Action A.2.02

Assessing the influence of forest management on carbon storage

METHODOLOGY REPORT

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1. Quantifying carbon in living and dead biomass

Plot distribution and dendrometric parameters

The field approach for quantifying carbon stock in old-growth and managed forests will be based on a sampling strategy able to convergence with the different sampling approaches available for describing old-growth attributes. Such an approach will be based on circular plots, with radius equal or larger than 10 m (>300 m^2), placed randomly or systematically using a regular grid. In case of previously established sampling schemes, larger plots can be used, and plots will be located according to established statistical approaches already in place, provided that they have been deployed for quantifying stand structure and overall stand biomass, and a quantification of the statistical error is associated to the estimates.

The final number of plots to be established in the field depends on their size and their density. It is important to report the uncertainty connected to each estimation, to be assessed using statistical techniques, such as the error propagation or Monte Carlo simulations, as reported by IPCC (2006).

Within each plot, the amount of carbon stored in living aboveground and belowground biomass will be quantified, as well as the amount of dead biomass.

Furthermore, a subsample of all measured plots will be statistically selected for collecting tree cores and soil samples. Tree cores will allow to analyze wood density in beech and, eventually, the main co-dominating conifer species in mixed forests. Wood density changes will be assessed in the light of tree size, forest naturalness and along the biogeographic gradients within the network.

Soil samples will serve for quantifying soil organic carbon (SOC) in organic and mineral horizons and evaluate how much C storage in soils is related to biogeography and aboveground structure and is affected by past management.

Aboveground Living Biomass

In each plot, all trees with at least half of the stem falling within the perimeter and with diameter at breast height (DBH) > 10(7) cm will be measured. The measurements may involve one DBH parallel to contour lines, or two orthogonal DBHs. Tree species will be recorded.

Within each plot, height will be measured on a subsample of all measured trees, chosen among healthy individuals with straight stem. At least 1-2 trees (depending on plot size)



will be measured per each of the large DBH classes: < 20 cm, 20-39 cm, 40-59 cm, 60-79 cm, >80 cm.

Belowground Living Biomass

The belowground portion of tree biomass will be estimated using tree size parameters and available allometric equations available from national level inventories or from species-specific equations from the scientific literature. A comparison among the two methods, when possible, will be performed to assess the variability in final C estimations.

Dead Biomass (Deadwood)

Dead trees will be divided in standing dead trees (SDT) and lying deadwood (coarse woody debris, CWD). A minimum diameter threshold of 10 cm will be considered, measured at 1.3 m from the ground in SDT, or corresponding to maximum diameter on CWD.
All standing dead trees within plots and with DBH above the minimum measuring threshold will be measured in DBH and height.

CWD may be measured entirely within the plot, by recording the end diameters and the length of pieces above the minimum size threshold, or by using the line intersect method. In the first case, pieces should have a minimum length of 1 m. If using the line intersect method, at least 4 lines with fixed directions (e.g. compass) and extending at least 20 m from the plot center must be established. All pieces intersecting lines with diameter above 10 cm will be counted. Species may be recorded, whenever possible.
Each deadwood piece will be assigned to a decay class. The decay classes considered will be 5, increasing from 1 to 5 with increasing decay, divided according to the progressive changes in tree original shape and wood consistency (Pyle & Brown 1998; Sippola & Renvall 1999; Heilmann-Clausen & Christensen 2004; Table 1a). Alternatively, a simplified method can be used aggregating the decay classes in 3 categories (Table 1b).

Table 1. Synthetic description of the main decay stages for dead biomass in hardwood dominated forests. The decay class is assigned according to the features occupying most of the volume of the snag or CWD. (a) Refined scheme with 5 detailed decay classes. (b) Alternative, simplified scheme based on 3 decay classes.

