
**Guidelines for the determination of
organic carbon and nitrogen stocks
and their variations in mineral soils at
field scale**

*Lignes directrices pour la détermination des stocks de carbone
organique et d'azote et de leurs variations dans les sols minéraux à
l'échelle d'une parcelle*

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 190, *Soil quality*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Soils comprise an important pool in the biogeochemical cycles of carbon (C) and nitrogen (N), and thus are critical for climate regulation either by emitting greenhouse gases (GHGs) or by sequestering C.^[1] Soils are the largest terrestrial reservoir of organic carbon, accounting for more carbon than contained in the atmosphere or biota. Consequently, relatively small changes in soil carbon stocks can equate to considerable exchanges with other actively cycling carbon pools, such as the atmosphere. Estimation of soil organic carbon stock changes is one of the main methods applied to determine long-term carbon fluxes and to design carbon sequestration strategies. Soil organic carbon (soil OC) is the balance between inputs (e.g. plant residues, manure, etc.) and biologically mediated losses. Information on soil total N stocks is valuable, because adequate N is critical for plant production while excessive N can be an environmental hazard. Leakage of nitrous oxide (N₂O) from terrestrial systems to the atmosphere (where it enhances radiative forcing and may catalyse stratospheric ozone (O₃) destruction) is one hazard associated with excessive soil N inputs. The ratio of organic C to total N stock can also provide insight into soil OC stability and potential for element retention in the soil. Climate policies promote actions regarding the protection and increase of soil OC stocks. Such measures require standardized methods to assess the current soil OC stocks at the relevant scale (e.g. plot, farm, region) and to verify the efficiency of soil carbon sequestration actions. This document provides guidance on the measurement of carbon and nitrogen stocks in soils and to the detection of their temporal variations.

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Guidelines for the determination of organic carbon and nitrogen stocks and their variations in mineral soils at field scale

1 Scope

This document presents a method to quantify the soil organic carbon and nitrogen stocks in mineral soils at plot scale. It also provides guidance on how to detect and quantify simultaneously the variations of carbon and nitrogen stocks over time in mineral soils at field scale. It is based on several documents already published [2], [3], [4], [5], [6], [7], [8].

This document does not apply to organic soils, soils with permafrost, wetland soils, or to soil layers prone to submergence below the groundwater table.

NOTE 1 The possibility of increasing soil C storage is viewed as a means to sequester atmospheric carbon dioxide (CO₂) and mitigate greenhouse gas (GHG) emissions. Information on soil nitrogen (N) stocks is crucial because it interacts with carbon cycling through plant nutrition and organic matter decomposition, and leakage of N is of environmental concern (e.g. N₂O emissions, NO₃⁻ leaching). Therefore, it is becoming increasingly important to measure accurately the impact of changes of land uses and practices on organic carbon and nitrogen stocks.

NOTE 2 While understanding changes in soil inorganic carbon it is important also to understand the land-atmosphere exchange of CO₂, measuring stocks of soil inorganic carbon is outside the scope of this document.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 16133, *Soil quality — Guidance on the establishment and maintenance of monitoring programmes*

ISO 18400-101, *Soil quality — Sampling — Part 101: Framework for the preparation and application of a sampling plan*

ISO 18400-105, *Soil quality — Sampling — Part 105: Packaging, transport, storage and preservation of samples*

ISO 18400-206, *Soil quality — Sampling — Part 206: Collection, handling and storage of soil under aerobic conditions for the assessment of microbiological processes, biomass and diversity in the laboratory*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

3.1

spatial composite sample

two or more individual soil samples (e.g. cores) separated laterally in space having the same volume, and coming from the same soil layer or depth increment

Note 1 to entry: Also called average sample or aggregated sample.

Note 2 to entry: Composite samples sometimes are collected to encompass more lateral variability and better represent the mean of the measurement (e.g. water content, or C concentration) than provided by a single soil core.

