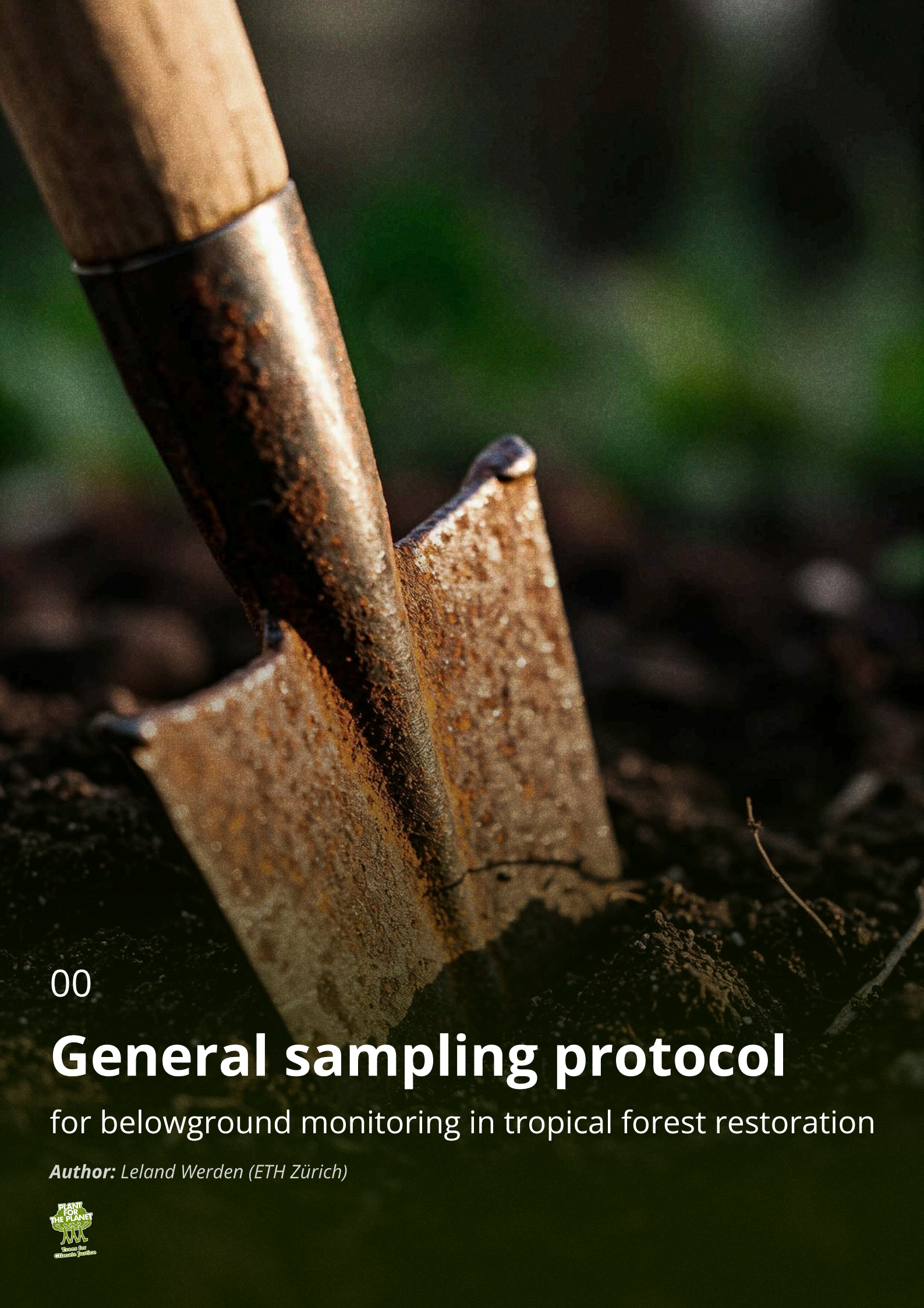




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General sampling protocol

for belowground monitoring in tropical forest restoration

Author: Leland Werden (ETH Zürich)

Why measure soil indicators?

Soil health determines whether a restored forest can sustain itself over time – but most restoration monitoring stops at the canopy. This protocol establishes a consistent, repeatable framework for sampling soil across six belowground indicators, so that field teams can detect real changes in soil recovery and connect them to restoration outcomes.

What indicators can be measured?