(a)

Decay Class	Stem shape	Branches	Surface	Bark	Consistency
1	Circular	Present	Bark	Present, firmly attached	Hard
2	Circular	Mostly missing	Partly with bark	Present, not firmly attached	Hard
3	Circular	Missing	Hard wood	Mostly missing	Hard



4	Mostly circular	Missing	Soft, wet wood	Missing	Mostly soft
5	Flat, flat elliptic	Missing	Spongy wood	Missing	Soft

(b)

Decay Class	Stem shape	Surface	Bark	Consistency	Correspondence with decay classes in Table 1a
1	Circular	Partly or entirely with bark	Present, not firmly attached	Hard	1-2
2	Circular	Hard wood	Mostly missing	Hard	3
3	Elliptic or flat	Soft, wet wood	Missing	Mostly soft	4-5

For converting volume data to biomass, in each decay stage a relative correction factor for wood density must be applied. Each partner will specify if correction factors are already available for deadwood decay stages, and if these were developed within national inventories. Alternatively, available data from the scientific literature will be used.



2. Collecting tree cores for wood density

In areas where tree coring will be allowed, trees of different size from the dominant species (i.e. beech) will be sampled for wood and bark samples to determine their density variations along biogeographic gradients and according to naturalness degree.
In each area, one old-growth and one managed forest growing under comparable ecological conditions will be cored using an increment borer. In each stand, the sampling will interest the dominant species (e.g. beech). At least 25-30 cores per forest will be sampled. In case of mixed stands with conifers, and depending on the budget available for coring, the same number of cores from the dominant conifer (e.g. spruce) will be collected, or biomass conversions will be based on allometric equations.

Sampling strategy for collecting tree cores

Within a randomly chosen subset of measured plots, 5 trees per plot will be cored. In particular, one tree will be cored in each of the following 5 DBH class: < 20 cm, 20-39 cm, 40-59 cm, 60-79 cm, >80 cm. Cored trees must have regular shape (i.e. straight stem) and not showing signs of decline/dieback or heart rot. Trees may be selected within the plot, or immediately outside the plot. In this case, the closest trees to the plot border falling within the required DBH classes will be sampled. If the sampling will interest trees within the plot, a random selection from measured data will be used.

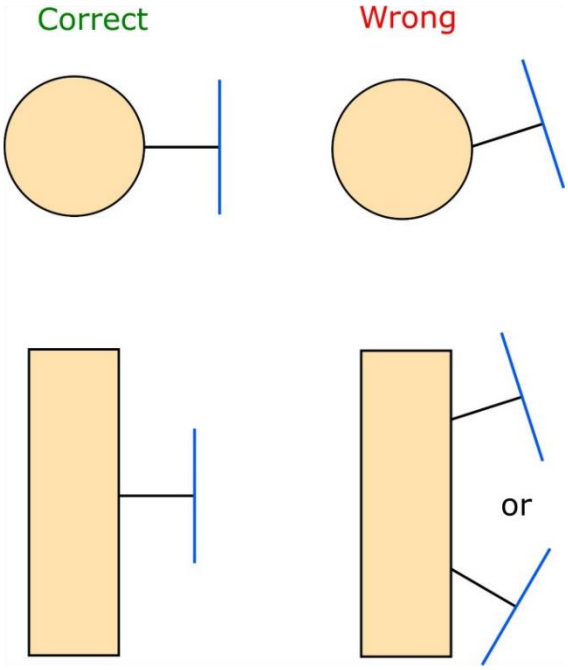
Field collection of tree cores

Field operations for tree core extraction must be performed by experienced personnel, with adequate knowledge on how to use increment borers correctly, collect and store cores in the proper way, select trees for the required aim. This is necessary to avoid the possibility of common errors in tree coring (e.g. Figure 1a) that may lead to inconsistent scientific results. Tree stems will be sampled radially considering both stem shape and crown geometry. It is highly recommended to use a starter to facilitate the borer insertion and avoiding that too much pressure is exerted on the external tissues of the stem (Fig. 1b).

Additionally, personnel expertise is required to allow that each extracted tree core is complete (see next sub-chapter), i.e. including both bark and wood, and ensuring that the entire wood radius is represented to the pith, or sufficiently close to it.



(a)



(b)



Figure 1. (a) Representation of some frequent mistakes in collecting tree cores with an increment borer by unexperienced operators, with view on the transverse (above) and longitudinal (below) stem sections. (b) Increment borer starter.