3.2

land cover

observed (bio)physical cover of the Earth's surface

3.3

land use

socio-economic purpose of the land

3.4

land management practices

approach taken to achieve a land use outcome - the 'how' of land use (e.g. cultivation practices, such as minimum tillage and direct drilling)

3.5

mineral soil

soil composed largely or entirely of mineral (inorganic) constituents

[SOURCE: ISO 14688-1:2017]

3.6

organic soil

soil in which the organic component is dominant with respect to the mineral component

Note 1 to entry: For the purpose of this standard, organic soils are taken to contain more than 50 % organic matter by volume or more than 30 % organic matter by weight, i.e. 17 % of organic carbon. The definition of 'organic soil' varies between different soil classification systems.

3.7

organic soil layer

horizon 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 top of either mineral or organic soils

3.8

permafrost

ground consisting of mineral soil and sediment, rock, ice, peat and other organic materials that remain below 0 °C for at least two consecutive years

3.9

sampling point

precise position within a sampling site or within each soil constituting horizon from which samples are collected

Note 1 to entry: The coordinates must include x and y dimensions to indicate lateral locations and may also indicate the elevation of the soil surface in m relative to sea level.

3.10

undisturbed sample

sample obtained from the soil using a method designed to preserve the soil structure

3.11

soil layer

layer of soil defined by its upper and deeper dimension (e.g. 0-30; 30-50 cm etc.) and/or by the sampling procedure and it may comprise, or intersect, one or more soil horizons

Note 1 to entry: An horizon is a layer in soil that is roughly parallel to the ground surface and which is distinguished from layers above or below it on the basis of physical, chemical or biological differences ([9], [10]).

Note 2 to entry: Horizon related sample: sample collected from and representing a defined soil horizon.

4 Principle

Organic carbon and nitrogen stocks in mineral soils reflect the balance between inputs and outputs of C and N to the soil over decadal spans of time. Soil is heterogeneous due to variations in climate, parent material, topography, organisms (including human activity) and time. Consequently, soil C stocks vary with depth, location in space and sampling time. A proper sampling strategy should be implemented to take this into account in order to get a representative estimate of C and N stocks. This generally entails collecting several soil samples at different depths and locations.

To estimate soil organic carbon (soil OC) and total nitrogen (TN) stocks, samples of a known volume shall be collected, and the following determinations made:

- dry mass of the entire sample;
- dry mass of coarse (> 2 mm) mineral fragments or stones;
- fine soil (≤ 2 mm) mass per volume sampled ("bulk density");
- carbon and nitrogen concentrations in the fine soil fraction.

In general, significant field variations in organic carbon and nitrogen stocks occur very slowly, often over a period of 5 to 10 years as a minimum, depending on climate and soil management practices. Careful consideration of the complex factors governing the distribution of carbon and nitrogen stocks is important for the sampling design over space and time to be able to differentiate spatial and temporal variations.

Each step of the procedure (e.g. sampling, analysis) is associated with uncertainties, which can be quantified in order to calculate the total uncertainties regarding stocks and stock variation values. However, it could suffice to collect replicate cores, recognizing that they will encompass variability in space as well as errors associated with all the steps. Separately quantifying analytical uncertainties can verify that properly implemented methods using modern elemental analysers have small errors.

5 Procedure

5.1 Site investigation strategy

Site and soil description are necessary to interpret soil carbon stocks measurements and provide a basis for extrapolation.

A site investigation strategy shall be prepared for the overall investigation. In addition to the sampling strategy prepared in accordance with 5.2. This might include:

- description of the area of interest;
- current and past uses (e.g. crops, livestock, natural vegetation, restoration works) and management (e.g. soil tillage, organic fertilization and amendment, cover crops, crop yields, crop residue removal);
- characterization of soils and profiles as deemed necessary, including for example soil type, layer thicknesses, and basic physical and chemical characteristics;

- using methods to record sampling locations that will permit precise positioning of subsequent (5 to 10 years in the future) sampling, including GPS coordinates with sub-metre resolution, distances to other permanent features, installation of an electromagnetic marker, etc.

