INSTRUCTIONS FOR

SOIL SAMPLING AND PREPARATION

for monitoring the soil organic carbon and nitrogen

Version	Release date	Summary of changes
20170530	20170530	Explained labeling of soil bags, correction of wrong reference to annexes.
20180322	20180322	Corrected the units of $SUMWA_{l,s}$ and $WA30_{i,l,s}$ (kg m^{-2}), suggested to exclude Post Office as carrier for samples transportation. Clarified that metadata and correct labelling of samples are mandatory for the analysis. Other minor textual editing.
20200319	20200319	Complete revision of the part regarding the organic layers (figure 17): *a)* clarified that also coarse elements should be removed from the fresh sample; *b)* added the weight of the holorganic fresh sample (W30_OH); *c)* added the possibility to weight also the coarse elements and living plant material (and specified the temperatures); *d)* removed the drying of the organic matter at 70°C; *e)* added the submission of the organic composite samples to the archiving facility. For the Mineral soil samples corrected the drying temperature of coarse elements (150 instead of 30) and all living plant material should be removed (not only roots). Added a table in the data submission section with the list of variables and BADM names. Other textual clarifications and changes
20201020	20201020	Clarified that sampling design (i.e. location of all the points and their verification) must be completed before starting the soil samples collection. Clarified how to correctly mane the samples collected in the different SP-II locations. Added a reference email address to contact to organize the shipment or soil samples.

20211109	20211109	Summary modified in order to highlight some crucial step needed before the sampling. Added information and clarification for the histic soils. Added instructions on how to submit the soil classification and description (pdf document) and the WRB class with the BAMD. Clarified that W70 R and W105_S should be always submitted even in case of all zeros. Mentioned to send 50 g aliquot from holorganic samples to the Central Soil Analysis Laboratory. Clarified how SOC and SON content and soil texture are measured (Annex 3 and 4). General revision of the document structure.

The ICOS protocols and the derived Instructions documents can be changed and amended in time, because new methods become available or to improve their clearness. For this reason, it is crucial to keep track of the versions and differences.

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SUMMARY

The soil is a major component of the terrestrial carbon cycle and monitoring the temporal changes in the Soil Organic Carbon (SOC) and Nitrogen (SON) Stocks is requested in order to close the ecosystem mass balance of these elements and to assess the role of terrestrial ecosystems in the global carbon cycle.

This document describes how to collect and prepare soil samples to monitor the SOC and SON stocks within the target area of an (Eddy Covariance) EC flux tower. The SOC and SON stocks are calculated for the first meter (0 to 100 cm) and is determined from simultaneous measurements of soil organic carbon, nitrogen content and bulk density. Although the soil organic carbon and nitrogen contents of soil samples are determined by the ETC central laboratory, the station team must measure the soil density. The methodology used to determine the SOC and SON stocks is based on the ICOS soil sampling and preparation protocol (Arrouays et al. 2018), and the ISO 10694 and ISO 13878 norms. The design based theory used for the sampling is described in the Instruction on Sampling Design and, together with the results of soil analysis and SOC and SON quantification in an example at the link: https://traitementinfosol.pages.mia.inra.fr/icos/DE-RuSCarbonReportv2.html. The soil texture is also measured on a selected number of locations, if not already known.

It is important to highlight that before going sampling in the field it is needed to contact the ETC Soil Team in order to ensure that all the points are clarified and that before shipping the samples it is mandatory to first submit all the BADM information that will be validated by the ETC. Only after the green light from the ETC it is possible to ship the material. The ETC Soil Team can be contacted at icos-etc-soil@inrae.fr keeping as always info@icos-etc.eu in CC.

This Instruction document is based on the following ICOS Ecosystem protocols and documents:

- Arrouays D, Saby N P, Boukir H, et al. Soil sampling and preparation for monitoring soil carbon. Int. Agrophys. 2018;32(4):633-643. doi:10.1515/intag-2017-0047.
- Jolivet et al. 2016 "Volumetric sample for the determination of bulk density". In *"Manual for the French soil monitoring network"*, version 1.1. Available here http://www.gissol.fr/wp-content/uploads/2018/03/Manuel_V_Num2.pdf
- International Standard ISO-11074-2:1998 (Soil quality – Vocabulary – Part 2: terms and definitions relating to sampling)
- Kruger et al,. 2016 "Calculating carbon changes in peat soils drained for forestry with four different profile-based methods", For Ecol Manag, 381, 29-36, 10.1016/j.foreco.2016.09.006.
- WRB IUSS Working Group WRB, 2015 "World Reference Base for Soil Resources 2014, update 2015, International soil classification system for naming soils and creating legends for soil maps". World Soil Resources Reports No. 106. FAO. http://www.fao.org/3/i3794en/I3794en.pdf

OBJECTIVES AND DEFINITIONS

Sampling Point: it is a point in the target area where sample collection will be operated. In ICOS guidelines, the soil sampling points correspond to SP-II and are defined by the ETC following the Instruction on Sampling Design.

Individual sample: sampling unit collected by a single operation of a sampling device, kept, and treated separately from others.

Composite sample: two or more individual samples mixed in appropriate proportions, from which the averaged value of a desired characteristic may be obtained.

Aliquot: known amount of a homogeneous material, assumed to be taken with negligible sampling error.

Organic layers: according to the World Reference Base (WRB) for Soil Resources, two organic horizons can be distinguished: the "O" horizons that are on top of a soil that is mostly mineral, and the "H" horizons that are dep organic soils with no or few mineral soils below.

Organic horizons "O" are dominated by organic material, consisting of undecomposed or partially decomposed litter, such as leaves, needles, twigs, moss, and lichens, which has accumulated on the surface; they may be on the surface of either mineral or organic soils, or at any depth below the surface if it was buried. O horizons are not saturated with water for prolonged periods. The mineral fraction of such material is only a small percentage of the volume of the material and generally is much less than half of the weight. The O horizons classification is as follows:

- Slightly decomposed plant material (**Oi**) are almost entire leaves and twigs
- Moderately decomposed plant material (**Oe**) are fragmented plant parts still identifiable as twigs, leaves, buds, etc.
- Highly decomposed plant material (**Oa**): not identifiable.

The "H" horizons or layers are organic layers dominated by organic material, formed from accumulations of undecomposed or partially decomposed organic material at the soil surface, which may be underwater and are saturated with water for prolonged periods or were once saturated but are now artificially drained.

SOIL SAMPLING OVERVIEW

To sample the relevant soil characteristics across the target area, the sampling strategy includes 20 discontinuous measurement plots (SP-I). Within each SP-I, a variable number (3 or 5) of sampling SP-II points are used for soil sampling. The Instructions for Sampling Design describes how these points are selected.

Determine if sampling is needed

As a preliminary step it must be determined whether the detection of a temporal change in the soil carbon stock is feasible and the present instructions are applicable. As a rule of thumb, temporal changes of the total SOC stock over the next 20 years expected to be less than 20-25% of its spatial standard deviation are unlikely to be detected. This is mainly the case for soils where the carbon stock is huge as compared with its expected changes over 20 years, typically the peat soils. This may apply also for the soil having a relatively large spatial inhomogeneity due for instance to stoniness (e.g. forests soils on glacial deposits). In such cases, it is important to describe the soil in order to evaluate the feasibility of detecting a temporal change and decide together with ETC whether these instructions can be applied or an alternative approach found.

Sampling design requirements

Before starting to organize the sampling it is important to have completed the procedure for the sampling design described in the relative Instruction. The procedure completed when the real position of all the SP-I and SP-II points are submitted to the ICOS ETC database and verified and confirmed by the ETC staff.

Soil description

The first core or pit of each location at the SP-I points should be described by a soil expert or a properly trained staff. When the number of 20 cores or pits is not achievable because of e.g. difficulty of access, it can be reduced to ten or five after discussion with the ETC. The soil description should be reported in a pdf document that is submitted to the ETC from the PI Area selecting “Other file” in the options and naming it “CC-###_Soil_Description.pdf” where CC-### is the official site code.

The following elements must be reported:

WRB name: (e.g., Albic Stagnic Luvisol (Cutanic, Endoclayic)) (see http://www.fao.org/3/a-i3794e.pdf), that must be also submitted using the ICOSBADM_Soil_Info template (see Submission section)

Horizons description: each horizon of the profile must be described using the following variables:

- Upper and lower vertical limits of the horizon

- Colour of the horizon using the Munsell color code chart and the format as follows:

  xxYY /vv cc (e.g., 10YR /3 4) where lower case are numbers and upper case letters coding for hue (*xxYY*), value (*/vv*) and chroma (*cc*) respectively. See for instance http://munsell.com/color-products/color-communications-products/environmental-color-communication/munsell-soil-color-charts/.

- Texture of the horizon (in Annex 1)

- Diagnostic horizon name (if applicable) according to the FAO WRB (http://www.fao.org/3/a-i3794e.pdf)

- Diagnostic material name (if applicable) according to the FAO WRB (http://www.fao.org/3/a-i3794e.pdf)

- Diagnostic property name (if applicable) according to the FAO WRB (http://www.fao.org/3/a-i3794e.pdf).

A picture of each pit or core showing the vertical profile of the entire soil from 0.0 to 1.0 m depth must be taken. A graduated scale must be positioned along the core or pit and must be visible in each picture. The upper and lower limits of the soil must be clearly identified when necessary. The picture should show the entire vertical profile of the soil so that the soil cores taken along the same profile must be reassembled accordingly. The pit wall chosen to be photographed must be well clean and lit, preferably by natural light (Figure 1).

Figure 1. (a) Example of photo of the soil profile with scale as reported with a pit. (b) Example of soil profiles as reported when cores are used (graduate scales on the right side).

Measurement methods

All the measurements and sampling are based on a set of standard depths measured from the surface of the mineral soil and specifically: 0-5; 5-15; 15-30; 30-60; 60-100 cm. Organic ("O") horizons are sampled separately, with no prescribed thickness. Their thicknesses correspond to the identified horizon. They are counted negatively (above the mineral soil), and usually vary between 0 and -15 cm. For peat soils, the standard depths are the same but supplemental cores can be sampled until reaching the mineral layer.

The International Standard ISO-11272:1998 describes three methods for the determination of dry bulk density of soils calculated from the mass and the volume of a soil sample. All three methods involve drying and weighing a soil sample, the volume of which is known (core method) or has to be determined (excavation method and clod method). At each sampling point, a series of core samples of known volume must be extracted. The core method (i) must be used for non-stony and coherent soils and the excavation method (ii) for stony soils.

(i) (i) In non-stony coherent soils, samples have to be taken as vertical undisturbed cores that are extracted with a motor hammer corer or a root corer. All five SP-II locations around each SP-I (n = 20 × 5) have to be sampled (Figure 2a). The core samples of known volume will be extracted from the layer depths defined above.

(ii) (ii) In stony or incoherent soils, only the first three SP-II locations around each SP-I have to be sampled (n = 20 × 3, Figure 2b). At each SP-II location, a soil pit is dug and individual volumetric samples are extracted in the pit from the layer depths defined above. Because volumetric samples thicker than 10 cm are practically difficult to extract in one piece, the layers 30-60 cm and 60-100 cm are sampled by means of two superposed samples of equal volume. The individual volumetric samples are taken either by the cylinder method or by the excavation method according to the International Standard ISO 11272:1998.