Bulk density (Protocol 01)
Aggregate stability (Protocol 02)
Soil organic matter and soil organic carbon (Protocol 03)
pH (Protocol 04)
Decomposition rate (Protocol 05)
Macrofaunal abundance (Protocol 06)

What these indicators capture

This document defines the shared sampling design – where, when, how many, and how deep – that underpins all six indicator-specific protocols (bulk density, aggregate stability, soil organic matter and carbon, pH, decomposition rate, and macrofaunal abundance). It does not describe indicator-specific laboratory or field measurement procedures. Those details live in protocols 01 through 06. Any sampling decision made here applies to all six indicators unless an individual protocol explicitly overrides it.

Sampling design

This is the core of the protocol. Every decision below directly affects the quality and comparability of your data.

When to sample

Baseline and long-term schedule

- **Collect a baseline before any intervention** – before planting, fencing, soil amendment, or any other restoration activity. This is the single most important sampling event. Without it, you cannot measure change.
- **Resample at minimum every 5 years.** The 0–10 cm soil layer changes measurably within the first 5 years of recovery (Toro et al. 2025). Sampling more frequently (every 2–3 years) during the first decade is valuable if resources allow.
- **Sample reference sites on the same schedule** as restoration sites so that comparisons remain valid over time.

Where to sample

Plot placement

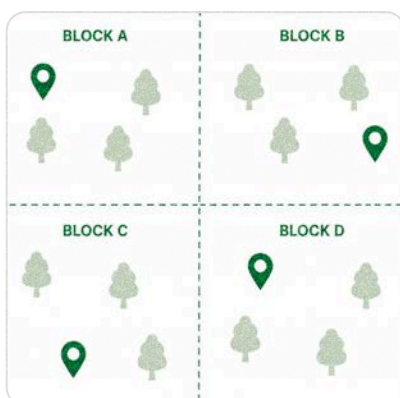
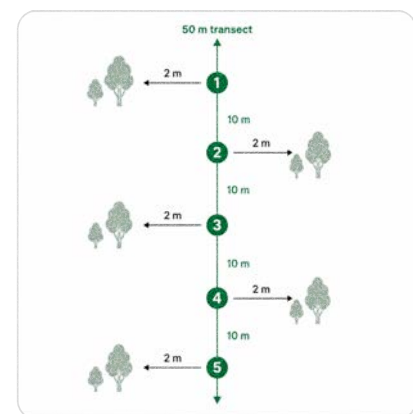
- **Nest soil sampling plots within existing aboveground vegetation monitoring plots.** This links belowground data to canopy cover, stem density, species composition, and other metrics already being collected. Do not create a separate spatial framework for soils.
- **Use a rule of thumb of 2–4% of the total project area for soil sampling areas** (Londe et al. 2022). This is a starting point, not a rigid prescription.
- **Recognize that sampling intensity reflects local variation, not total project size.** A small, highly heterogeneous 10-hectare site with multiple soil types and land-use histories may require sampling effort comparable to a uniform 10,000-hectare project. Let site variability drive your design.

Sample point placement within a plot

- **Place 3–10 sampling points per plot, spaced at least 5 m apart.** Aim for 5–10 points when feasible; use 3 as the absolute minimum for any statistical comparison.
- **Distribute points along a transect or use a stratified random layout within the plot.** Transects are simpler to navigate in dense vegetation. Stratified random designs capture more spatial variation but require pre-planned coordinates.
- **Sample biological indicators during the growing (wet) season.** Decomposition rates, macrofaunal abundance, and even pH fluctuate seasonally, tracking peaks in biological activity. Sampling during the growing season captures the system near its functional maximum.
- **Where possible, add a nongrowing-season sampling round** to characterize seasonal range. This is especially valuable for pH and decomposition, which can shift substantially between wet and dry periods.
- **Be consistent across years.** If you sample in March during year one, sample in March in subsequent years. Seasonal consistency matters more than hitting the exact biological peak.

Transect approach:

Lay a measuring tape along the longest axis of the plot. Place sampling points at equal intervals (e.g., every 10 m on a 50 m transect), offsetting alternately 2 m left and right to avoid creating a compacted trampling path.

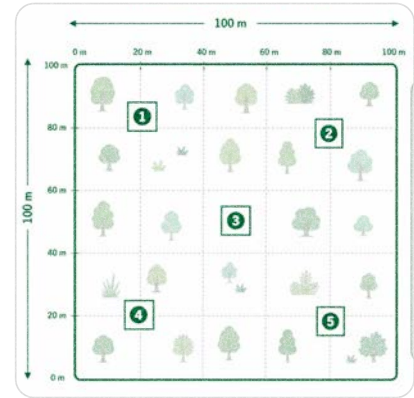


Stratified random approach:

Divide the plot into equal-area blocks (e.g., quadrants or strips). Randomly generate one or two sampling coordinates within each block using a random number table or phone app.