Particular care shall be taken when developing the overall strategy and the sampling strategy so that samples can be collected from the same sampling locations in future years to monitor changes in soil organic carbon stock (in accordance with ISO 16133 relating to monitoring sites). Sufficient and appropriate information on the site/area and soils should be collected to enable comparisons with the results for other areas, when this is required.

NOTE 1 ISO 18400-202 gives detailed guidance on desk studies and site inspections (preliminary investigations) and ISO 18400-205 gives further guidance relating to natural, near natural and cultivated sites. ISO 18400-205 gives specific guidance on sampling in orchards etc. and wooded areas.

NOTE 2 ISO 25177 provides guidance on site and soil descriptions.

5.2 Sampling

5.2.1 Sampling objectives

The goal of soil sampling is to collect volumetric samples that represent the area of interest, and that estimate mean soil OC and TN stocks (element masses per unit area to a specified soil depth and mass), including estimates of variability (i.e. scatter or dispersion of the data) about the means.

Determination of the soil organic carbon and total nitrogen stocks for a defined area (e.g., plot, field) thus requires the boundaries of the area of interest to be delineated and the depth (range) of interest to be decided.

The mass of soil also shall be stated. Assuming negligible geomorphological processes, comparisons among soil OC or TN stocks should preferably be based on an equivalent soil mass, rather than on a fixed volume.

It is also necessary to know the moisture content so that the results can be expressed on a dry weight basis. The analytical measurements for C and N are made on the less than 2 mm fraction. It is therefore necessary to know the mass of material (e.g. rocks, organic fragments) in the soil that is > 2 mm.

All organic matter in representative soil samples must be quantified, including the coarse (> 2mm) organic fraction. Such materials can be ground or chopped to < 2 mm and included for analysis with the entire < 2 mm mineral soil sample, or they can be isolated (e.g. as particulate or light fraction OM) and analysed independently, but they must not be discarded.

NOTE 1 Scale is discussed in ISO 18400-104, 5.6 and Annex E.

NOTE 2 Since sample processing and chemical analyses account for relatively small cost increments on sample collection, it usually is preferable to perform independent analyses on separate sampling points and soil layers. This provides important information on variability in three dimensions. In addition, when samples are taken on at least two different collection dates, it makes it possible to distinguish temporal variability from spatial variability.

NOTE 3 Depending on the specific program objectives, the coarse organic fraction could be determined (and its C-N content measured) separately from the mineral soil to assess the time dynamics for specific purposes (short term change of stocks, effect of a specific practice of OM management etc.). Particulate or light fraction OM is often sensitive to management changes, and measuring > 2 mm fractions could provide early and valuable indications of forthcoming changes in soil OC stocks.

5.2.2 Sampling plan

A sampling plan shall be prepared in accordance with ISO 18400-101.

This should describe what is to be done to obtain the required samples and the practical requirements for carrying out the work (i.e. how to implement the sampling strategy, see [5.2.3](#)).

Whatever the methods used to collect or form samples, their form and how they are to be taken should be prescribed in the sampling plan.

5.2.3 Sampling strategy

5.2.3.1 General

A sampling strategy should be prepared in accordance with the guidance in ISO 18400-104 having regard also to the guidance in ISO 18400-205. Usually based on the site investigation ([5.1](#)), the site may be stratified in different zones using as a minimum the following variables^[11]:

