaches from the bag). Clean all equipment (sieves, bowls, etc.) carefully with water to avoid contamination.

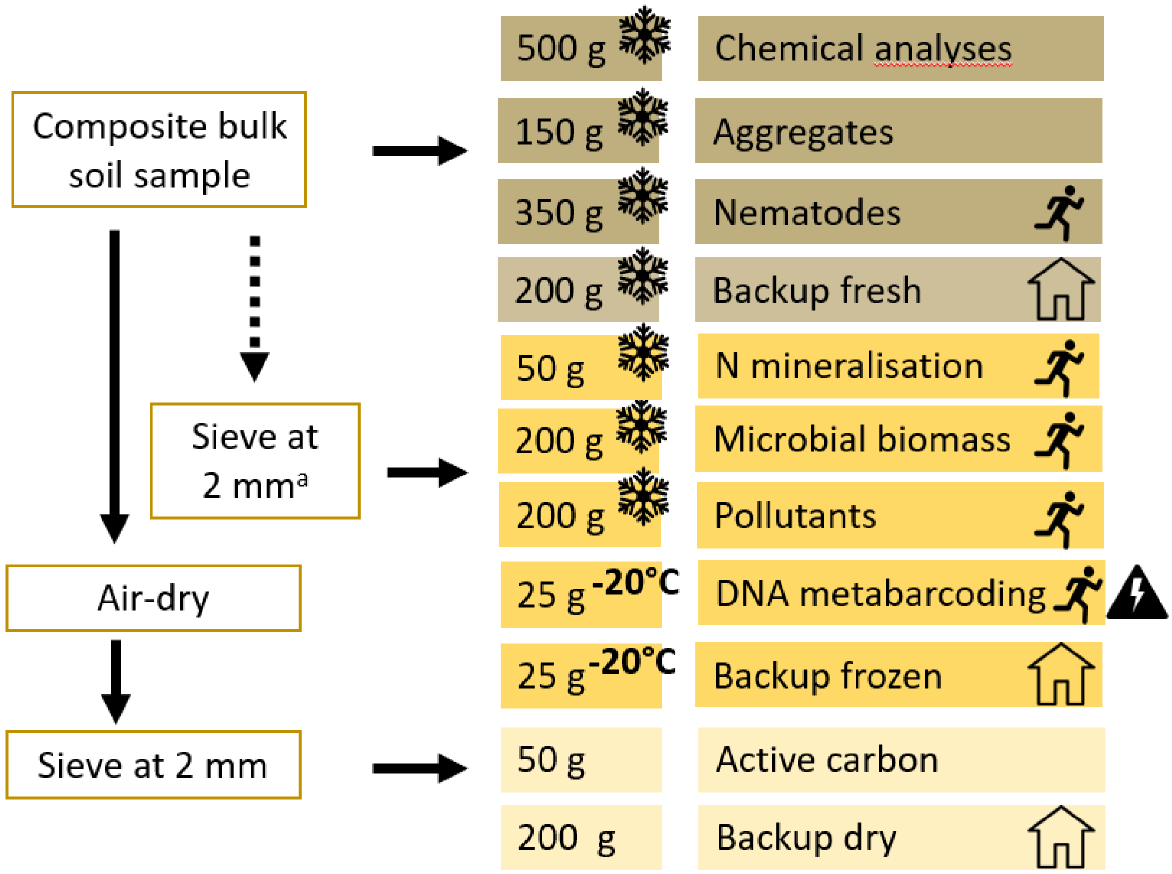

Figure 4. Overview of bulk soil processing and shipping. Bulk soil processing steps required sample volumes and storage conditions and shipping conditions are shown. The dashed arrow indicates that only a subsample of the fresh bulk soil sample is sieved at 2 mm (a or at 5 mm in case of clay-rich/peat soils) before drying. Snowflakes indicate that samples need to be stored at 4 °C until shipping, -20°C indicates storage at -20°C, runner indicates express shipping with cooling packs, runner with flash indicates express hipping on dry ice, house indicates that backup samples are stored locally at the partner institution in case they are needed later. N, nitrogen.