For macrofauna specifically:

Randomly assign five 25 cm x 25 cm quadrat locations within a 1-hectare area (see Protocol 06 for details).



Reference sites

- **Always pair each restoration site with a nearby reference site** – an intact, mature, or late-successional forest on similar soil type, slope, and parent material. The reference represents the recovery target.
- **If no suitable reference exists nearby**, establish a time-zero baseline at the restoration site itself and track change over time. This is a valid alternative, but note that it cannot tell you how far the site is from full recovery – only whether it is moving in the right direction.
- **Sample the reference site using the same design, depth, timing, and methods** as the restoration site. Any difference in protocol between paired sites invalidates the comparison.

How many samples – replication

- **Collect at least 3 independent sampling points (biological replicates) per plot.** Each point must be a physically separate location, not a repeated measurement of the same spot.
- **Distinguish biological replicates from technical replicates:**

1. Biological replicate: An independent soil core or pit at a different location within the plot. These capture real spatial variation.

2. Technical replicate: A repeated measurement on the same sample (e.g., running a pH reading twice on the same soil slurry). These capture measurement error but do not increase your effective sample size for site-level conclusions.

- **Prioritize fewer indicators measured well over more indicators measured poorly.** If resources are limited, measure fewer properties with higher intensity (more samples, higher frequency) rather than spreading effort thin across all six indicators.

Depth

- **Standard sampling depth: 0–10 cm** for all physical and chemical properties. This top layer is where soil responds fastest to restoration, holds a high concentration of soil organic carbon, and is most sensitive to disturbance.
- **Add a 10–20 cm layer in areas with a history of intensive agriculture** – plowing, heavy machinery, or prolonged cultivation – where compaction and carbon loss extend below 10 cm (Keller and Or 2022).
- **For soil organic matter (SOM) and soil organic carbon (SOC) specifically:** Sample 0–20 cm as the priority depth for organic horizon characterization. For SOC stock calculations, extend to 30 cm where feasible, since carbon accounting standards often require deeper profiles.
- **Record the exact depth of every sample.** Do not assume the target depth was achieved – roots, rocks, and compacted layers can prevent full-depth insertion of the corer.

Decision tree – quick reference

Use the following as a starting checklist when planning a new sampling campaign:

Decision	Default	Adjust if...
Sampling points per plot	5–10	Reduce to 3 minimum for very small plots; increase if site is highly variable
Spacing between points	≥ 5 m	Increase on large plots to cover more ground
Depth	0–10 cm	Add 10–20 cm for former agricultural land; extend to 30 cm for SOC stocks
Frequency	Every 5 years	Every 2–3 years during the first decade if budget allows
Seasonal timing	Every 5 years	Add a dry-season round for pH and decomposition if resources allow
Reference site	Paired nearby intact forest	Use time-zero baseline if no reference available

Sample handling and transport

Labeling

- Label every bag **before collecting the sample**. Write directly on the bag with permanent marker, and place a duplicate paper label inside the bag.

- Each label must include:

Site name

Plot ID

Sample number (e.g., P1-S03 for Plot 1, Sample 3)

Depth (e.g., 0–10 cm)

Date (YYYY-MM-DD format)

Sampler initials



Storage

- Place soil samples in sealed resealable plastic bags. Squeeze out excess air before sealing.
- If processing within 24 hours:** Store at ambient temperature in a shaded, cool location. Avoid direct sunlight or vehicle dashboards.
- If processing will be delayed beyond 24 hours:** Store samples at 4°C (standard refrigerator). Do not freeze unless the specific analysis explicitly requires it – freezing disrupts soil structure and kills organisms.
- For chemical analyses (SOM, SOC, pH):** Air-dry samples as soon as possible. Spread soil thinly on clean paper or trays in a well-ventilated area out of direct sunlight. Air-drying halts biological activity and stabilizes the sample.
- For biological samples (macrofauna):** Transfer organisms into 70% ethanol immediately in the field. Label the vial with the same information as soil samples.