For each cored tree, DBH (two orthogonal measures, along isolines and maximum slope) and height will be measured. Crown extent will be described by measuring the length of 4 radii along the cardinal directions, considering only the sun-exposed portion of the crown. Additionally, metadata regarding species, plot number, tree ID (if available), GPS position will be recorded. Not all plots may have trees in all DBH classes. In that case, additional plots will be selected for additional sampling.

Storing tree cores in the field and laboratory analyses

Both bark and wood will be collected and stored in plastic tubes or straws to avoid breakage (Figure 2). In case of rotten cores, cores broken in more than 2 pieces, cores that missed to get close to the stem pith, another core must be collected from the closest tree pertaining to the same DBH class. If bark or core pieces are lost during the coring process, another core must be collected below (approximately 10 cm) the first one.

Once collected and stored, cores from the dominant species in the old-growth and managed stand from the same area will be shipped to UNITUS, where each of them will be analysed for density.

After the density analyses the cores will be shipped back to each laboratory for the tree-rings measurements.

Additionally, tree-ring widths from available cores may be analysed by local tree-ring laboratories to reconstruct growth dynamics under the light of climate change and process-related metrics connected to natural dynamics.

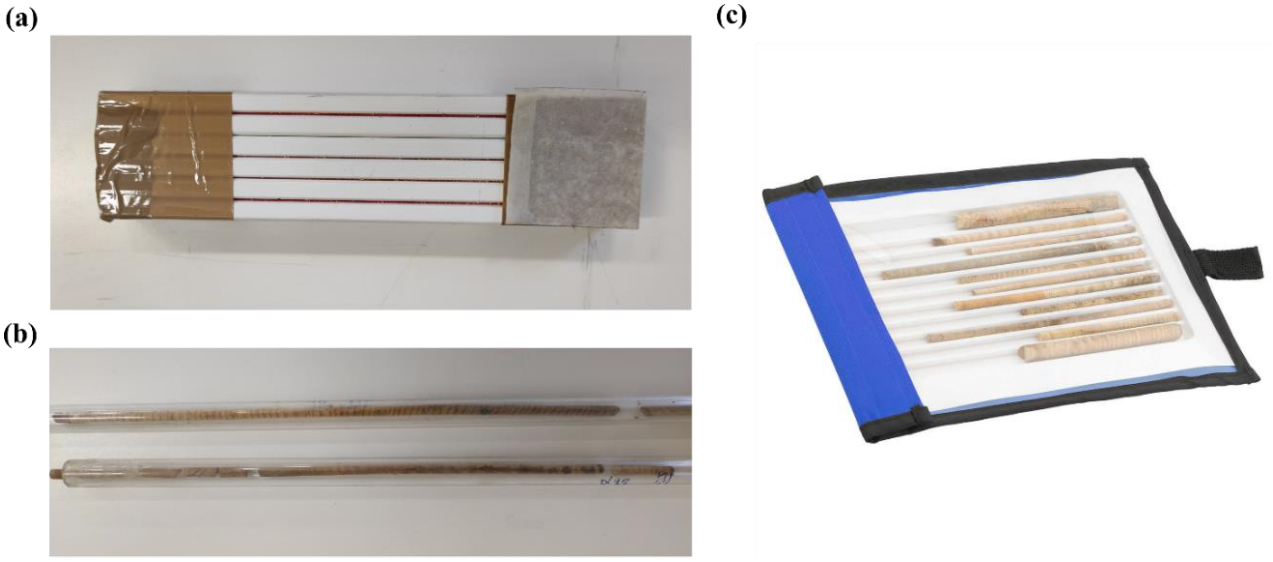


Figure 2. Possible ways to store wood samples. (a) Plastic tubes. (b) Straws. (c) Plastic core case (source: Forestry Suppliers).