Store soil cores from BENCHMARKS bulk density sampling in the cold room for max. one week until further processing. Weigh the cylinder to determine soil fresh weight (mf), place the cylinder containing the soil sample in an oven at 105 °C for 48 h until constant mass is reached. Transfer cylinders from oven into desiccator and allows to cool for 4 h. Weigh the cylinders after removal from the desiccator (mt). Remove the soil, clean cylinder and weigh empty cylinder (ms). Calculate bulk density as follows: ρ (g cm^{-3}) = (mt-ms)/V.

Store soil cores from BENCHMARKS hydraulic properties sampling in the cold room and ship them max. one week after sampling. Pack and ship the cores to protect them from vibration, shock, extreme heat and freezing. To do so, wrap the cores in cushioning material with a minimum thickness of 2.5 cm around the sample and 5 cm on the bottom. If necessary, protect against heat or cold by shipping it in styropor/thermo box. To facilitate handling, it is recommended that packages are not made too large or heavy.

Store cylinders from BENCHMARKS mesofauna sampling in a cold room (4–10 °C) and keep plastic bags open for aeration. Ship cylinders max. five days after sampling by express with cooling packs. Do not place cylinders directly onto the cooling packs (add a layer of isolation material) and avoid compacting the cylinders by directly staking them on top of each other, add a layer of buffer material (polystyrene or buffer foil with air cushion) on the first layer of cylinders and then add a second layer of cylinders.

Store earthworms from BENCHMARKS earthworm sampling at room temperature in a fume hood or an open and well aerated place. Change ethanol after 24 h to avoid ethanol dilution due to the water content in the earthworms.

Store samples from BENCHMARKS plastic sampling at 4 °C and process them at the latest 48 h after sampling. Freeze a subsample of 200 g at -20 °C in an aluminum coated paper bag or in a paper bag placed inside a plastic bag as backup. Dry the remaining samples, including the plastic-free control at 40 °C in an oven until constant weight. Avoid contamination by i) cleaning the oven before use, ii) avoid contact with plastic material, iii) try to wear no/few synthetic cloths during sample handling and iv) avoid possible sources of dust in the room with the oven and only keep the soil exposed to the environment during drying. Directly after drying, store samples in aluminum coated paper bags to avoid further contamination at room temperature until further processing. Sieve samples at 2 mm metal sieve into a metal container. Transfer 2 x 200 g soil into a labeled paper bag and double pack each sample for transportation.

An overview of shipping details including sample properties and volume and shipping conditions (regular, express, express dry ice) is shown in Table 1. For each package, fill out an analysis order (template provided in Appendix 1.3) and proforma invoice (template provided in Appendix 1.4) and send it together with the samples (the analysis order inside the package and the proforma invoice attached outside of the package). Before shipping the samples, inform the recipient and pass over the tracking number of the parcel as soon as the parcel has been posted.

Table 1. Overview of shipping requirements, sample properties and volumes per analysis type.

Analysis	Sample property and volume	Shipping
Chemical properties	Moist, 500 g	Regular
Active carbon	Air-dried, 50 g	Regular
Pollutants	Moist, 200 g	Express[a]
Nitrogen mineralisation	Moist, 50 g	Express[a]
Nematodes extraction and morphotyping	Moist, 350 g	Express[a]
Biological analyses	Moist, 200 g	Express[a]
DNA metabarcoding	Moist-frozen, 20-30 g	Express dry ice[b]
Mesofauna extraction and morphotyping	Moist, cores from mesofauna sampling	Express[a]
Aggregates	Moist, 150 g	Regular-cautious[c]
Earthworm morphotyping	Earthworms in >90% Ethanol	Regular
Soil hydraulic properties	Moist, undisturbed cores of hydraulic property sampling	Regular-cautious[c]
Plastic analyses	Air-dried, 400 g from plastic sampling	Regular; in paper bag

[a]Express: Ship samples in styropor box with ice packs by express courier (e.g. Fedex, DHL) and provide tracking number to recipient.
[b]Express on dry ice: Ship samples in styropor box with dry ice by express courier (e.g. Fedex, DHL) and provide tracking number to recipient. Only ship samples Mondays or Tuesdays.
[c]Regular-cautious: Pack and ship soil cores to protect them from vibration, shock, extreme heat and freezing.