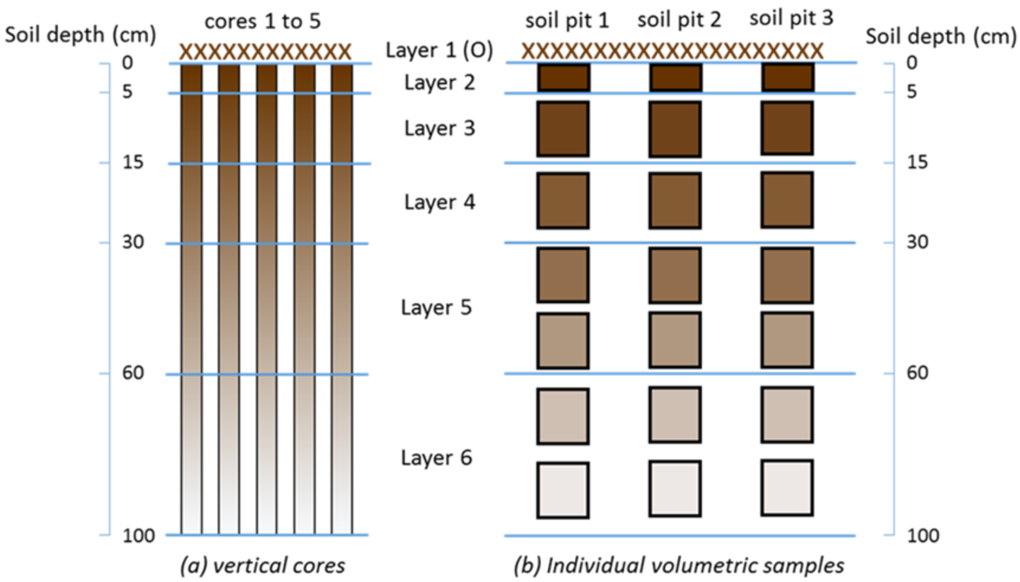


Figure 2. Soil sampling at each SP-I location, extracting either (a) 5 undisturbed vertical cores or (b) individual volumetric samples from 3 soil pits.

Timing of soil sampling

The soil sampling should be carried out at a time when the conditions of humidity are close to the field capacity and in a stabilized ground, not recently ploughed, to ensure qualitative sampling. The groundwater table should be below the soil depth to be sampled. In croplands, the sampling must be carried out at least four weeks after the last ploughing at a time when the upper soil layers are firm and wet and allows using a soil auger or automated corer. In addition, periods following cultivation operations (tillage, disking, seeding, fertilisers' application etc.) must be avoided. The total duration of field operations can be estimated between 2 and 4 weeks depending on the number of staff and soil characteristics. It must not exceed three months. When several sampling teams are in charge of sampling they must be trained first together in order to achieve the best achievable comparability.

OVERALL SOIL SAMPLING PROCEDURE

For sampling mineral and organic horizons, the upper surface of the soil at the interface between "O" horizons and the upper mineral horizon must be carefully identified. Then, at each individual sampling location, the organic horizons O must be collected using a frame (25x25 cm²) and separated between the Oi, Oe, and Oa layers. Then, mineral soil cores can be taken from sampling depths 0-5, 5-15, 15-30, 30-60, 60-100 cm for organo-mineral and mineral horizons (see Figure 2). Whatever the technique used, it is essential to obtain for each sample collected i) the sample volume, V in cm3, ii) the sample vertical thickness, LT in cm, and iii) the sample horizontal cross sectional area, A. Cores have to be typically about 10 cm in diameter. The size of the pits has to be adapted to local conditions and in any case kept as small as possible. If core sampling is impossible to achieve (i.e., randomly distributed stones in depth) it is recommended to try to reach at least a depth of 30 cm by resampling once 1 m away from the theoretical point. In case a depth 30 cm can again not be reached, the deepest sampling point will be retained, and the soil depth will be considered to have been reached. For cores deeper than 30 cm, any impossibility to dig down to a depth less than 1 m, i.e. because of shallower bedrock, will define the soil depth. In case the soil depth is in between the standard depth values (e.g. it was possible to arrive to 55 cm, in between the standard range 30-60 cm) the layer between the upper standard depth and the soil depth has to be sampled (e.g. 30-55 cm is considered as last layer instead of 30-60 and 60-100). Pits have to be re-filled after digging, respecting the vertical order of layers. In addition, before extracting the core from the auger or starting to sample from the pit, a picture of each soil core or pit must be taken, with the site code, date and location in the name (e.g. FR-Bil_20161202_01-03.jpg).

Because of the numerous samples, and to ensure a precise identification, it is mandatory to use special plastic bags with pre-printed forms that will be provided by the ETC, and to label them carefully according to the following cases.

Sample identification codes and labelling

Code for SP-I mineral horizons and histic soil samples

Mineral sample identification code must follow the scheme 1:

CC-SSS_YYYYMMDD_S1-S2_UD_LD (1)

Where:

- CC-SSS is the ICOS station code,
- YYYYMMDD is the sampling date,
- S1 is the SP-I identification number as defined in the PLOT_ID (e.g. two digits, like 01, see ICOS BADM on Sampling scheme),

- UD and LD are respectively the theoretical upper and lower depths in cm. Values must be integer (it is only an ID, exact depths are provided in the metadata of SP-II samples) and using the minimum number of digits (e.g. 5 cm must be reported as 5 and not as 05).

This identification code has to be reported on the label on the bag and duplicated on a plastic label to be inserted inside each plastic bag for avoiding mistakes. Additional information such as the name of the person collecting the sample, type of sample, sampling method and sample volume, thickness and area must be recorded and subsequently transferred to the ETC.

Code for SP-I organic horizons

Organic horizons samples must be collected in the same kind of plastic bags. In this case the identification code UD is substituted by the name of the horizon (O, or eventually Oi, Oe and Oa when appropriate), while LT will have to report the average thickness of the horizon collected in cm, as an integer without decimal values. Note that this is not valid for the histic soil samples that must follow the mineral soil samples identification). The sample identification code must follow the scheme 2:

CC-SSS_YYYYMMDD_S1 _XX_LT *(2)*

Where:

- CC-SSS is the ICOS station code,
- YYYYMMDD is the sampling date,
- S1 is the SP-I identification number as defined in the PLOT_ID,
- *XX* is the name of the organic horizon *Oi, Oe* or *Oa,*
- *LT* is the layer thickness (integer in cm).

Example: Labels for the samples collected at the SP-I point 05 of the FR-Bil station on December 1st 2016 and where there are two organic horizon Oi and Oa, of respectively 2 and 3 cm thickness plus the standard 5 depths in mineral soil will be:

FR-Bil_20161201_05_Oi_2

FR-Bil_20161201_05_Oa_3

FR-Bil_20161201_05_0_5

FR-Bil_20161201_05_5_15

FR-Bil_20161201_05_15_30

FR-Bil_20161201_05_30_60

FR-Bil_20161201_05_60_100

Hence, for a station with 3 organic layers (Oi, Oe, Oa) and 5 mineral soil layers, a total of [3 + 5] × 20 SP-I =160 samples should be sent for analysis.

Code for SP-II mineral and organic horizons

For the SP-II single samples, it is needed to define an SOSM_SAMPLE_ID for reporting several pieces of information, even if they are not shipped to the laboratory. The samples ID in this case must follow the same structure as the SP-I explained above, with the only difference that instead of the SP-I value, the code of the SP-II should be added separated by dash:

Mineral horizons: CC-SSS_YYYYMMDD_S1-S2_UD_LD (3)

Organic horizons: CC-SSS_YYYYMMDD_S1-S2_XX_LT (4)

Where

- CC-SSS is the ICOS station code,
- YYYYMMDD is the sampling date,
- S1-S2 is the SP-II identification number as defined in the PLOT_ID (e.g. 01-01, see ICOS BADM on Sampling scheme),
- UD and LD are respectively the theoretical upper and lower depths in cm,
- XX is the name of Oi, Oe or Oa,
- LT is the layer thickness (cm).

Code for the organic soils

For soils that are only organic, the same sampling strategy as mineral soil is used. However, it is recommended to take contact with the ETC staff to clarify any misunderstanding.

Also organic horizons samples must be collected in the same plastic bags and in the identification code UD is substituted by the name of the horizon (O, or eventually Oi, Oe and Oa when appropriate) while LD will have to report the thickness of the horizon collected in cm.

CC-SSS_YYYYMMDD_S1-S2_XX_LT

where XX stands for Oi, Oe or Oa and LT for the layer thickness (cm).

Before extracting the core from the auger or starting to sample from the pit, a picture of each soil core or pit must be taken, with the site code, date and location in the name (e.g. FR-Bil_20161202_01-03.jpg).

Soil sampling equipment

Generic equipment

- Shovel, spade, pickaxe, moil
- Knife, Berthelet trowel
- Spatula and trowels
- Pruning shears, scissors, handsaw (to cut the roots)
- Folding rule
- Labels, elastics, pencils, markers, paper labels
- 500 to 800 Plastic bags (provided by ETC) for field collection. The exact number of bags requested depends on the sampling scheme and the presence of organic layers.
- Portable field scale with 0.5g precision.

Specific equipment for the frame technique

- Metallic square frame with 25 x 25 cm inner dimensions and equipped with two 10 cm long iron rods for plugging.

Specific equipment for the cylinder technique

- Steel cylinders or rings (with an inner volume of approximately 500 cm^3) with one sharpened edge (homemade or commercially available, see for instance at https://en.eijkelkamp.com). The cylinder dimensions can be adapted to the thickness of the layers.
- Hitting plate or cylinder
- Small plate to place the cylinder on
- Small and large mallet

Specific equipment for the water technique

- Spirit level (20 cm) to verify the flatness of the prepared surface
- Annular template of inner diameter long enough to take at least a 1000 cm^3 sample
- Nails to fix the annular template
- Tablespoon, ladle, paintbrush
- Clear, thin and robust plastics bags
- 500 ml measuring cylinder graduated by 5 ml
- 5 litres of water
- Funnel

Specific equipment for the sand technique

- Graded sand with sand size particles between 500 and 700 µm

SOIL SAMPLING METHODS

The manual frame method (organic and uppermost soil layer)

This method is compulsory for organic horizons (above the uppermost mineral soil layer) and recommended for the upper mineral layer (0-5cm) as it is difficult to core precisely the surface horizons particularly with crumb or granular structures. It is based on the collection of a volumetric sample by excavating the organic layer or soil within a square frame, and in a given thickness. The volume extracted can be calculated from the dimensions of the cavity (frame area × thickness). As the difficulty of maintaining a proper geometrical shape can alter the precision of the method, the use of a large frame of at least 25 x 25 cm is recommended. The depth of the dug out cavity is measured in order to allow volume calculation.

Step-by-step operation mode

1. Sampling is realized directly from the soil surface. Install the frame on the soil by pushing the two iron rods in the ground (Figure 3A).
2. Collect the O layers on the whole frame surface and throughout their entire thickness. Use a knife to cut the sample along the inner edges of the frame and collect the material with a hand spade or spoon. Put the material in a labelled sample bag and insert a plastic label with sample identification code inside. All vegetation debris inside the frame must be collected (Figure 3B).
3. Measure the thickness of the cavity (mean from 5 to 10 measurements in case of irregular depth) and note it on the sample bag and on the field report (Figure 3C).
4. For the 0-5 cm layer, proceed in the same way by collecting the soil layer on the whole frame surface, using a knife to cut the sample along the inner edges of the frame (Figure 3D).

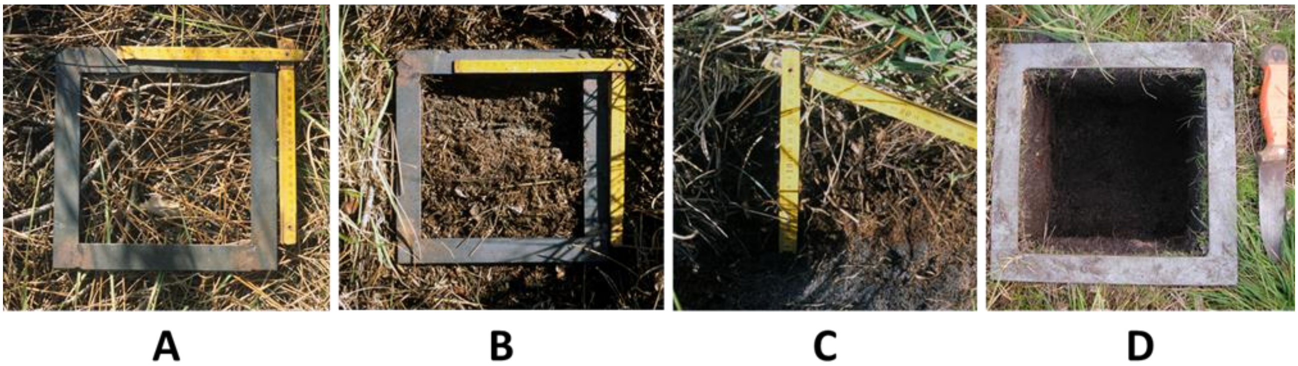


Figure 3. Steps of the manual frame technique for organic and uppermost soil layer sampling. A: frame installation; B: organic layer sampling; C: organic layer thickness measurement; D: first soil layer sampling.