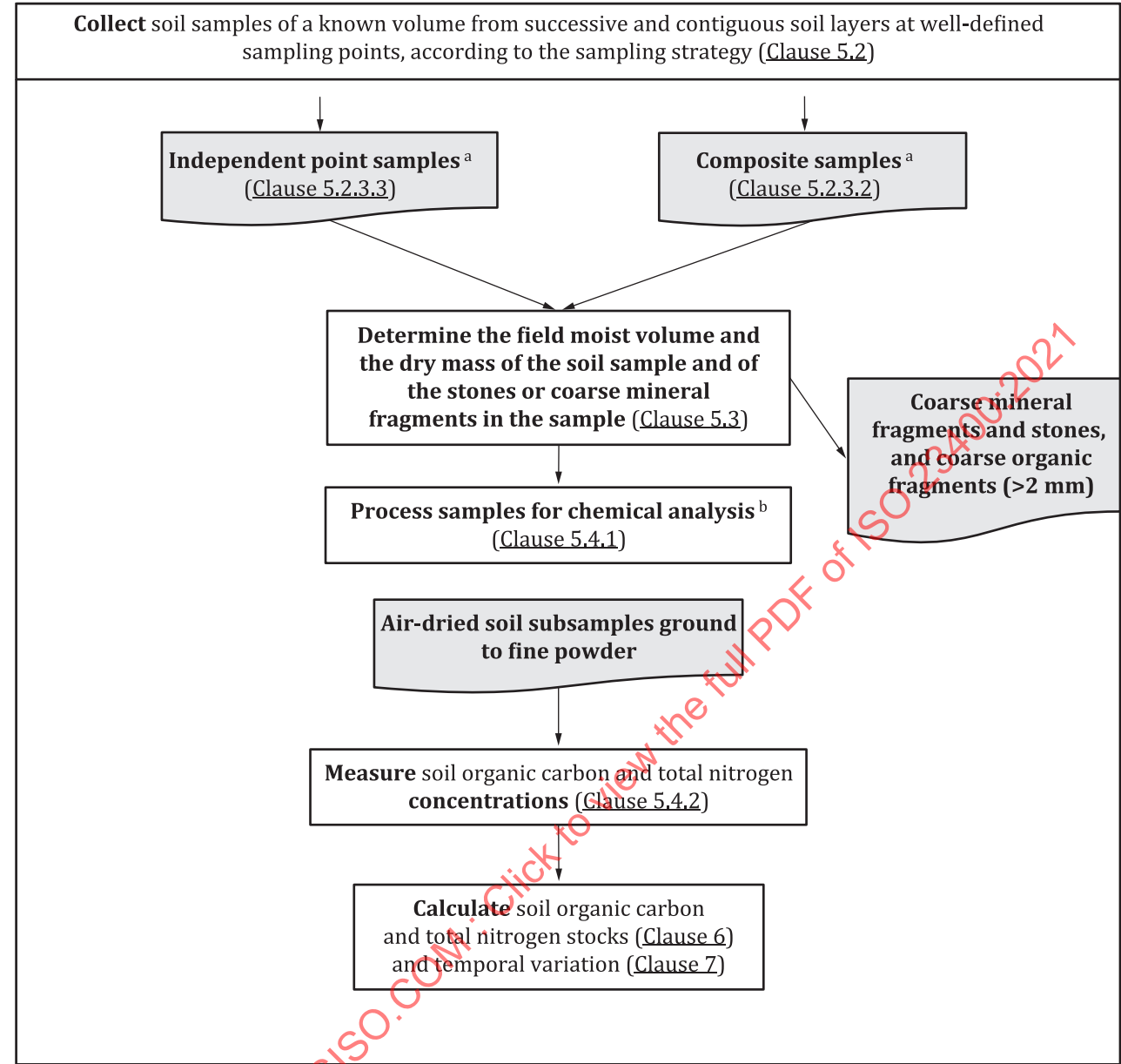
- land location (nearest settlement or roadway), legal land description, GPS coordinates;
- typical soil texture, parent material, solum thickness, soil classification;
- topography and landscape morphology (e.g. slope position, surface shape (concavity/convexity), erosion forms, drainage and water regime);
- biome, ecodistrict (if known), remote sensing images, vegetative cover, land use and management.

The sampling strategy should also:

- include all sampling activities that are to be undertaken;
- determine how to collect volumetric samples that represent the area of interest and that estimate mean soil OC and TN stocks including estimates of variability about the means;
- provide information on spatial variation at the desired scale if required.