4. BENCHMARKS soil health indicator catalogue

For basic characterisation, and depending on the specific challenges of a site, a set of chemical, physical, and biological SHIs is selected for analysis (Table 2, Table 3, Table 4). Each SHI is analysed in the same laboratory using standardized methods to ensure data comparability.

Table 2. Methods used for the analysis of chemical soil health indicators in BENCHMARKS soil samples.

Soil health indicator	Methods	Reference
Cation exchange capacity	Extraction in 0,1 mol/l $BaCl_2$ followed by ICP-AES	ÖNORM L 1086-1
Electrical conductivity	Metal electrode in a 1:5 (W/V) suspension of soil in H_2O extract	ISO 11265:1994
pH	Glass electrode in a 1:5 (W/V) suspension of soil in 0.01 M $CaCl_2$ extract	ISO 10390
Total nitrogen	Elemental analysis using a CNS at 1250 °C	ÖNORM EN 16168
Plant available phosphorus	Sodium hydrogen carbonate extraction followed by spectral photometry	ISO 11263
Plant available potassium	Calcium-acetate-lactate extraction followed by flame photometry using a Segmented flow Analyser SAN	ÖNORM L1087; Schüller, 1969
Copper, Iron, Manganese, Molybdenum, Nickel, Zinc	Aqua Regia extraction followed by ICP-OES	NEN 6961: 2014; NEN 6966: 2005
Soil organic carbon	Dry combustion at 900-1500 °C	ÖNORM EN 15936
Active carbon	Pyrolysis Rock-Eval coupled with the PARTYSOC model	Cécillon et al., 2018; Cécillon et al., 2021;
POM:MAOM	Rapid particle size fractionation to determine labile vs. stable cycling soil organic carbon	Baldock et al., 2013; Lavallee et al., 2020; Poeplau et al., 2018; Sanderman et al., 2013
Metals	Aqua Regia extraction followed by ICP-MS	Rotter et al., 2017
Pesticides	QuEChERS method followed by LC-MS and GC-MS	Geissen et al., 2021; Svobodová et al., 2018; Lehotay et al., 2005
Persistent organic pollutants	Determination of persistent organic pollutants by GC-MS	Tombesi et al., 2017; Llanos et al., 2022
Plastics	Extraction of microplastics from soils with subsequent µ-FTIR analysis	Foetisch et al., 2024

POM:MAOM, ratio of particulate organic matter and mineral associate organic matter.

Table 3. Methods used for the analysis of physical soil health indicators in BENCHMARKS soil samples.

Soil health indicator	Methods	Reference
Soil texture	Calculated from the mass and the volume of sole cores taken with rings of known volume	ISO 11277:2020
Aggregate fractions	Wet sieving method	Elliot et al., 1986; Six et al., 1998
Bulk density	Cylinder (gravimetric) method	ISO 11272:2017
Soil water retention, un-saturated soil hydraulic conductivity	Wind's evaporation method, HYPROP2	Arya, 2002; Basile et al., 2006; Van Genuchten, 1980. Bin Shokrana and Ghane, 2020
Saturated soil hydraulic conductivity	Constant head and falling head method (lab method), adapted single ring infiltrometer method (field method)	Reynolds et al. 2002

Table 4. Methods used for the analysis of biological soil health indicators in BENCHMARKS soil samples.