3. Soil sampling approach

3.1. Glossary / definition of the terms used

- **Study Area**. Territory hosting the forests to be sampled (e.g. Parco Nazionale d'Abruzzo, Kalkalpen National Park).
- **Forest**. The ecosystems to be compared within each study area (e.g. old-growth vs. managed forest).
- **Plot**. Areas sampled for stand structure within each forest, of about 2,000 m2. The sites correspond to the study areas where local information/metadata will be collected, and where internal replicates of soil samples will be collected.
- **Sampling Area**. Location for collecting the soil cores in relation to the center of each plot.
- **Sampling Points or Replicates**. Number of soil cores and place of collection at each sampling area. Three cores will be taken at each sampling area, and the content at the same depth from each replicate will be mixed to produce average data for each sampling area.
- **Soil Core**. Soil sample collected across the entire prescribed depth (see below) at each sampling area, by manual augering (drill) or pits (hole large some decimeters, manually dug by spade).
- **Depths**. Soil core is collected in sequential drills/excavations, thus dividing the whole soil sample into fixed, prescribed depths (see below).
- **Soil Sampling Sheet**. Pre-printed document for reporting the prescribed metadata and measures associated to every soil sample.

3.2. Forest Selection

In each study area, available maps of the strata will be used to select 20-30 plots (preferably paired plots) in managed and long unmanaged parts of the research site.
An effort must be made to select plots that clearly differ in terms of old-growth-status but grow under comparable ecological conditions (bedrock, elevation range, aspect, slope).
The selected managed forest must preferably cover the full rotation cycle.

3.3. Locating the soil sampling areas within each forest

Available plot descriptions and metadata will be used to stratify the research site for collecting soil samples. The 'research site' encompasses both managed and unmanaged forest areas (e.g. UNESCO old beech forest and its managed buffer zone)



Within each forest, the candidate sampling areas for soil analysis will be extracted among plots within the different strata (see before) and selected using a stratified random approach based on local geomorphological conditions, i.e. plots falling in marginal or extreme conditions, like mountain tops, ridges, rocky outcrops, narrow valleys, gorges (Figure 3) will be excluded. The selection will be made possible using plot metadata on geomorphology, also including elevation, slope, exposure, rockiness and stoniness (%).
The number of sampling areas per site will be at least 2x10 (if possible 20-30, in accordance with the available budget).

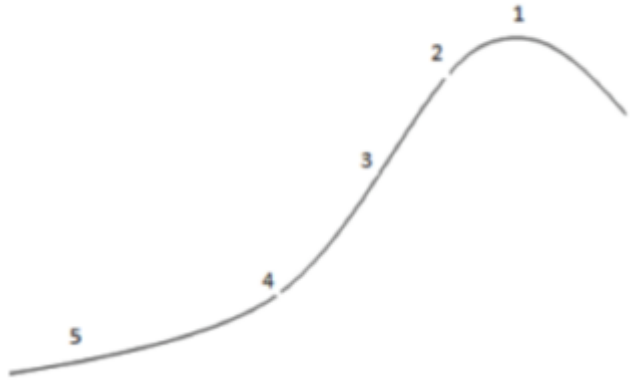


Figure 3: Potential slope position attributed in the metadata description of biomass plots. 1: mountain top/ridge; 2- upper slope; 3: mid slope; 4: bottom slope; 5 valley/plain).

3.4. Locating the replicates within each sampling area

At each selected soil sampling area, three sampling points (replicates) will be collected.
Each replicate will be located along the azimuth directions 0°, 120° and 240°, at 2.5 m outside the border of the plot established for quantifying stand structure (Figure 4).
If the sampling point falls closer than 2 m to a tree's base, the first among any successive points falling along the same direction (outward from the plot centre) at intervals of 2.5 m will be used (I.e. at 15m, 17.5m, ...).
A short description for vegetation cover and soil surface, using a reduced version of the metadata collection sheet used for stand structure, will be compiled for the surrounding of each sampling point.