Chain of custody

- Maintain a sample log (paper or digital) that records:
 - Sample ID
 - Date and time of collection
 - Person who collected it
 - Date and time of handoff to the lab
 - Person who received it at the lab
 - Storage conditions during transport
- This log accompanies the samples from field to lab and is kept with the final dataset.

Data recording

Record the following metadata for every sampling event. Missing metadata reduces the value of the data – sometimes to zero.

Recommended metadata (every sample)

Field	Format	Example
GPS coordinates	Decimal degrees (WGS 84)	-3.4521, 28.8310
Date	YYYY-MM-DD	2026-03-15
Site name	Text	Kigoma-North
Plot ID	Text	KN-Plot-03
Sample number	Text	P03-S05
Depth (cm)	Numeric range	0–10
Sampler name	Text	J. Mwangi
Soil type	Text (local or FAO classification)	Highly weathered tropical soil
Vegetation type	Text	Secondary forest, 8 years
Land-use history	Text (brief)	Cassava cultivation until 2018

Extra metadata (record when known)

Field	Format	Example
Forest age (years since planting or abandonment)	Numeric	8
Recent weather / rainfall	Text or mm	35 mm rainfall 2 days prior
Slope and aspect	Degrees	12 degrees, NW-facing
Elevation	m.a.s.l.	1,140
Canopy cover (%)	Numeric	65
Photo reference	Filename	KN-P03-S05_photo1.jpg

Recording tips

- Use a standardized field data sheet (paper or tablet) with pre-printed column headers.
- Back up digital data daily to a second device or cloud storage.

Important considerations

- **Be consistent.** A simple, repeatable design applied identically across sites and years produces better data than a complex design applied inconsistently.
- **Mark your sampling points permanently** (e.g., buried magnets, rebar, or georeferenced coordinates) so you can return to the same locations in future sampling rounds. Resampling near – but not at – previous locations avoids disturbing already-sampled soil while staying within the same microhabitat.
- **Avoid sampling after heavy rain.** Saturated soil is difficult to core cleanly, and recent rainfall can temporarily alter pH and nutrient concentrations. Wait at least 48 hours after heavy rain if possible.
- **Do not sample within 2 m of trails, roads, or plot boundaries.** Edge effects and trampling introduce bias.
- **Calibrate and test equipment before each field campaign.** A corer with a bent tip produces unreliable bulk density measurements.
- **Train all field staff on the full set of protocols before the first sampling day.** Practice on non-target soil. Standardizing technique across samplers is critical.
- **Record deviations from the protocol.** If you could not reach 10 cm depth because of rocks, write it down. If a sample bag tore, note it. Unrecorded problems become invisible errors in the dataset.
- **Plan your logistics backwards from the lab.** Know how long you have before samples degrade, and schedule field days so that transport to the lab (or to processing) fits within that window.
- **Composite samples reduce cost but obscure variability.** If you combine 5 cores into one bag, you get a plot-level average but lose the ability to assess within-plot variation. Keep individual samples separate unless budget constraints make compositing necessary, and document when compositing is used.

Glossary of key terms

Aggregate stability – The ability of soil clumps (aggregates) to resist breaking apart when exposed to water or mechanical stress. Higher stability indicates better soil structure and resistance to erosion.

Biological replicate – An independent sample collected from a separate location within the plot. Biological replicates capture real spatial variation in the soil. Contrast with technical replicate.

Bulk density – The mass of dry soil per unit volume (g/cm^3), including pore spaces. Low values indicate loose, well-structured soil; high values indicate compaction.

Decomposition rate – The speed at which organic material (e.g., leaf litter) breaks down in the soil, typically measured as percent mass loss per day using buried mesh bags (litterbags or tea bags).

Litterbag – A mesh bag or a teabag containing a known mass of organic material, buried in the soil to measure decomposition rate over time.

Loss on ignition (LOI) – A laboratory method for estimating soil organic matter content by weighing a sample before and after burning it at high temperature (typically 550°C). The mass lost during combustion approximates the organic matter content.

Macrofauna – Soil-dwelling invertebrates visible to the naked eye (body width greater than 2 mm), including earthworms, termites, ants, beetles, and millipedes. Their abundance and diversity reflect soil biological health.

Organic horizon – The uppermost soil layers enriched in organic matter, including surface litter and partially decomposed material. In tropical forests, this layer is often thin but biologically active.