Soil health indicator	Methods	References
Potentially mineralizable nitrogen	Anaerobic incubation	ÖNORM L1204
Microbial biomass carbon and nitrogen	Chloroform-Fumigation Extraction	Vance et al., 1987
Earthworms	Hand sorting and morphological identification	Sims and Gerard, 1985; Marcel-B. Bouché, 1972
Microarthropods	MacFadyen extraction and morphological identification	Macfadyen, 1962; Parisi et al., 2005; Vandewalle et al., 2010
Microarthropods	DNA metabarcoding of microarthropods	Shokralla et al., 2015; Elbrecht et al., 2019
Nematodes	Extraction, counting and morphological identification	Bongers, 1994; Oostenbrink, 1960
Nematodes	DNA metabarcoding of nematodes	Stoeck et al., 2010 ; Shokralla et al., 2015
Microorganisms (bacteria, fungi)	DNA extraction, 16S and ITS fragment PCR amplification and sequencing using Illumina or PacBio platforms	Lori et al., 2023; Labouryie et al., 2023
Bacterial abundance	qPCR of 16S marker gene	Caporaso et al., 2012; Han et al., 2023
Fungal abundance	qPCR of 18S marker gene	Vainio and Hantula, 2000; Han et al., 2023
Nitrifying archaea	qPCR of ammonia monooxygenasea (moA) functional genes	Leininger et al., 2006; Schauss et al., 2009; Han et al., 2023
Nitrifying bacteria	qPCR of ammonia monooxygenasea (moA) functional genes	Rothauwe et al., 1997; Han et al., 2023
Nitrous oxide reducing bacteria	qPCR of nitrous oxide reductase (nosZ, nosZII) functional gene	Henry et al., 2006; Han et al., 2023
Proteolytic bacteria	qPCR of alkaline metallopeptidase (apr) and neutral metallopeptidase (npr) functional genes	Bach et al., 2001; Han et al., 2023
Urea-hydrolizing bacteria	qPCR of urease (ureC) functional gene	Gresham et al., 2007; Han et al., 2023

5. Field-based assessments

Document each sampling site by photography from each cardinal direction. In addition, record information about climate and weather conditions, site description including information on landscape, land use, ground cover and human influence and describe soil surface characteristics such as coarse surface fragments, signs of soil erosion or soil surface sealing, etc. using the BENCHMARKS field observation protocol (Appendix 1.2). Also report deviations from the original protocol.

If the soil is characterised by a high quantity of stones, take a sample of 20 cm x 20 cm x 20 cm with a spade to quantify the amount of stones (in kg).

If possible, take additional on-site measurements, depending on the research focus and availability of tools (Table 5).

Table 5. Overview of field-based assessments.

Soil health indicator	Methods	Reference
Soil profile	Guidelines for soil description	FAO (2006) Guidelines for soil description
Soil erosion	Visual observation	FAO (2006) Guidelines for soil description
Surface sealing	Visual observation	FAO (2006) Guidelines for soil description

6. Identification and registration of samples

Sampling points are identified by unique sample identifiers (IDs) previously assigned based on the geo-reference points. The sample IDs are composed of a site ID, treatment ID and profile ID. To distinguish individual samples collected at the same sampling point (profile ID), sample IDs are further amended by layer ID and a sample type ID (BULKS for bulk soil sample; BDENS for bulk density sample, HYDRA for hydraulic property sample, PLASTIC for plastic sample, MFAU for mesofauna sample, EWORM for earthworm sample). To distinguish samples collected in different years, the sampling date is added at the last position of the label ID. These sample IDs are used in each sampling campaign to record agro-environmental data related to each point on the data management platform. At each sampling point, surveyors document agro-environmental observations by filling in the BENCHMARKS field observation protocol and by taking photographs. All the data is then stored on the data management platform.

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Appendix 1

1.1. Sampling and sample processing materials

Common material and equipment for sampling

Sampling

- GPS (will be brought by sampling core team)
- Camera (mobile phone also fine)
- Field observation protocol (one for each sampling point, or only one + notebook to write down the in-field assessments)
- Paper tape
- Tape measure
- Wooden scale of 2 m
- Compass
- Knife
- Trowel/ hand shovel
- Marker sticks: large (n=no of sampling points), small (n=no of sampling points x3)
- Rope (min. 2.5 m)
- Scissors

Sample packaging and transport

- Labelled plastic bags
- Permanent markers
- Cooling boxes (enough to fit all samples)
- Freezer packs
- Hand scale

Cleaning

- Lab gloves
- Water
- Tissue paper
- Brush

BENCHMARKS bulk soil sampling

- Soil corer (min. 2-5 cm diameter)
- Wooden device to get the soil core out of the corer
- Big rubber/plastic hammer (when soil is dry)
- Buckets (min. 1 per sampling depth + some spare)
- Metal spoons