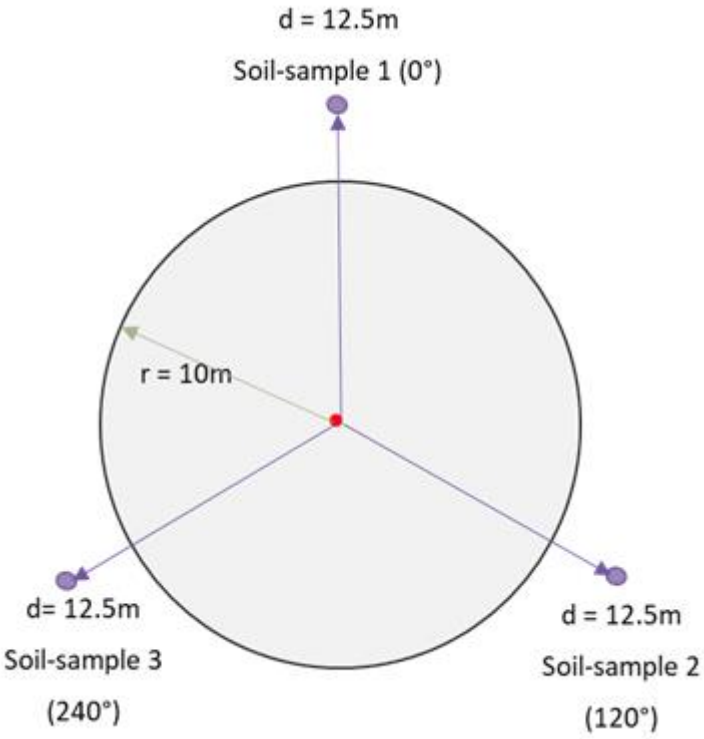


Figure 4: Location of soil sampling points within each selected sampling area (grey dots) with reference to plot center.

Collecting litter and soil samples in the field at each replicate

Soil samples will be collected at each sampling point with different approaches regarding litter and organic horizon, versus mineral layers.

At each selected sampling plot, 3 replicate samplings are performed. At each point, both litter and soil samples are taken. For the soil, samples are taken at fixed depth (4 mineral horizon depths 0-10, 10-30, 30-60, 60-100 cm; see below).

The tree replicates per fraction (organic, fixed depth of soil) are merged to one composite sample, and bagged. Whenever possible, the mixing of the samples and taking the subsample for chemical analysis is best performed in lab conditions. Only if the composite sample is too heavy, the sample is thoroughly mixed in the field and a subsample is bagged.

Add a waterproof label that describes the plot identity and the depth of sampling in the bag.

Sample analyses will be divided in two steps: a preparatory procedure performed in local laboratories (sample preparation); and laboratory analysis performed in a single selected soil laboratory (at INBO - Belgium) for density and chemical analyses.

3.5. Litter (O_L + O_F) and organic horizon (O_H)

The organic layer combines the Litter layer (OL+OF), combined with the Organic horizon (OH). All three are sampled together, as the subdivision is not always straightforward, and is not essential for the assessment of the Carbon stock.

The organic horizon must clearly show a content in organic matter higher than mineral content (SOM > 70%). Usually, the O_H horizon can be easily divided from the lower mineral horizons.

Litter and organic layer are sampled together. They will be collected at each sampling point by using a circular or square sampling frame (side or diameter ≥ 25 cm; Figure 5). At each sampling point, the full organic layer thickness is measured with a ruler within the sampling frame and registered on the Soil Sampling Sheet.

All organic matter within the frame is now manually collected and bagged. The sample will include all dead leaves, fine woody debris with diameter < 1 cm and decayed organic matter. Live roots, woody debris with diameter ≥ 1 cm, rocks, and all fruits (cones, ackorns) and bark > 1cm diameter will be removed.

The litter samples at the three sampling points are compiled to one sample (3 replicates) into an airtight plastic bag (LDPE plastic), adding a plastic label with the sample code and, after removing most of the air, will be taken to the local laboratory.



Figure 5: Example of a frame for collecting litter.

3.6. Mineral horizons (A)

Samples from the mineral horizons will be collected at fixed depths:

- 0-10 cm
- 10-30 cm
- 30-60 cm
- 60-100 cm (only in optimal cases, in deeper soils, without stones; in case the core is stopped between 60 to 100 cm, mark the maximum depth you reached)

Mineral horizons could be sampled using soil auger (Figure 6) or excavating pit.