The “Core” method with mechanical sampling (motor hammer corer)

Mechanical sampling can be used for coring the mineral part of the soil (after removal of the organic layer as seen previously). The Annex 1 of this document presents the different systems: Jack hammer or drillers, and a method to extract the gouge from the soil. The soil coring must be operated at the same location than organic layers sampling. The corer must be driven vertically.

Soil sample preparation in the field

Once the gouge is extracted, in order to avoid errors due to soil compaction, measure the soil depth and the depth of the gouge (Figure 4A) and note them a logbook, then carry the gouge to a “soil preparation spot” (Figure 4B), where you have prepared the labels, aluminum trays and a knife. At the soil preparation spot arrange the trays and plastic labels in the necessary order, open the window of the gouge (Figure 4C) and measure the length of the soil core (Figure 4D) taking note of it.

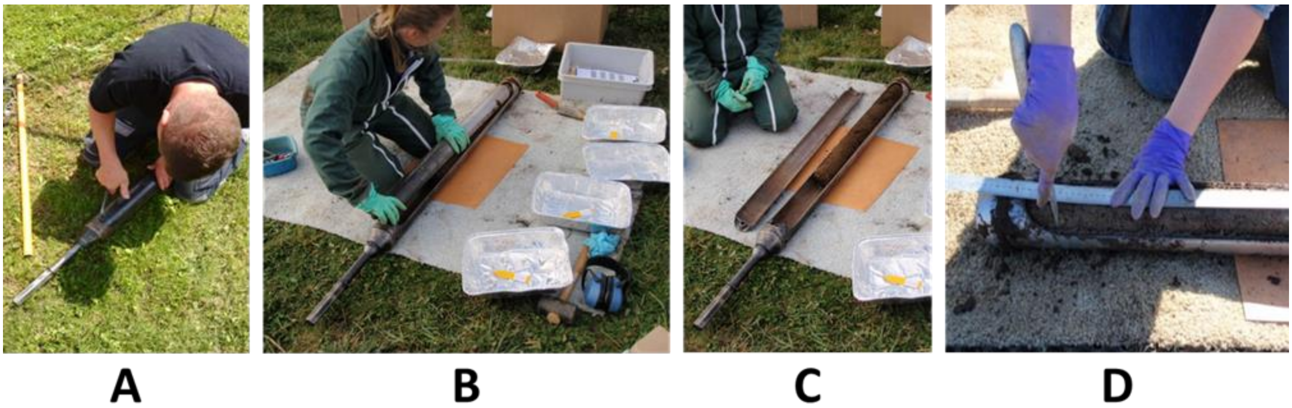
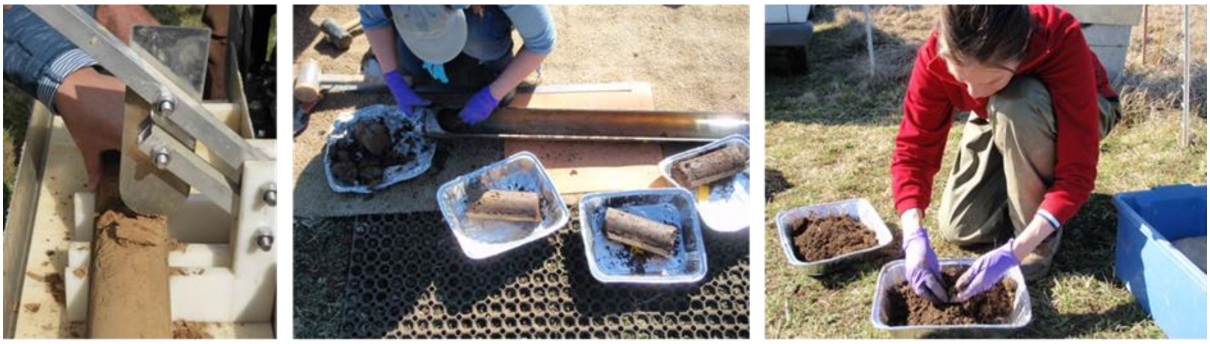


Figure 4. Soil sample preparation with “Core” method. A: measure the depth of the gouge; B: organize a soil preparation spot with all the material needed; C: open the window of the gouge; D: measure the length of the soil core.

Cut the soil column into the predefined slices (0-5, 5-15, 15-30, 30-60 and 60-100 cm) (Figure 5A) and transfer the soil slices into the corresponding trays (Figure 5B). Once the whole soil column is emptied, crumble and dissect the soil in order to disaggregate the soil aggregates (Figure 5C). Put the soil into the ICOS plastic bag properly labeled, insert its plastic label inside.

Repeating the sample identification code, close the bag evacuating the air inside as much as possible and store the plastic bag for the transport to the lab. In case a scale can be used in the field, weight the fresh soil sample. Otherwise the weighing must be done within 5 days after bringing the samples back in a cold chamber between 4 and 10°C. At this stage, the soil sample volume (V) and fresh weight (W0) must have been determined.



A B C

Figure 5. Soil sample preparation with "Core" method. A: cut the soil column at the predefined depths using a homemade slicer in this case; B: transfer soil slices into the corresponding trays; C: crumble and dissect the soil.

The "Core" method with semi-mechanical sampling (root corer)

The use of a root corer (Figure 6A) could be an alternative to the use of the motor hammer corer described above. Typically, such a corer is provided with a sampling cylinder of 15 cm length and a diameter of 8 cm that is a volume of about 750 cm^3. The corer is driven vertically in the soil either by manual rotation or by percussion using a mallet, the latter being useful in the compact horizons. The crank is used to remove the sample from the cylinder. The handle of the corer is graduated each 15 cm to check the depth of sampling.

The root corer can be easily used to sample the 5-15 cm layer (one 10 cm long core), the 15-30 cm layer (one 15 cm long core), the 30-60 cm layer (two successive 15 cm long cores), and the 60-100 cm layer (three successive cores: 15, 15 and 10 cm long). The sample preparation is the same explained for the mechanical sampling apart from the extraction of the sample from the corer that is done using the crank (Figure 6B).

A B

Figure 6. Soil sample preparation with "Core" method using a root corer (A). B: extraction of the sample

Excavation method (cylinder, water and sand techniques)

Three excavation techniques dedicated to soil pits are described here: the cylinder technique, the water technique and the sand technique. For organo-mineral and mineral layers, the most appropriate technique will be selected according to the soil characteristics (consistency and thickness of the soil layer, stones and roots contents).

The water and sand techniques must be used when the cylinder technique is difficult to use: in the presence of gravels, pebbles and roots, or in the very clayey horizons. These techniques are not suited to sample small volumes. The main difficulty of the water technique is to obtain a soil surface flat enough to fit exactly the water level to the surface of the ground. That is why for soil on steep slopes, the sand technique is more suitable.

Cylinder technique

The cylinder technique allows collecting undisturbed volumetric samples of predetermined volume. This technique is described according to the ISO 11272:1998 International Standard. It is suited for soils with a small content of stones, gravels and roots. Large steel cylinders are recommended (500 cm^3 e.g. 8.4 cm inner diameter × 9 cm height) to allow extraction of large volumes and minimize relative errors. Small cylinders (250 cm^3 e.g. 8.4 cm inner diameter × 4.5 cm height or 100 cm^3 e.g. 5.3 cm inner diameter × 4.5 cm height) can be used to characterize the thinner soil layers (5-15 cm and 15-30 cm). It is not recommended to use cylinders with diameter larger than 10 cm unless the soil is particularly plastic and coherent. In addition, cylinders with height larger than 10 cm must also be avoided wherein removing the sample from the soil matrix can be very difficult. For thick soil layers e.g. 30-60 cm and 60-100 cm, it is necessary therefore to take several samples superposed to cover the whole layer thickness. As shown in Figure 2b, one soil sample must be taken for each depth level sampled from 5 to 30 cm (i.e. 5-15 cm and 15-30 cm), two superposed for the 30-60 cm level and three superposed for the 60-100 cm level.

Step-by-step operation mode

Sampling is realized from the soil surface for the uppermost sampling depth (Figure 7A) and from stair-stepped surfaces in the soil pit for the deeper sampling depths (Figure 8C). Horizontal surfaces are shaped by using the trowel or the Berthelet trowel. The cylinder (Figure 7B) is hammered vertically in the soil using one or two mallets on the hitting plate (an aluminum half cylinder in Figure 7C). Gently remove the cylinder from the soil by means of a knife, a spatula or a trowel digging out the soil all around the cylinder. Cut the soil below the cylinder’s underside with a well sharpened knife and insert a spatula below the cylinder and remove the cylinder + spatula, ensuring that no soil falls out of the cylinder (Figure 7D)

A B C D

Figure 7. Cylinder technique phases: A) prepare the surface and the cylinder (B), C) hammer the cylinder vertically in the soil and then extract it (D).

Once extracted, place the cylinder on the plate and carefully level both sides of the cylinder with a knife (Figure 8A-B). Empty the cylinder in the labelled sample bag adding the plastic label inside. Remember to measure the sampling depths of the cylinder (top and bottom) and note them on the sample bag and on the field report.

A B C

Figure 8. Cylinder technique phases: A and B preparation of the sample in the cylinder; C: stair-stepped surfaces in the soil pit.

Note: driving the cylinder vertically is recommended but driving it horizontally through the pit wall is allowed, particularly in deep horizons.

Water and Sand techniques

The water technique and the sand technique allow both to collect volumetric samples of varying volume by excavating soil and measuring the volume of the cavity with either water or graded sand poured into in the cavity covered by a plastic film. The water technique is adapted from the standard sand technique (ISO 11272:1998) by replacing the graded sand with water. The stonier the soil and the larger the pebbles (stones or blocks), the more important is the volume of the cavity. So to improve the precision of the measurement, it is recommended to collect samples of more than 2000 cm^3.

Step-by-step operation mode

Sampling is realized from the soil surface or in the deep horizons of a pit, by fitting stair-stepped surfaces (Figure 9A). Horizontal surfaces are shaped by using the trowel or the Berthelet trowel. A perfect leveling is needed so place the annular template on the soil surface, fix it with the nails and check the leveling with the level (Figure 9B). Start to dig a cavity at the center of the annular template with a knife, a tablespoon and a ladle, according to the wetness of the soil. The diameter of the cavity has to correspond to the annular template and the depth of the cavity must be adjusted according to the thickness of the layer to sample. All of the extracted earth must be collected in the labelled sample bag with the corresponding plastic label inside (Figure 9C). The extracted earth must be extracted without losses (collect the possible losses fallen on the template and the small aggregates remaining at the bottom of the cavity by means of the paintbrush). Be sure take at least a 1000 cm3 sample. The shape of the cavity does not need to be a perfect cylinder but its walls must be as regular as possible: avoid recesses and projections of sharp stones or roots susceptible to drill the thin bag used to coat the cavity. When a sufficient amount soil is sampled, place the thin bag in the cavity and stick it onto the walls (Figure 9D).

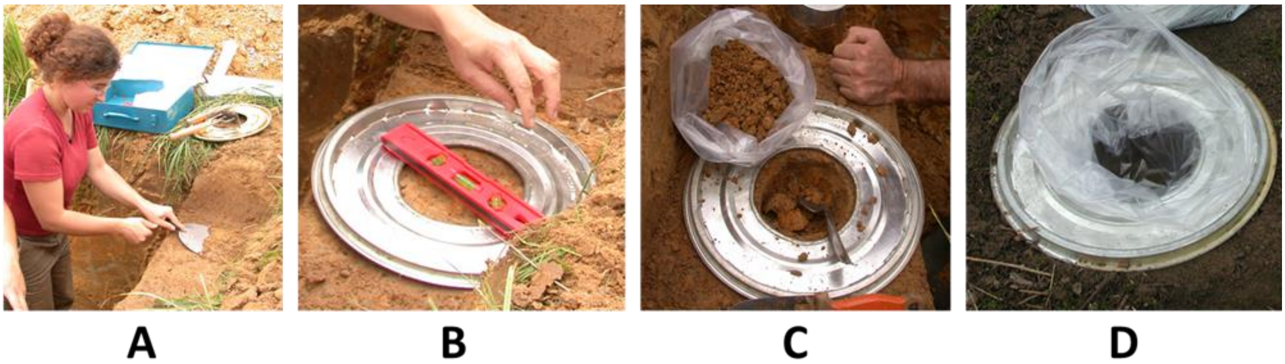


Figure 9. Water technique. A) Prepare the stair-stepped surface; B) place the annular template perfectly horizontal; C) sample the soil using a spoon and a ladle; D) place the thin bag in the cavity.