Particulate organic matter (POM) – Fragments of partially decomposed plant material (0.053–2 mm in size) found within the soil. POM is a relatively fast-cycling fraction of soil organic matter and an early indicator of recovery.

pH – A measure of soil acidity or alkalinity on a scale from 0 to 14, where 7 is neutral, values below 7 are acidic, and values above 7 are alkaline. Measured in a soil-water slurry, pH has no units.

Reference site – A nearby area of intact, mature, or late-successional forest used as a benchmark for the recovery target. It should share similar soil type, slope, climate, and parent material with the restoration site.

Replicate – See biological replicate and technical replicate.

Soil organic carbon (SOC) – The carbon component of soil organic matter. SOC is typically about 58% of SOM by mass and is the standard metric for carbon accounting. SOC stocks (kg/m²) require both concentration and bulk density data.

Soil organic matter (SOM) – All organic material in the soil, including decomposed plant and animal residues, living organisms, and humus. Expressed as a percentage of dry soil mass.

Soil structure – The arrangement of individual soil particles (sand, silt, clay) into aggregates, and the pattern of pores between them. Good structure supports water infiltration, root growth, and gas exchange.

Supernatant – The liquid that sits above a settled solid after a soil-water mixture is allowed to stand. Relevant in pH measurement (the electrode is placed in the supernatant) and in aggregate stability tests.

Technical replicate – A repeated measurement on the same sample, used to quantify measurement error. Technical replicates do not increase the effective sample size for site-level conclusions. Contrast with biological replicate.

Transect – A straight line along which sampling points are placed at regular intervals. Transects provide a systematic spatial layout and are practical in dense vegetation.

Walkley-Black method – A wet chemistry laboratory method for estimating soil organic carbon by oxidizing the carbon with potassium dichromate and sulfuric acid, then measuring the amount of dichromate consumed. Widely used in tropical soils research but involves hazardous chemicals.

Reporting units summary table

Property	Unit	Notes
Bulk density	g/cm ³	Mass of oven-dried soil per unit volume of the intact core
Aggregate stability	% stable aggregates or SLAKES index (0–7)	SLAKES app provides the 0–7 index from a smartphone video; wet sieving gives % stable aggregates
Soil organic matter (SOM)	% of dry soil mass (or mg/kg)	Via loss on ignition at 550°C
Soil organic carbon (SOC) stocks	kg/m ²	Requires both SOC concentration (%) and bulk density (g/cm ³) to calculate
pH	Unitless (standard scale 0–14)	Measured in 1:2.5 soil-to-water slurry; report to one decimal place
Decomposition rate	% mass loss per day	Report both total % mass loss and the daily rate; specify incubation period
Macrofaunal abundance	Individuals per m squared	Standardized to area; report separately by major taxonomic group if possible

Expected direction of change during restoration

As a degraded site recovers following restoration, expect the following general trends over years to decades (based on Toro et al. 2025, Figure 1):

Indicator	Expected direction during recovery
Bulk density	Decreases (soil loosens as roots and organisms create pore space)
Aggregate stability	Increases (organic matter and fungal hyphae bind particles)
SOM / SOC	Increases (litter inputs and root turnover add organic material)
pH	May increase toward neutral if previously acidified by degradation
Decomposition rate	Increases (growing community of decomposer organisms)
Macrofaunal abundance	Increases (habitat and food resources improve)

Recovery rates differ across indicators. Bulk density and macrofaunal abundance can show measurable improvement within 2–5 years. Soil nitrogen may recover within a decade. pH, soil organic matter, and decomposition rates often take several decades to approach mature-forest values (Veldkamp et al. 2020; Poorter et al. 2021; van der Sande et al. 2023). Changes in years 5–10 are especially important to capture.

These are general patterns. Individual sites may deviate depending on starting conditions, restoration method, climate, and soil type. The critical question is not whether a single value is “good” or “bad” but whether the trajectory over time is moving toward the reference condition.