- Manual augering generates semi-undisturbed sampling (Figure 7). For bulk density, the core diameter should be between 50- and 100-mm to avoid excessive soil compression. Alternatively, the rotative auger (root auger or traditional auger) can also be used, with particular attention to determine the right sampling depth. Alternatively, an excavation pit will be dug (if possible/allowed), necessary under those soil conditions not appropriate for the auger (e.g. rocky soils or dry sands).



Figure 6: Root augers, with accessories.



Figure 7: Examples of ring samplers for bulk density.

When using the auger, 3 samples are taken at fixed depths :
0-10 cm
10-30 cm
30-60 cm
60-100 cm.
At one of the auger samples, an additional sample for bulk density is taken at mid-depth (approx. 5 cm, 20cm, 45 cm and 80cm) using a ring at known volume (usually Kopecky rings, 100 or 110 cm^3). Try to fill the whole cylinder avoiding compaction of the soil. To avoid compaction, use ring sampler that push into the soil only the border of the ring (AVOID WOOD TABLES, because the risk to compact the soil into the ring is high!)
The fixed-depth soil samples of the 3 corings are combined to one sample, bagged and labelled. The bulk density samples are bagged and labelled separately.

What if full depth is not reached?
This is noted on the sampling document, mentioning the depth that was attained and reason for not reaching the full depth (e.g. hitting bedrock)
If one or two of the corings is not reaching full fixed depth, the remaining sample(s) is/are selected and bagged as the sample for this depth. Do not (!) add a sample that did not

reach full depth of a fixed-depth section (as you will oversample the upper part of that fixed depth, and you don't know whether you reached bedrock, or just a large stone)

In case of a profile pit: You dig one pit, carefully separating the different fixed depths. If full depth is not reached, note the depth achieved, and, if you reached bedrock, you can still consider a non-complete depth sample (carefully noting bedrock depth). Take the soil samples that are relevant depending on this reached depth. Depending of the fraction of the horizon reached, a soil sample may or may not be taken.
e.g.; depth reached = 48 cm: sample for 30-48 cm is relevant; if depth is 34cm, insufficient material can be sampled, so this makes no sense
Take the bulk-density samples in the profile-side of the pit at depths of 5, 20, 45 and 80 cm, by pushing in a Kopecky ring horizontally.

Pre-processing of the samples (in each country)
Each soil sample (litter, organic horizon, up to 4 mineral horizon depths 0-10, 10-30, 30-60, 60-100 cm) from each sampling area will be carried to local laboratory for preliminary treatment, before being further shipped to the laboratory for chemical analyses.

Pre-processing of the organic layer – samples
Organic layer samples are weighed (fresh weight) upon arrival in the office/lab. They are inserted in the oven at 105°C for 24 h or until the weight is stable, and weight (dry litter). Both values are carefully recorded.

The samples are reinserted in a sealed bag, labelled and sent to the central lab for further analysis.

Preprocessing of the mineral layer-samples.
First, the composite samples are weighed (fresh weight), sieved through a 1-cm sieve, to separate stones and coarse roots with diameter >1 cm. Each separated fraction will be weighted again and the % volume occupied within the soil sample will be estimated (Figure 8).

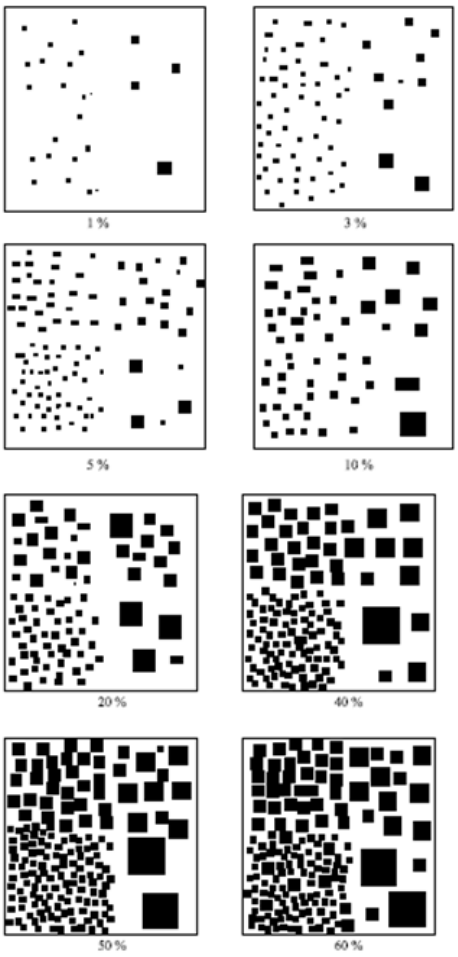


Figure 8: Examples of volume percentages occupied by the coarse fraction within the soil.