Fill the bag with water (Figure 10A) by using a measuring cylinder until the exact surface level of the cavity is reached (Figure 10B). Check that the plastic film adheres well to the walls of the cavity by rectifying the adhesion by means of the spoon. The volume of extracted soil corresponds to the total volume of water poured into the cavity. Note the measured volume of the cavity on the sample bag and in the field report. Measure also the sampling depths of the cavity (top and bottom) and note them on the sample bag and in the field report (Figure 10C).

In the sand technique, the water is replaced by the graded sand, which is used to measure the volume of the cavity. This technique is described in the International standard ISO 11272:1998.

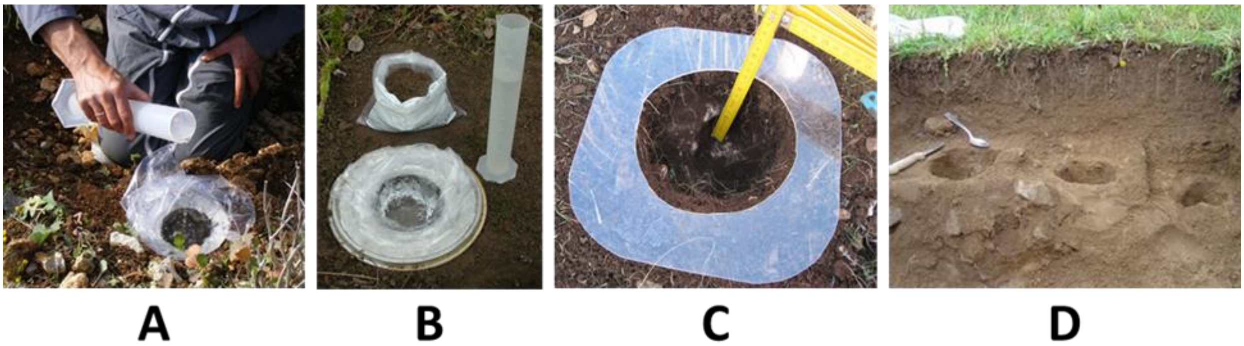


Figure 10. Water technique. A) Fill in the cavity with water; B) measure the total volume of water poured into the cavity; C) measure the depth of the cavity; D) multiple cavities can be used.

Special instructions for peat soils

Acknowledging that the assessment of temporal changes in the total SOC and SON stocks in virgin mires using repeated measurements based on cores is not achievable and that the carbon export by lateral fluxes is a major component of carbon balance of mires. Therefore, it can be proposed to the ETC to not apply the sampling scheme described in this document in these sites.

Either alternative method can be recommended, especially in actively drained peatlands and shallow peat soils where temporal changes might be significant and detectable, or the determination of the temporal changes of C and N stock simply ignored. Since the main objective of the soil sampling strategy is to close the ecosystem mass balance of carbon, the alternative method proposed should consider also determining the discharge fluxes of carbon and nitrogen.

However it remains compulsory in all sites to sample the soil peat down to its lower limit that is its transition with the mineral subsoil, as far as this is technically feasible. Sampling must be carried out at a total of 5 to 10 SP-I locations, selecting one point per location, in order to provide an overall estimate of the total SOC and SON stocks and a general description of the peat vertical profile as part of the general site description.

In case the cores are sampled deeper than 1 m, the portion until 1 m must follow the standard layer sizes (0-5, 5-15, 15-30, 30-60, 60-100 cm), while the part exceeding 1 m is sampled in layers of 50 cm (e.g. 100-150, 150-200, 200-250 cm etc.).

To collect the samples in peat, it is recommended to use a corer adapted for these ecosystem such the Wardenaar corer (Figure 11), an apparatus for sampling intact, undisturbed peat profiles in peat lands up to a depth of 1 meter. The peat profile sampler consists of a rectangular stainless steel box casing, divided lengthwise into two halves, with very sharp specially shaped cutting edges at the base. The handgrip hinging with both halves allows both halves to be pushed into the soil alternatingly. A clamp mechanism on the grip allows the profile to be clamped in the sampler when it is extracted from the soil. Then, in the field, the peat core is cut with a sharpened blade and scissors to obtain samples at the required depths (0-5, 5-15, 15-30 cm, etc.). If the peat layer thickness is less than 1.0 m, the Wardenaar corer can be used down to this depth and a standard corer can be used for collecting soil samples at deeper levels.

Figure 11. The Wardenaar corer designed to sample peat profiles

SAMPLE PREPARATION AND PROCESSING

This step describes the operating modes to obtain the composite samples that will be sent for chemical analysis and archiving and the instructions for the bulk density and soil texture determination. The following operating modes are adapted from the International Standard ISO-11464:1994. Please refer to this document for further details. All the used equipment must be clean and dry, the worktop must be cleaned each time a sample has been processed to avoid cross contamination between samples. Every sample must be clearly and precisely identified at each step of the sample processing.

Required equipment

- Latex gloves
- Plastic trays (typically 25 x 25 cm), about one or two for each sample
- Sieve with 2mm mesh size with round holes
- Porcelain pestle and mortar
- Pinch tweezers for roots manual removal
- Metallic containers for stones /gravel and roots
- Flat circular plate with removable vertical sides (type springform pan)
- Small plastic shovel
- Knife blade
- Ventilated oven with temperature control (70 and 105°C)
- Drying room (~30°C and 30% of humidity) [optional]
- 100 to 160 additional plastic bags (provided by ETC) for shipment. The exact number of bags requested depends on the sampling scheme and the presence of organic layers.
- 160 plastic containers of 500 cm^3 for sample packaging for C and N analysis. Example of recommended containers: https://www.grosseron.com/flacon-carre-a-large-ouverture-simple-et-double-fermeture-cape-jointee_48-132-1-317-1-748.html
- Precision electronic balances (0-2000g with <0.01g error and 0-200g with <0.001g error)
- Large desiccators (20 dm^3) with dry silica gel
- Grinder, crusher, blade or bead mills for organic material

Maintenance of equipment

The calibration and uncertainty of the scales used for weighing the soil and organic horizons samples are given by the accredited companies, which should verify them once a year.

The thermometer used for oven temperature control must be calibrated by a certified organism to the nearest 0.5°C.

Mineral soil samples

The mineral soil samples preparation and processing can be divided in 3 main steps that are described here below and summarized in Figure 16.

Step 1 - Drying and sieving each individual sample to obtain fine earth, coarse elements and living plant material mass values

The following processing should be applied on each individual sample: use this method for all the samples of each soil layer.

1. Weigh the moist field sample, either in the field or in the laboratory with the precision balance (W0).

2. Dry the soil in a heating chamber or a drying room at 30°C ± 5°C. To do this prepare the plastic trays to spread samples. Verify the cleanliness of the trays. Stick on every tray the identifiers of the sample (Figure 12A), to be able to recognize it unequivocally during the following steps, by writing on a label stuck on the front of the tray with the following information:

    - [The identifier of the sample] as CC-SSS_YYYYMMDD_S1-S2_UD_LD (see Sample identification codes and labelling section above)

    - [The tray number (n/nt)], nt is the total number of trays for a single soil sample. Optional when nt =1.

    With latex gloves on, spread the samples in the trays, ensuring a good mixing of the sample at this stage (Figure 12B). Every sample is distributed in a sufficient number of trays to allow fast drying: clumps of earth are to be reduced to small aggregates before spreading them in a thin layer not thicker than 20 to 25 mm. Depending on the soil texture, the drying time takes between 5 to 10 days, until a constant weight is reached.

3. Use the precision balance to weigh the sample after drying, paying attention to weigh all the trays for each sample and to subtract the weight of the trays.

4. Sample sieving at 2mm mesh

    - **Crumbling.** The crumbling step is requested depending on the soil sample structure. It consists in breaking the clods or aggregates and in separating the coarse elements (stones, gravels and concretions) of the fine earth by means of a porcelain mortar and pestle. Soil clods or aggregates are manually disintegrated without grinding the elements like concretions. Be careful in the case breakable elements as concretions or pieces of limestone are present in the sample, as these should not be grinded.

    - **Sieving.** The loosened earth is then sieved through a 2mm mesh size sieve to obtain the fine earth. Stones, gravels and roots or other plant residuals constitute the refusal > 2 mm (residue on the top sieve). They are removed manually with the pinch tweezers (Figure 12C) and put aside in metallic containers for weighing (coarse elements) and further drying (living plant material). The sieving is finished when the refusal elements are clean; a criterion to judge sufficient sieving is to check the colour of the face of the stones (Figure 12D).

5. Weighing of fine earth, coarse elements and living plant material

   5.a Weigh the fraction of fine earth (W30_E) using a precision balance.

   5.b The living plant material fraction must first be further dried at 70 °C before weighing (see box 1 below).

   Determine the dry weight using a precision balance (W70_R - if there is no living plant material, set the values to zero)

   5.c Dry at 105°C and weigh the fraction of coarse elements using a precision balance (W105_ S - if there is no coarse elements, set the values to zero).

Figure 12. Sample preparation. A) drying the sample; B) crumbling clods and aggregates; C) removing coarse elements and living plant material during sieving step; D) fine earth.

BOX 1. Drying and weighing procedure

This procedure must be applied for drying and weighing soil mineral and organic samples at a given temperature. Drying at 30 ±5 °C can be operated in a well ventilated room. Drying of samples at 70°C or 105°C must be done in a ventilated, temperature-controlled oven until a constant weight is reached. For sake of practicality, the duration requested to reach a constant weight can be determined prior to the experiment, typically 48 or 72 hours. The temperature of the oven should be controlled by a calibrated sensor and kept constant at the target temperature ±1°C.

After drying, the samples must be taken from the oven into a desiccator filled with dry silica gel for cooling down to room temperature while avoiding the re-humidification of the sample. Alternatively, soil samples may be left cooling down in the oven closed providing a sufficient volume of fresh silica gel have been placed inside the oven for avoiding the sample to re-humidify. Each sample is then weighed to the nearest 0.01 g. Dry weight uncertainty is calculated using the uncertainty of the scales used to perform the weighing. The uncertainty of the scales is given by the accredited company that verifies them once a year.

At the end of this step of the operating mode one should obtain for each individual sample:

- The weight of fine earth, ***W30_E***
- The weight of coarse elements (stones and gravels), ***W105_S***
- The weight of living plant material, ***W70_R***

Step 2 – Combining fine earth of individual samples to obtain a composite sample in two steps

The composite sample is a representative sample obtained by mixing the five (or three) fine earth samples of the same layer and SP-I location. This step is thus repeated for each mineral soil layer (0-5, 5-15, 15-30, 30-60, 60-100) at each of the SP-I locations (n=20). This step must be done in two steps that are described here below.

First step

The proportion of each individual sample in the composite sample is calculated using the apparent density of each sample (eq. 1):

$$Q_{i,l,s} = \frac{WA30\_E_{i,l,s}}{SUMWA_{l,s,}} \qquad \text{(eq. 1)}$$

where

- $Q_{i,l,s}$ is the proportion of soil dried at 30 °C to be used from the sample *i* (*i*=1 to 3 for pits or 1 to 5 for cores), layer *l* (*l* = 0-5, 5-15, 15-30, 30-60, 60-100), SP-I location *s* (*s* = 1 to 20)
- $SUMWA_{l,s}$ is the sum of the mass of fine earth ($WA30\_E_i$) of all the samples to combine for the layer *l* and the SP-I location *s* (in kg m^{-2}).
- $WA\_30E_{i,l,s}$ is the mass of fine earth of sample *i* per unit area (in kg m^{-2}).