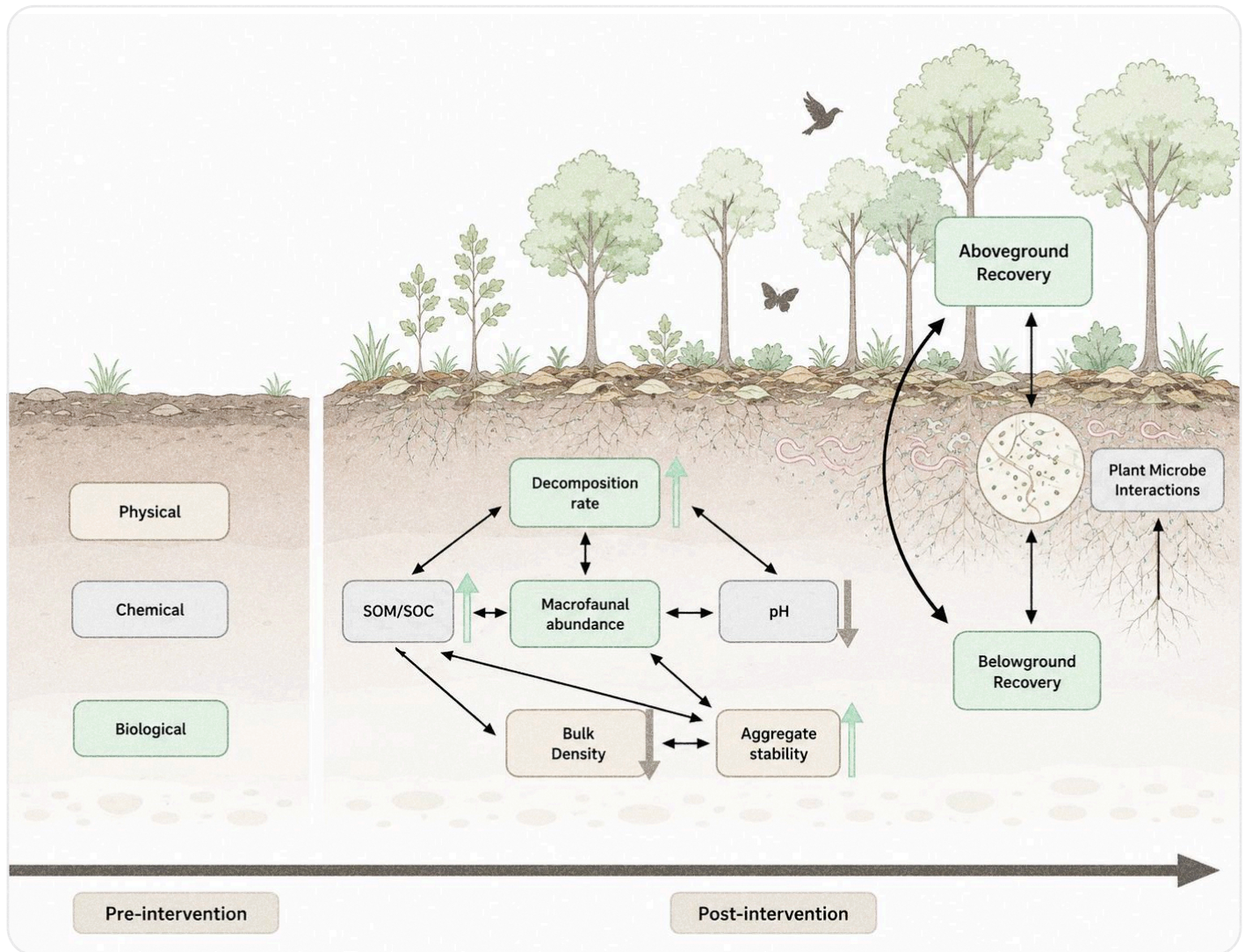


Figure 1. From Toro et al. 2025, included here with permission from the authors.

Safety and ethical considerations

Personal safety

- Wear gloves when handling soil, especially on sites with unknown contamination history.
- Wear splash-proof safety glasses and nitrile gloves when handling 70% ethanol (macrofauna preservation). Ethanol is flammable – keep away from open flames and heat sources.
- Carry a first aid kit to every field site. Ensure at least one team member has basic first aid training.
- In remote sites, carry a charged phone or satellite communicator and share your field plan and expected return time with someone not on the team.
- Be aware of venomous snakes, spiders, and stinging insects when digging soil pits. Check pits before reaching in.

Ethical considerations

- Obtain permission before sampling.
- Share results with local communities and landowners in an accessible format.
- Acknowledge local knowledge about soil conditions and land-use history – field teams and community members often hold information that no remote dataset captures.
- If hiring local assistants, compensate them fairly for their time and expertise.

References

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Useful resources

- FAO Global Soil Partnership – resources on soil monitoring: <https://www.fao.org/global-soil-partnership/en/>

Questions or Feedback?

Please contact

info@plant-for-the-planet.org

ltoro@mobot.org

Many thanks to our partners for their contributions to the belowground restoration monitoring protocols series



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