The sieved samples will be air dried (alternatively at 30°C for 24h in oven with fan), labelled and sealed in plastic bags and sent to the analytical lab.

If the sample is too large or heavy, a subsampling will be performed first: the sample is mixed intensely and then subsampled using the quartering method (Figure 9), a representative aliquot of 150 g will be sent to the laboratory selected for chemical analyses. The rest may be preserved in each local laboratory.

For **Bulk density** (BD), the fresh samples are weighed, then dried in the oven at 105°C for 24h and then weighted again (dry weight).

3.7. Laboratory analyses

3.7.1. Organic layer and litter:

The dried litter/organic layer samples are cut-up in a cutting mill (RetschSM2000) or knife mill (GRINDOMIX GM 200), until a size of <0.2mm is reached and homogenized. A subsample is taken (using a splitter), and ready for analysis. Total Carbon (TC), inorganic C (TIC) and

total nitrogen (N) are determined through combustion methods. The total organic carbon (TOC) is then the difference between TC and TIC.

3.7.2. Chemical analysis in the selected laboratory

The air-dried samples are further dried until stable weight (in the oven at 40°C), and grinded to fraction sizes of < 2mm using a 'jawbraker'-mill. For futher milling to sizes < 0.02 mm (needed for total inorganic carbon content) a planetary ball mill (type Pulverisette 6 classic line) is used.

Total, organic, and total nitrogen (N) are determined through combustion methods on the fine earth fraction (soil materials passing a 2 mm sieve), and for inorganic C, the pulverised sample is used. Total Carbon (TC), inorganic C (TIC) and total nitrogen (N) are determined through combustion methods. The total organic carbon (TOC) is then the difference between TC and TIC.

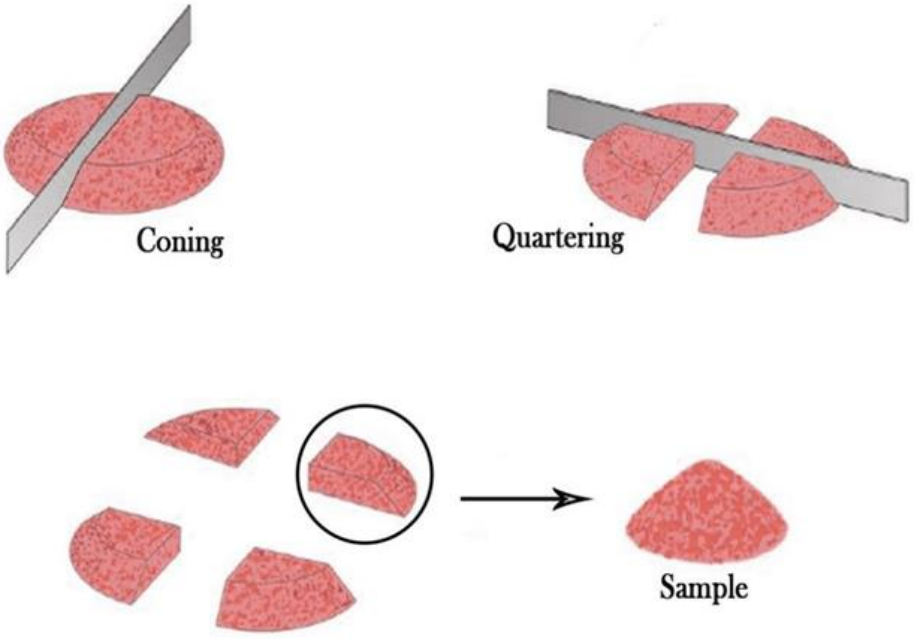


Figure 9: The quartering method for subsampling soil samples.

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