The mass of fine earth per unit area for each sample can be calculated as (eg. 2):

$$WA30\_E_{\ i,l,s} = LT_{\ i,l,s} \times \frac{W30\_E_{i,l,s}}{V_{i,l,s}} \times 10 \qquad \text{(eq. 2)}$$

Where

- $W30\_E_{i,l,s}$ is the mass of fine earth of the sample (in g),
- $LT_{i,l,s}$ is the sample thickness in cm,
- $V_{i,l,s}$ is the volume of the sample in cm^3.

The layer thickness may vary among samples with excavation methods. The mass requested from each individual soil sample, $QE_{i,l,s}$, (in g) is given as (eq. 3):

$$QE_{i,l,s} = Q_{i,l,s} \times TW_{l,s} \qquad \text{(eq. 3)}$$

Where

- $TW_{l,s}$ is the target weight (in g) of the final composed sample for layer *l*, SP-I location *s* (1500-2000 g).

An Excel file that allows the calculation of the mass needed from each individual sample is provided by the ETC in the repository (fileshare).

Second step

The mass requested of each individual soil sample, is obtained with the quartering method. This method reduces the mass of a soil sample without introducing a sample bias and is based on a series of sub-sampling (Figure 13):

1) Using a flat circular plate with removable vertical sides, spread the sample mass in order to form a circular and uniformly flat "pie" over the bottom area. The surface should be dry and clean with enough space;
2) Divide the pie in 4 quarters of the same size by drawing 2 perpendiculars with a knife blade;
3) Remove two opposite quarters. The remaining mass will be about half the original mass;
4) If the remaining soil mass is more than the double of the calculated required mass for the composite sample, $QE_{i,l,s}$, repeat the previous step: homogenise the remaining fine earth sample, on the circular plate and remove this time the alternate opposing quarters;
5) Repeat the previous steps again in the presented order until the remaining mass is less that the double of the calculated required mass. The number of repetitions of the quartering method that is needed can easily be determined in advance.

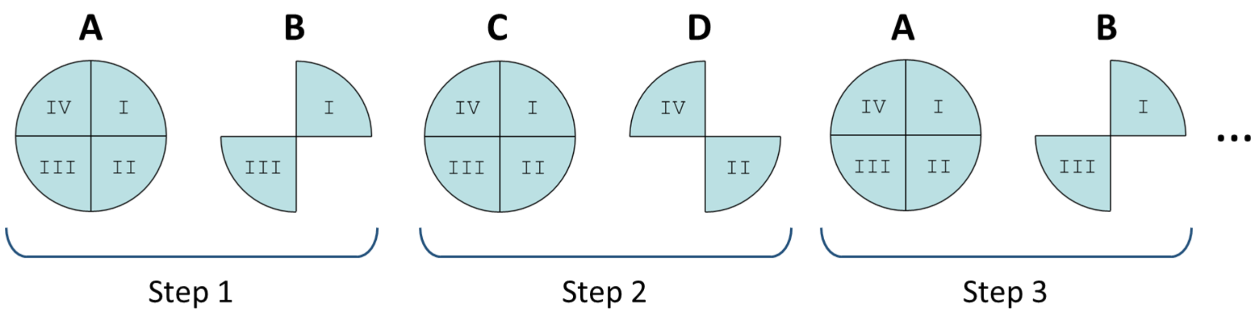


Figure 13. Quartering method to reduce the mass of a soil sample. Homogenise the soil in a circular plate and split it in 4 quarters (Step1 A); Remove two opposite quarters (Step1 B). If the soil mass is still more than double the needed amount homogenise is again (Step2 C) and remove the alternate quarters (Step2 D). Continue with the sequence (Step3 and so on) until the desired mass is almost reached.

The final mass of each sample obtained with the quartering method will be probably close to the requested amount but not the exact amount. In order to reduce it to the exact value QE i,l,s, put the well mixed sample in a plastic container on an electronic balance and remove soil with a spatula until the target mass value is reached.

The obtained required quantities of the five (or three) individual samples of the same SP-I location and soil layer, are then combined and mixed thoroughly to create the composite sample for the SP-I location *s*, and depth *l*. Each composite sample will have a minimum dry mass of 1500g.

Although the quartering method is recommended to reduce the sample mass, using a riffle splitter could be an alternative to obtain a non-biased sub-sample.

Step 3 – Extracting three aliquots from the composite samples

The obtained composite samples (one for each SP-I location and layer) must be split in three aliquots:

1) A 50±10 g aliquot for determination of bulk density and soil texture. This aliquot must be weighed (WX30_E), then dried at 105°C following the procedure described in Box 1, and then weighed again (WX105_E) to calculate the residual humidity and the fine earth mass.

BOX2. Samples for measuring mineral soil texture

The analysis of the mineral soil texture will be done by the CSAL for three SP-I locations at each mineral soil layer (0-5, 5-15, 15-30, 30-60, 60-100). The analysis will be done on these 15 samples that were used for bulk density measurement. Hence, these samples must be kept after drying for being sent to the CSAL for soil texture determination. The Texture analysis will be done using the method described in Annex 4. In addition, the soil texture samples must be labelled and coded according to the following rule:

Code for the soil texture samples

Samples used for the soil texture determination are those used for determination of dry weight (WX105_E). The whole 50 g sample should be placed in a container and labelled as the corresponding SP-I, with the addition of "TEX" at the end of the name (CC-SSS_YYYYMMDD_S1_UD_LD_TEX). Altogether, there should be 3*5 = 15 samples. Soil texture is only measured for mineral layers.

2) A 300±30 g aliquot that must be send to the Central Soil Analysis Laboratory (CSAL) for soil organic carbon nitrogen and residual water content analyses; packaged in a 500 cm3 plastic container (Figure 14 left). The SOC and SON analysis will be done using the method described in Annex 3.

3) The remaining sample (not exceeding 1700 g) that must be sent to INRAE Orléans for archiving in the ETC Archive (European Conservatory of Soil Samples), packaged in plastic bag provided by the ETC (Figure 14 right).

Figure 14. Example of plastic containers for the sample sent to the CSAL (left) and plastic bags for the soil to be sent to the archive (right)

The splitting of the composite sample in the three aliquots must be done following the steps 1-6:

1) Divide the composite sample(1500-2000g) into 4 trays;
2) Take a sample with the plastic small shovel along the diagonal of each of the 4 trays and put these 4 samples in a new clean and dry tray (Figure 15);
3) Mix the composed sample in the new tray in order to create a homogeneous sub-sample;
4) If the obtained quantity is not sufficient, homogenise the remaining earth in each of the original four trays again and use the shovel again to take additional samples, this time along the opposite diagonal.
5) If needed, repeat the previous step until the requested amount is reached.
6) Finally, use a precision balance to obtain the exact amount of the 50 g and 300 g aliquot.

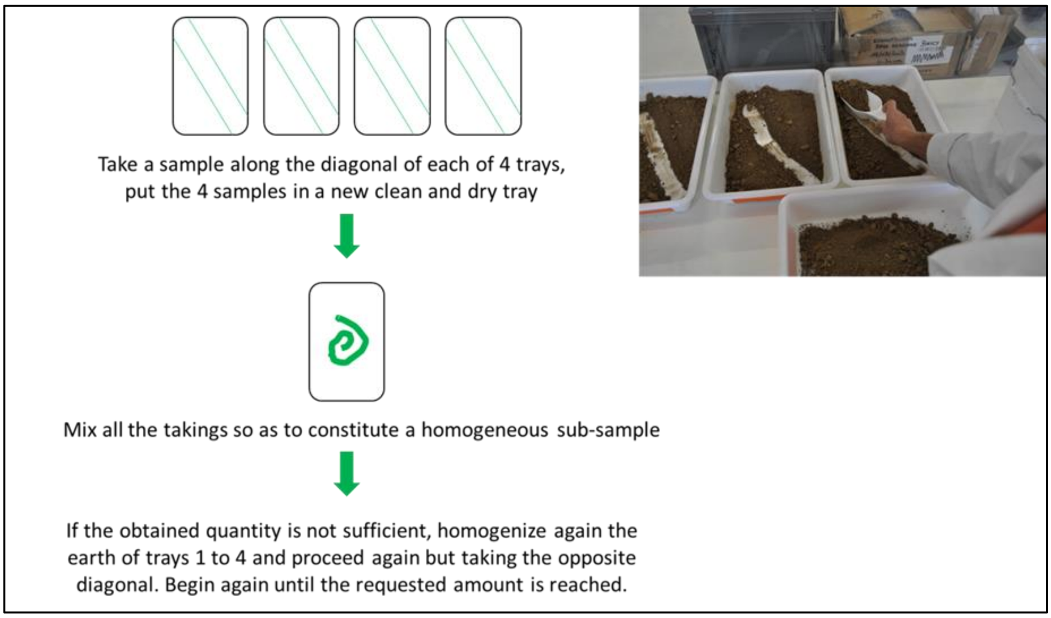


Figure 15. Splitting of the composite sample. The 4 trays with earth and sampled along the diagonal (top of the photo) and the samples put in a new clear and dry tray (bottom of the photo)

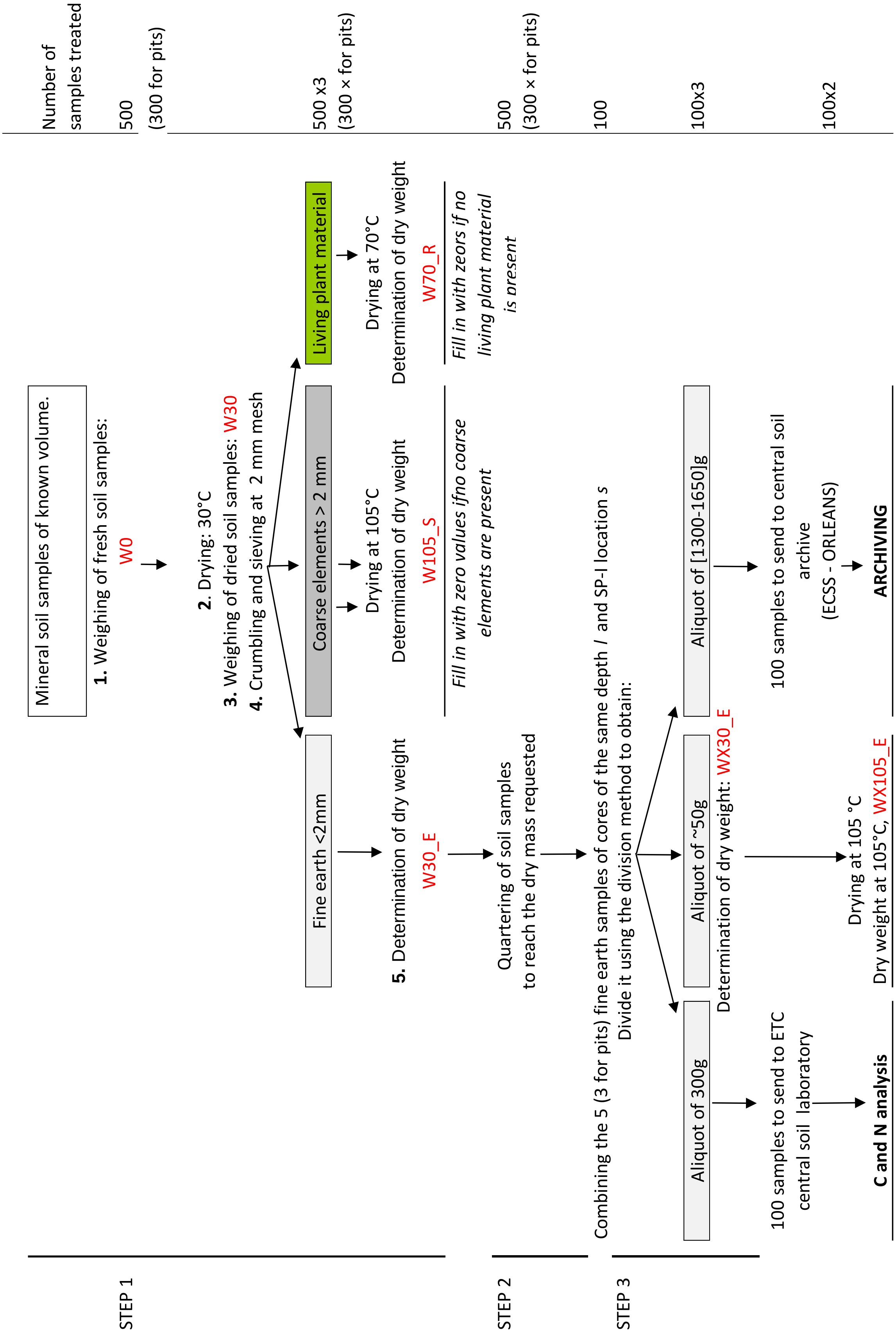


Figure 16. Scheme of the preparation and processing of mineral soil samples. For details on the different steps refer to the text.