BENCHMARKS bulk density sampling

- Large spatula
- Trowel
- Rubber/plastic hammer
- Wooden blocks (min. 4 cm thick)
- Steel cylinder (5 cm diameter * 5 cm height)
- Vaseline
- Plastic film
- Knife

BENCHMARKS soil hydraulic properties sampling

- Same as for bulk density sampling
- Steel cylinder (7 cm diameter * 7 cm height)

BENCHMARKS earthworm sampling

- Spate
- Large and stable plastic bags or large trays for hand-sorting of the earthworms
- Tweezers
- Labelled sample containers (e.g. Falcon tubes, or 100 ml beaker with air-tight lid) filled with 90% EtOH

BENCHMARKS mesofauna sampling

- PVC cylinders (5 cm x 5 cm) for undisturbed soil cores
- Split corer for litter
- Rubber/plastic hammer
- Wooden block (min. 4 cm thick)
- Knife
- Plastic film
- Needle

BENCHMARKS plastic sampling

- Soil corer (2-5 cm diameter)
- Wooden or metal device to get the soil core out of the corer
- Aluminium bowl/metal bucket
- Hand shovel
- Aluminium containers (n=no of sampling points) (example here)
- If soil is dry: wooden blocks (min. 4 cm thick) + steel hammer

Common material and equipment for sample processing

- Sieves 2 mm OR 5 mm (clay or peat soils)
- Bowls
- Balance
- Spoons
- Aluminum trays or similar for air-drying the soil

- Water
- Tissue paper
- Lab gloves (S/M/L)

- Labelled bags
- Additional bags

- Cooling boxes
- Cooling packs

- Thermoboxes
- Bubble wrap
- Cooling packs

1.2. BENCHMARKS field observation protocol

Benchmarks field observation protocol

Sample ID: .... Date: .... Sampler: ....

Sample site location

Site ID: .... City: .... Country: .... Date: ....

Coordinates (in degree, minutes, seconds)

Longitude: .... °/ ....'/............'' Latitude: .... °/ ....'/............''

Altitude (m): ....

Climate and weather conditions (see Table 2 in Guidelines for soil description, fao.org)
Monthly mean temperature: .... Monthly mean precipitation: ....

Present weather conditions: .... Former weather conditions: ....

Site description (see Table 4, 8-11 in Guidelines for soil description, fao.org)
Landscape/Topography: .... Land use: ....

Ground cover/crops: .... Human influence: ....

Soil description (see Table 14-20 in Guidelines for soil description, fao.org)
Coarse surface fragments: .... Surface cover: .... Size classes: ....

Soil erosion: Category: .... Area (%): .... Degree: ....

Surface sealing: Thickness (mm): .... Consistency: ....

Sample description

Texture: ◻ Sandy ◻ Sandy-loam ◻ Loamy ◻ Clayey-loam ◻ Clayey ◻ Clay ◻ Peat

Sample humidity: ◻ Dry ◻ Moist ◻ Wet

Coarse fraction (%, in case of a high quantity of stones): ....

Remarks/deviation from sampling protocol:

....

....

....

Photographs

Sample ID	Sampling site photograph
North facing photograph	East facing photograph
South facing photograph	West facing photograph
Additional photograph	Additional photograph

1.3. Template – Analysis order with shipping information

Analysis order

Recipient information:
Institution: ...
Address: ...
Name: ...
Phone: ...
Email: ...

List of soil analysis:

- ...
- ...
- ...

Required sample volume: ...
Soil conditions: ...
Shipping conditions: ...

List of soil samples

Site ID	Land use type	Sample ID	Institution	Sampler	Sampling date	Weather condition	Sample weight (g)

1.4. Template – Proforma invoice

Sender Institution: Name: Address: Phone: Email:	**Recipient** Institution: Name: Address: Phone: Email:
Date:	

Proforma invoice

Commission:	Name of sender / *Institution*
Content:	Soil samples / For soil analyses / Scientific research
Weight samples:	.... kg
Weight packaging:	.... kg
Value:	Euro 1.00
	No commercial value, for laboratory analysis only
Country of origin:	....

Add name here and signature above

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