Organic samples (including histic soil in peats)

The organic samples preparation and processing can be divided in three main steps that are described here below and summarized in Figure 17.

Step 1 - Sorting, drying and weighing each individual sample to obtain fine holorganic material, coarse elements (gravels and stones), roots and other living material mass values

The following processing should be applied on each individual sample: use this method for all the samples of each organic layer (3 to 5 samples according to the sampling method or the single sample in case of peat soils – see the specific section above).

1) Weigh the moist field sample either in the field or in the laboratory (W0).
2) Remove coarse elements (stones and gravels) and living roots, mosses and other living plant material, if present in the sample. Dead wood fragments and died roots must not be removed from the sample as they are part of the organic horizon.
3) Weigh the holorganic material fraction, (W0_OH). Optionally, as indicated in the blue frame, the fresh mass of coarse elements (W0_OS) and living plant material (W0_OR) and their dry counterpart at 105°C and 70°C, W105_S and W70_R respectively may also be determined (If there is no coarse elements and plant materials, set the values to zero in the BADM).
4) Dry the holorganic fraction in a drying room at 30 °C ± 5°C . To this end, use plastic or aluminium trays to spread the samples in a thin layer and clearly identify the samples with labels. The drying time could be between 2 to 5 days, until reaching a constant mass. Use the precision balance to weigh each dried fraction paying attention to weigh all the trays for each sample and to subtract the weight of the trays (W30_OH).

Step 2 – Grinding and combining fine holorganic material of individual sample to obtain a composite sample

1) Grind each sample of holorganic material to obtain fine holorganic material (< 2mm). This may be done using either a blade mill or a bead mill as long as no contamination from the instrument is guaranteed.
2) Pool together the entire mass of holorganic material of each layer from the 5 or 3 locations of the same SPI (not valid if you are in a peat soil with a single location sampled).

Step 3 - Packaging and sending the samples (i) to the Soil Analysis Laboratory and (ii) the ICOS soil archive

- A 50 g aliquot (*combining fine holorganic material of individual sample to obtain a composite sample*) that must be send to the Central Soil Analysis Laboratory (CSAL) for soil organic carbon, nitrogen and residual water content analyses, packaged in a 500 cm^3 plastic container (Figure 14 left).

- The remaining sample (not exceeding 1700g but at least 150g) that must be sent to INRAE Orléans for archiving in the ETC Archive (European Conservatory of Soil Samples) packaged in plastic bag provided by the ETC (Figure 14 right).

In case the holorganic soil layers are very thin and the available fine material after preparation is less than 50 g, send the entire available samples to the Central Soil Analysis Laboratory (CSAL). In that case, please contact the ETC for information.

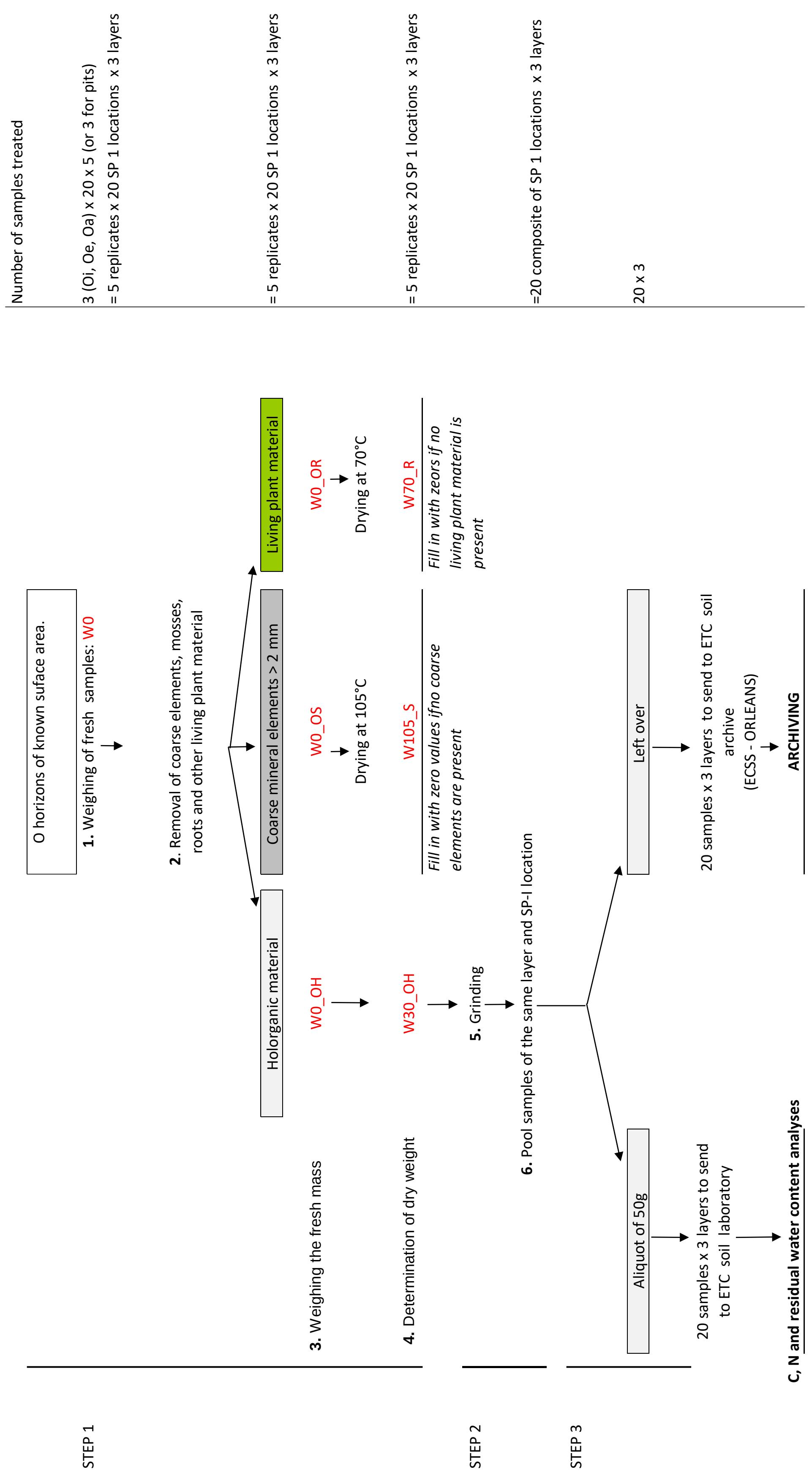


Figure 17. Scheme of the preparation and processing of organic samples and histic soils. For details refer to the text. Note: for peat soils the samples for the archive and analysis are 5 to 10 x 6 layers.

DATA SUBMISSION

Soil sample packaging for laboratory analysis

Each sample, that is always related to a SP-I location, must be put into a plastic container with a screw-on cap and duly labelled twice with the sample identifier shown below: once using a sticker to be put on the container side and second with a paper label to be placed inside the container. Great care must be taken to mark each unit clearly before sending it to the CSAL. Samples must be packed and carefully labelled otherwise they cannot be processed. Moreover, the identification code for each mineral and organic horizon at the SP-I and SP-II locations must follow the same rules given in the Sample identification codes and labelling section.

When ready for shipment, an email must be sent to icos-etc-soil@inrae.fr with attached the list of the samples that will be shipped. The ETC staff will check that all the relevant and mandatory information in the metadata are correctly imported and after the check give the green light for the shipment (see the "BADM data submission" section for the list). In case some information will be missing or to be confirmed, a message will be sent to the station team and the shipment can not start until all is solved, because the metadata are crucial for the analysis.

After receiving the green light, the samples can be sent to the Central Soil Analysis Laboratory (CSAL) of the ETC to the following address:

INRAE - Laboratoire d'Analyse des Sols
ETC Central Laboratory
273 rue de Cambrai
62000 ARRAS
France

In order to ensure a smooth shipment and reception, please send an email to inform when the samples are shipped to analysis lab and storage facility to: icos-etc-soil@inrae.fr

Soil sample packaging for archiving

The same procedure is applied as above and the shipment can happen only after a green light has been received. Each sample must be put into a plastic bag, hermetically closed, and duly labelled twice with the sample identifier shown in Table 1: first using a permanent black marker on the bag itself and second with a plastic label to be placed inside the bag.

Table 1. Example of data to be put on each plastic bag for acrhiving.

DATE OF SAMPLING	*20161201*
SAMPLER'S NAME	*Kirk DOUGLAS, Marlene DIETRICH*
SAMPLE TYPE	*Mineral / Organic*

SAMPLE IDENTIFICATION	*FR-Bil_20161201_01_5_15*
SAMPLING METHOD	*Soil corer (diam = 10 cm)*
MIN and MAX DEPTHS OS SAMPLING (cm)	*5-15*
SAMPLE VOLUME (cm^3)	*785.4*
ICOS STATION IDENTIFIER	*FR-Bil*

Great care must be taken to mark each unit clearly before sending it to the Central Soil Archive that is the ESSC (European Soil Samples Conservatory in Orléans). Also in this case altogether up to 160 samples should be sent for archiving for each sampling date. Samples should be grouped in a single parcel, sent by any surface transporter avoiding the public French Poste Office (Colissimo) to the ESSC at the following address:

INRAE - Conservatoire Européen des Echantillons de Sol
ICOS-ETC
2163 avenue de la Pomme de pin
CS 40001 Ardon
45075 Orléans Cedex 2
France

BADM data submission (including soil description)

All the data and information needed to correctly process and document the samples shipped to the ETC must be submitted using the BADM system (SOSM group). Data and metadata cover information about the method used, the sample type, size, position and all the different weights measured. All these are needed to identify and characterize each sample shipped.

All the metadata are mandatory and needed before the samples analysis and for this reason must be submitted as soon as the samples are ready to the shipped. Without metadata the analysis cannot be done.

Referring to the schemes in figures 16 and 17, the measurements to submit are reported in red and in the table here below there is the correspondent BADM variable to be used

Mineral samples	
Variable measured	**BADM variable**
W0	SOSM_W0
W30	SOSM_W30
W30_E	SOSM_W30_E
W105_S	SOSM_W105_S
W70_R	SOSM_W70_R
WX30_E	SOSM_WX30_E
WX105_E	SOSM_WX105_E

Organic samples	
Variable measured	**BADM variable**
W0	SOSM_W0
W0_OH	SOSM_W0_OH
W0_OS	SOSM_W0_OS
W0_OR	SOSM_W0_OR
W30_OH	SOSM_W30
W105_S	SOSM_W105_S
W70_R	SOSM_W70_R

In order to allow the correct processing and archiving of the samples, there are a set of variables that are needed and mandatory before the shipment of the samples. The mandatory variables are function of the type of soil and sampling location. Even if only samples related to SP-I locations are shipped, information about the SP-II samples are also needed, according to the table 2.

Table 2. mandatory and optional variables to be reported for each SP-I and SP-II function of the soil nature. Note that SP-I samples are the one shipped to the labs while SP-II samples are the individual samples not shipped but where information are needed to calculate the soil stocks. Organic is intended the organic horizon lying on top of mineral layers.

Soil nature	**SP type**	**Mandatory**	**Optional**
Mineral (M)	I	SITE_ID SOSM_PLOT_ID SOSM_SAMPLE_ID SOSM_SAMPLE_MAT SOSM_UD SOSM_LD SOSM_WX30_E SOSM_WX105_E SOSM_DATE	
Mineral (M)	II	SITE_ID SOSM_PLOT_ID SOSM_SAMPLE_ID SOSM_SAMPLE_MAT SOSM_UD SOSM_LD SOSM_VOLUME SOSM_AREA SOSM_W30_ SOSM_DATE	SOSM_W0 SOSM_W30 SOSM_W105_S SOSM_W70_R
Organic (O, Oi, Oe, Oa)	I	SITE_ID SOSM_PLOT_ID SOSM_SAMPLE_ID SOSM_SAMPLE_MAT SOSM_DATE	

Organic (O, Oi, Oe, Oa)	II	SITE_ID SOSM_PLOT_ID SOSM_SAMPLE_ID SOSM_SAMPLE_MAT SOSM_THICKNESS SOSM_VOLUME SOSM_AREA SOSM_W30_OH SOSM_DATE	SOSM_W0 SOSM_W0_OH SOSM_W0_OS SOSM_W0_OR SOSM_W105_S SOSM_W70_R
Histic (H)	I	SITE_ID SOSM_PLOT_ID SOSM_SAMPLE_ID SOSM_SAMPLE_MAT SOSM_UD SOSM_LD SOSM_DATE	
Histic (H)	II	SITE_ID SOSM_PLOT_ID SOSM_SAMPLE_ID SOSM_SAMPLE_MAT SOSM_UD SOSM_LD SOSM_VOLUME SOSM_AREA SOSM_W30_OH SOSM_DATE	SOSM_W0 SOSM_W0_OH SOSM_W0_OS SOSM_W0_OR SOSM_W105_S SOSM_W70_R

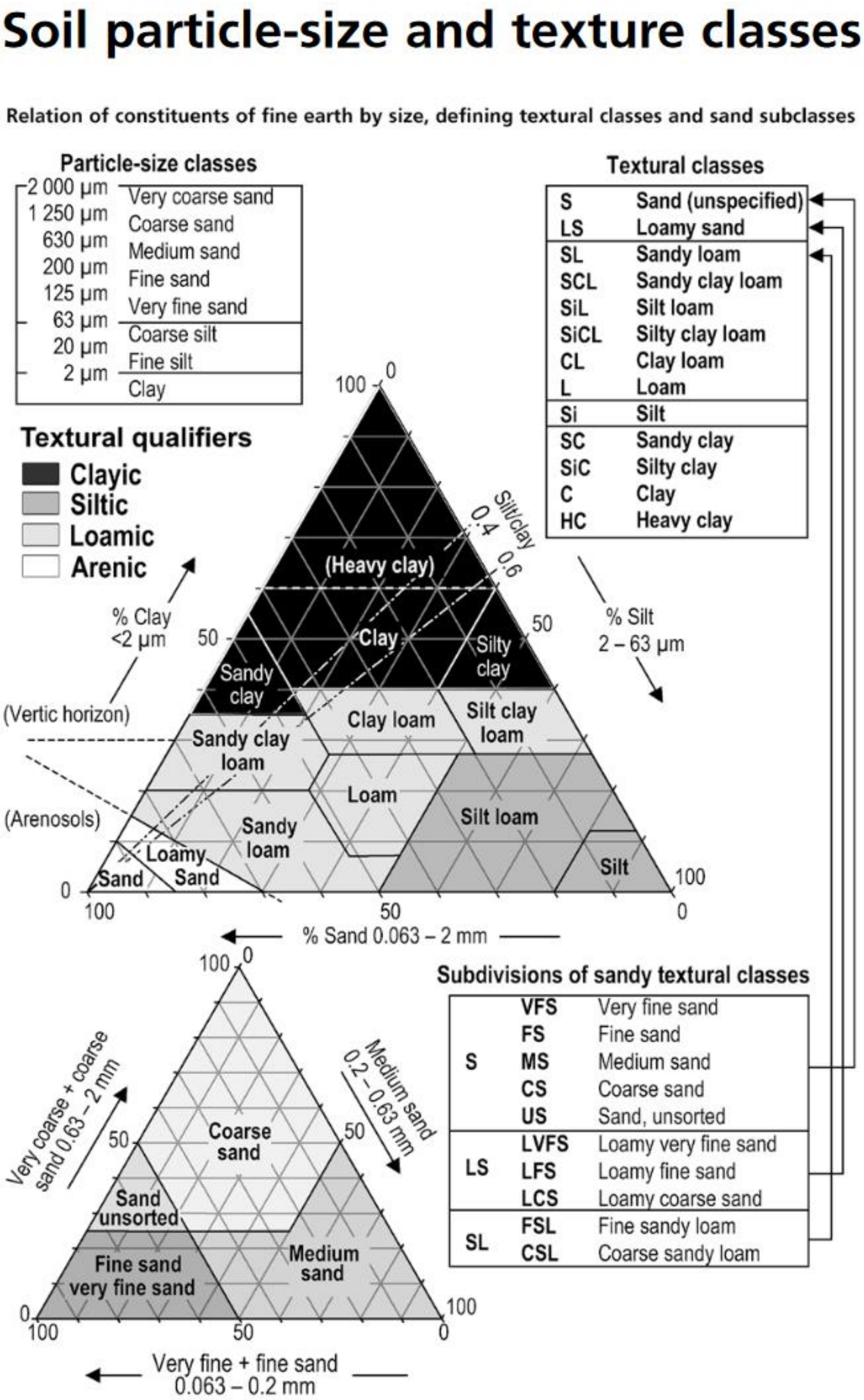
Soil description

The soil description, prepared as described in the Soil sampling overview section, must be prepared in a pdf document that is then submitted to the ETC from the PI Area selecting “Other file” in the options and naming it “CC-###_Soil_Description.pdf” where CC-### is the official site code.

In addition, the WRB class must be submitted using the ICOSBADM variable SOIL_WRB_GROUP available in the ICOSBADM_Soil_Info template. In the same template other classifications are also available that can be also submitted as well as soil texture data that, in case the station does not use the service for the texture determination offered by the ICOS ETC, must be communicated using the SOIL_TEX group.

ANNEX 1: SOIL TEXTURE CLASSES

Classes according to Annex 4 of the "World Reference Base for Soil Resources 2014", p 192



Source: Adapted from FAO (2006): Guidelines for Soil Description

ANNEX 2: MOTOR SAMPLING SYSTEM

(From *Mode opératoire* for soil sampling, INRAE, *Clermont-Ferrand*. K. Klumpp et al.)

Sampling and drilling systems

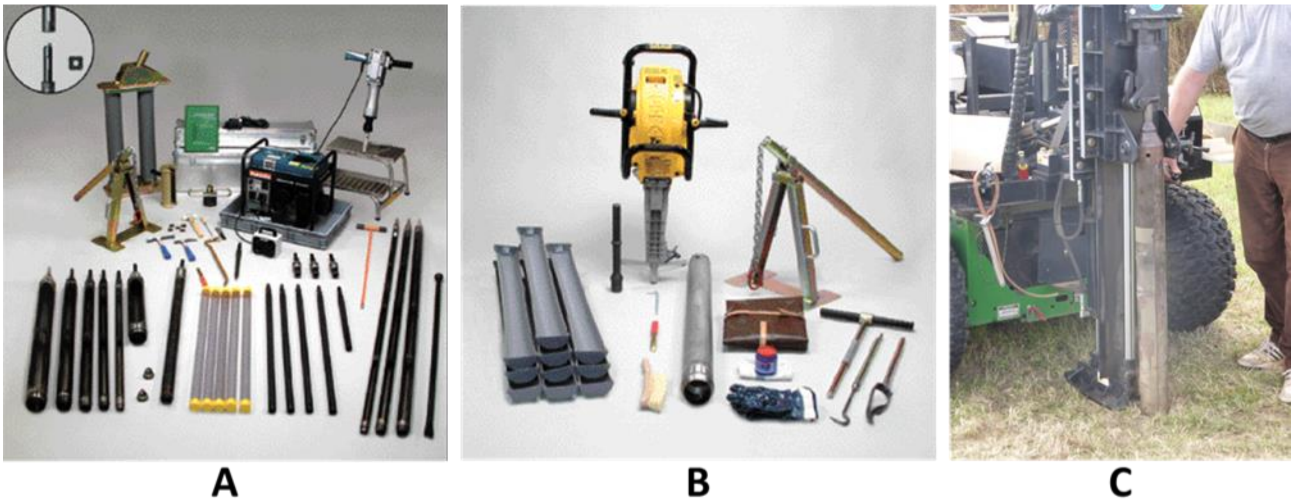


Figure A1. A: Percussion hammer Cobra TT powered; B: Percussion /jack hammer with gasoline; C: Hydraulic soil corer

Material

For more information see description of Eijkelkamp.com.

- Tripod or squad / pickup with adapted fixation to lift the jackhammer to the necessary height
- Hoist/winch with chains to fix jackhammer
- Percussion /jack hammer with gasoline or powered percussion hammer Cobra TT
- rod puller extension for the extraction
- lifting jack with lever and chain
- stairs to be at working height
- Percussion gouges (combination type for various types of soil) in sufficient diameter (e.g. larger than 5 cm) and length (100cm). See examples in Figure A2.

Make sure your gouges have a window to close in order to determine bulk density, and a cutting end at the bottom to go through root and stones. Some coring system may alternatively be equipped with a thin-walled, disposable transparent liner that is put inside the core before digging, and thereafter extracted once filled with soil sample. The corer is loaded from one end with a new liner which is firmly fixed before every drill. After coring, the liner with the core (see Figure A2) is taken out afterwards. Systems exist from various manufacturers in various thicknesses including near and above 10 cm liner diameter e.g. www.roehren-hamm.de, www.geoprobe.com.

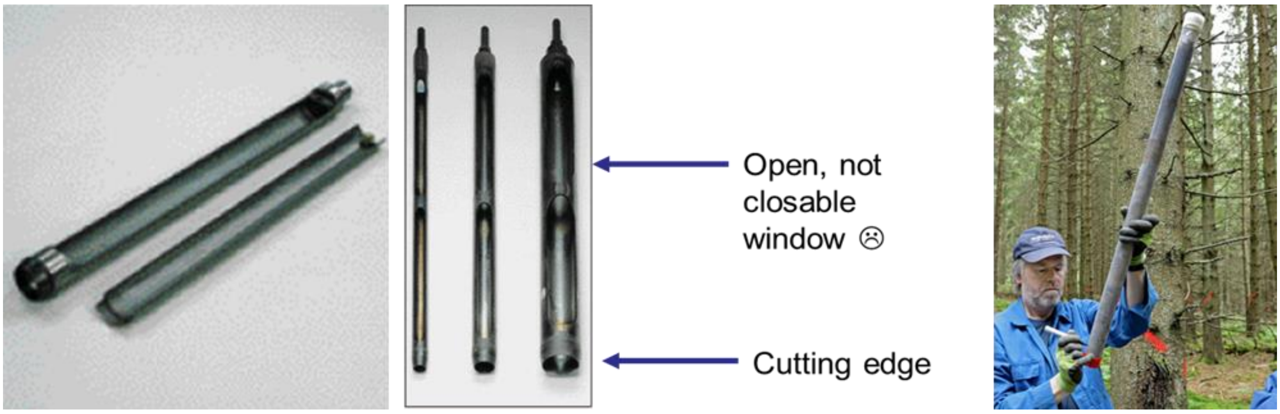


Figure A2. Examples of gouges

Methods

There are different ways to drill, either without uses of any accessories to facilitate the work and lighten the weight of the used material (see Figure A3) or by uses of a hydraulic system. Using the more simple version, there are two types of percussion hammer, depending on the number of points to sample, ecosystem (i.e. forest, arable, grass, etc.) and soil texture (i.e. stones, …):

- The light electrical percussion hammer has a beating power of 31.7 Joule at 1200 beats per minute, a net weight of 17 kg and an overall length of 613 mm. The sound power level acc. to 84/537/EEC is not more than108 dB (A).
- The heavy model has a beating power of 55.3 Joule at 1000 beats per minute, a net weight of 29.1 kg and an overall length of 825 mm. The sound power level is not more than 108 dB (A).

Using these hammers there are no petrol vapors and exhaust gasses directly over the sample.

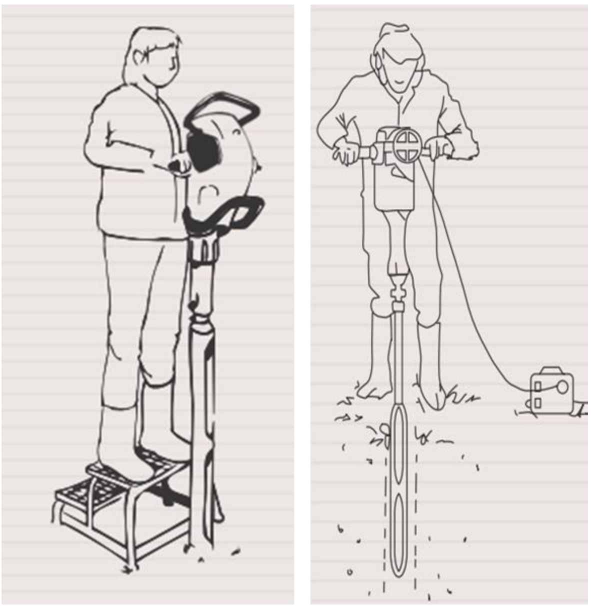
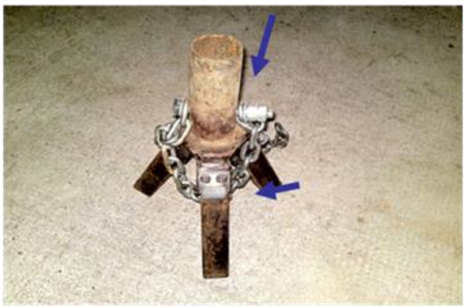


Figure A3. Examples of drills

Tripod System to suspend jackhammer

In order to ease the work you may use the help of hoist/winches, a tripod or other to lift of the jackhammer and gouge

Fixing head for the 3 legs, so you can slip iron square chunks (See slide below)
Rod with chain to hook the winch / hoist



Tripod set with legs to about 2/3 pieces
Length legs about 3,30m.

Example of commercial systems (light and heavy versions):

Light tripod

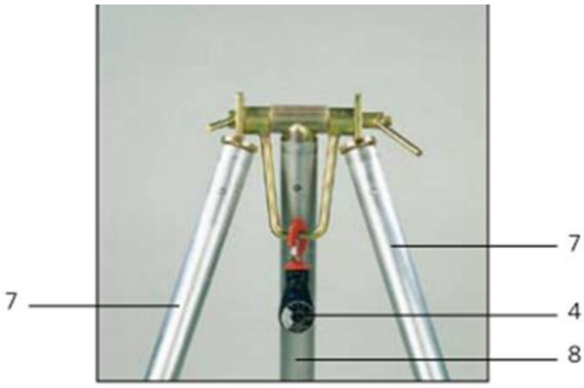
Article no.	02.01.01
Max. tensile force	6,5 kN
Total leg length	3 x 1,5 m
Working height	Ca. 3,9 m
Net weight	44 kg
Legs	Ø 50 mm
Snatch block	20 kN

Heavy tripod

Article no.	02.01.04
Max. tensile force	20 kN
Total leg length	3 x 1,65 m
Working height	Ca. 4 m
Net weight	108 kg
Legs	2 single Ø 70 mm 1 double Ø 80 mm
Snatch block	20 kN

Heavy tripod set-up procedure:

1. The heavy tripod is delivered in pieces. Attach the legs' top ends as shown in the figure at right.
2. Fold out the legs of the snatch block (6) and lay them wide apart on the ground.
3. Slide the extension pieces (9) over the legs' top ends. Lock them with bolts and nuts.
4. Attach the end-pieces with point (10) to the extension pieces (9). Mount the frame (11) on the Ø80-mm leg (8). Lock with bolts and nuts.
5. Attach the pulley with hook to the snatch block (6). Make sure the hook control is on.



How to use a tripod

Winch / hoist to suspend and hold jackhammer

Chain to hook the winch / hoist to the tripod (See 1.diapo)

Winch / hoist

On the main chain of the winch fixing carabiner + for double security

Adapt a jack Hammer to a tripod

The jackhammer has been modified (Figure A4). An iron frame has been added so that jackhammer can be attached at the tripod in order to better distribute the weight and to assure the security once suspended.

- Jackhammer is suspended with a winch hook / hoist in order to move/lift up and down easily.
- To this iron frame we welded a metal ring on which is hooked the winch hook / hoist.
- Jackhammer length 1m

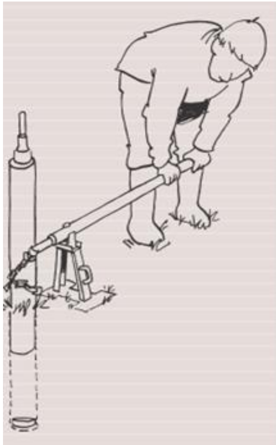
Figure A4. Modified jackhammer

Drilling

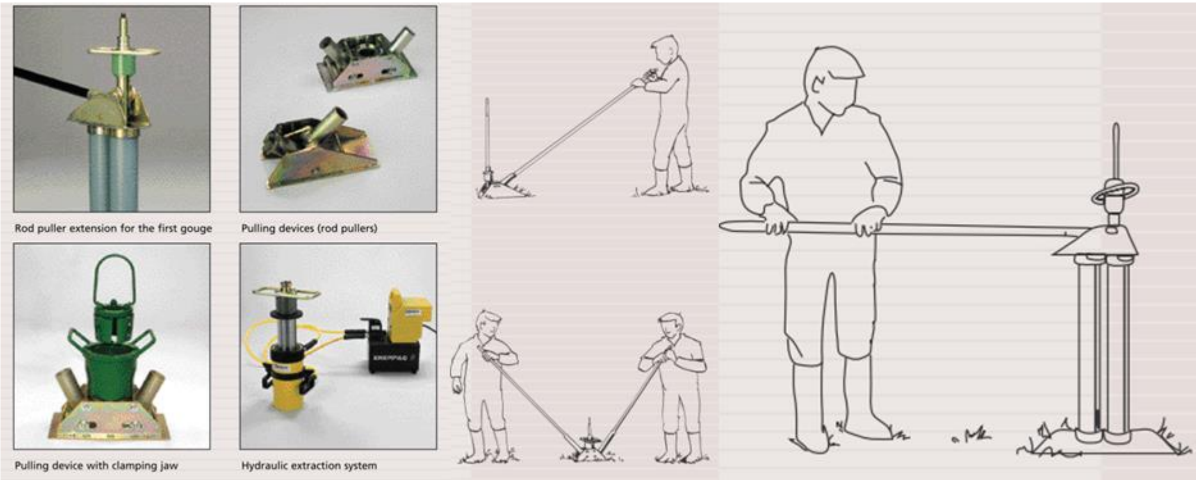
- Once the sampling point is prepared (e.g. depth, litter, stones, etc.), pull up the jackhammer with help of the hoist.
- Then insert the gouge into the jackhammer and start to drill.
- Before and during drilling, when using percussion gouges with window and cutting segment, make sure the window is closed properly and the cutting part of your gouges is well attached
- Control the drilling angle.

Once the necessary drilling depth is achieved, there are several ways to remove the gouge:

- A very basic and laborious way is the steel lifting jack and lever.
- A chain is tightly placed around the soil column (by scale) in order to pulls up the column.
- Before removing the soil column with the tripod/chain – shake/row with the gouge by inserting a metal bar to the top of the gouge. While "rowing" the gouge comes off the soil more easily.



- Another way is to uses of a rod puller which is either one-man operated extractor, two- man operated extractor or hydraulic extractor



By uses of a tripod or other setup using a hoist/winch, you may attach the gouge to the hoist and then by slightly “rowing” and up-moving the gouge with help to the chains you extract the gouge.

ANNEX 3: DETERMINATION OF SOC AND SON STOCKS

For each sample, the SOC and SON stocks (t C, N ha^{-1}) should be determined, by considering of the soil organic carbon and nitrogen contents, the soil depth at which sample was taken, and the bulk density of the fine earth along the pit. Bulk density will be determined by dividing the weight of the oven-dried fine earth by the volume of the cores. Total nitrogen (TN) and total carbon (TC) will be determined using the dry combustion method according to the ISO 13878 and ISO 10694 International Standards, respectively, and the determination of total carbonates according to ISO 10693. In this case, all the carbon and nitrogen compounds present in the sample are transformed into carbon dioxide (CO_2) and nitrogen gas (N_2) by heating the sample in an oxidation column at a temperature of at least 1000°C in the presence of the flow of oxygen-containing gas. Quantities of CO_2 and N_2 released are then measured with the thermal conductivity detector after chromatographic separation.

SOC stocks

As mentioned before, the measurement of TC will be performed on the soil samples by converting carbon to CO_2 at a temperature of 1000°C in a combustion tube. Under this condition, all carbon compounds are completely decomposed and transformed into CO_2. If the soil does not contain mineral carbon (carbonates), such as acidic soil, then the total carbon will be equal to organic carbon. While, if the soil contains mineral carbon, the total carbon measured will be the sum of organic and inorganic carbon. Thus, to access to organic carbon fraction, the inorganic carbon fraction should be quantified. Inorganic carbon can be separately determined by calcimetry analysis, (ISO 10693) and then deduced from the TC. In this case, the following correction must be done:

$$\%OC = \%TC - \%CaCO_3 * 0.12 \qquad \text{(eq. 4)}$$

Nevertheless, if the CaCO3 value is > 70%, to avoid degrading the accuracy, we either do a soil decarbonation treatment before measuring the organic carbon (after decarbonation, the total carbon measured is equal to organic carbon), or we do 3 to 5 repetitions of total carbon measurements to improve the accuracy.

SON stocks

The dry combustion will be carried out simultaneously with the SOC analysis, and the organic N stocks in the samples will be calculated according to the same procedure for organic carbon. In addition, the ratio of organic carbon to total nitrogen and the value of organic matter will be reported in the result.

These methods are described based on the general analytical catalogue (https://www6.hautsdefrance.inrae.fr/las/content/download/3327/33888/version/1/file/catalogueLAS2021_V1.pdf). All equations of soil analysis (like Bd, SOC, SON and C/N) and an example of soil analysis result can be accessed here:

https://traitementinfosol.pages.mia.inra.fr/icos/DB_Theory.html

https://traitementinfosol.pages.mia.inra.fr/icos/DE-RuSCarbonReportv2.html

ANNEX 4: SOIL TEXTURE DETERMINATION

Soil texture is the relative proportion of clay, silt and sand sized particles that make up the mineral fraction of the soil, and thus the range of particle size distribution in soil is from the smallest clay particles to the largest sand particles. To find the texture of a soil sample, It is recommended to use the Pipette method with five fractions (Coarse sand: 0.2 - 2 mm; Fine sand: 0.05 - 0.2 mm; Coarse silt: 0.02 - 0.05 mm; Fine silt: 0.002 - 0.02 mm and Clay: <0.002 mm) as described by ISO 11277 International Standard. In this method, both organic matter and CaCO3 must be removed before analysis. Therefore, soil texture will be determined according to the following steps:

1) Take an oven-dried fine earth sample (105 °C) then mix it with the deionized water (1 litre).
2) If organic matter and carbonates are present, remove them by hydrogen peroxide (H_2O_2) and hydrochloric acid (HCl), respectively.
3) Add dispersant agent [$(NaPO_3)_6$ + Na_2CO_3 and stir thoroughly.
4) Use the pipette to collect 10 ml of the particle solution at time and depth calculated from Stokes's Law (Figure AP.1).
5) Dry and weigh the fractions taken and determine the proportions of the different particle size.

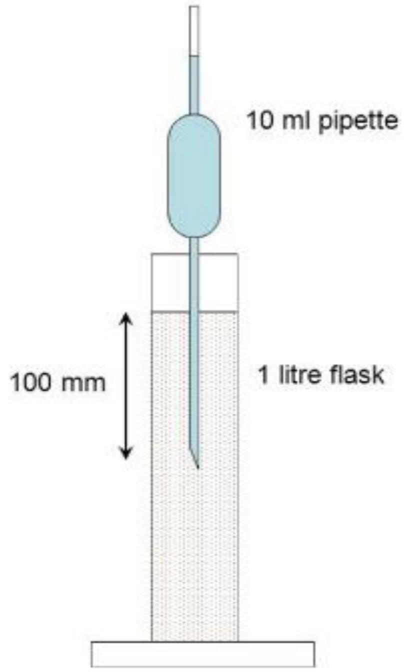


Figure A 5. Scheme for determination of soil texture using Pipette method