Mean elemental stocks of the area can be determined using composite sampling (see [5.2.3.2 – Figure 1](#)) or by averaging the stocks from independent sampling points (see [5.2.3.3 – Figure 1](#)). The latter is preferred because it will yield information on variability at the scale of the sampling pattern and it allows pairing of sampling points from different sampling times to improve assessment of temporal changes (see [Clause 7](#)). However, depending on the budget, compositing can be required. [Figure 1](#) gives an overview of the different steps needed from sampling to calculating to obtain a mean elemental value of the stock of an area.

Bulk density measurements and carbon and nitrogen concentrations should all derive from the same core sample to determine the soil OC and TN stocks for that sample.



Key



action



soil sample

^a Averaging the stocks from independent point samples is preferred because it will yield information on variability at the scale of the sampling pattern and it allows pairing of sampling points among sampling times to improve assessment of temporal changes. However, depending on the budget, compositing can be required.

^b Depending on the specific program objectives, the coarse organic fragments could be determined (and its C-N content measured) to assess the time dynamics for specific purposes (short term changes of stocks, effect of specific practice of organic matter management, etc.). Coarse fragments of plant roots and shoots and other organic materials may respond to management changes. Consequently, these organic materials could be crushed or chopped to <2 mm and included with the < 2 mm mineral soil.

Figure 1 — Steps of soil sample collection, preparation and analysis for soil organic carbon and total nitrogen stocks determination

NOTE 1 A pre-sampling (e.g. with augers and/or soil pit description) can be performed to produce a first qualitative assessment of soil carbon and nitrogen distribution and organize the sampling campaign.

NOTE 2 Composite sampling can also provide some information on variability depending on how it is applied. The simplest way is to collect several (at least 2) composite samples.

NOTE 3 Initially it could be useful to collect samples from more points than actually required so that the minimum number of samples required to attain the minimum detectable difference can be calculated. Furthermore, some samples might be lost.

NOTE 4 Mechanization of soil sample collection (e.g. using hydraulically driven soil core tubes) increases the likelihood of obtaining the samples required for statistically significant determination of soil OC and TN stocks. Furthermore, such mechanization typically minimizes site disturbance, allowing subsequent samples to be collected near (in space) to the initial ones. This might decrease the influence of spatial variability and increase the detectability of temporal changes. However, the practicality of using such equipment, which is commonly in the form of portable but heavy hand-operated gear or a self-propelled tracked rig, will depend on the location where it is to be used (e.g. topography, vegetation).

5.2.3.2 Composite sampling

When desired, to reduce analytical costs, composite sampling may be carried out in accordance with ISO 18400-104 which, among other things, provides guidance on:

- how to form composite samples;
- how many composite samples to take from an area of a given size (see [Table 1](#) and ISO 18400-104:2018, 7.3.2).

Table 1 — Number of zones for composite sampling in relation to the total area of the site (after ISO 18400-104:2018, Table 4)

Area <i>A</i> ha	Minimum number of Zones <i>n</i>
0 to 2	1
> 2 to 5	2
> 5 to 10	3
> 10 to 15	4
> 15 to 20	5
> 20 to 30	6

NOTE 1 For areas larger than those given, the following equation should be used to specify the number of zones to be sampled: $n = 1 + \sqrt{A}$.

NOTE 2 The underlying assumption is that properties are generally uniform within the area to be investigated – if this is not the case, the number of samples should be increased to ensure that each area with generally uniform soil properties is sampled separately, while also ensuring that the number of zones is not less than the minimum stated in the table.

- judgements to be made irrespective of the size of the area of interest (e.g. whether to zone the site in response to site observations such as topography, health of crops, evidence of flooding etc.).

NOTE 1 ISO 18400-104:2018, 6.5.4 provides guidance on the preparation and use of composite samples. For the reasons explained in ISO 18400-104:2018, B.2 a “W” pattern is generally favoured. Increments are taken from at least 25 locations in the area to be sampled. This applies irrespective of the size of the area, whether it is a small garden bed or a 2ha field (above this size the site is to be subdivided into at least 2 zones).

5.2.3.3 Using independent point samples

Average properties can be determined with reasonable confidence by taking the mean of values determined on point samples taken using a probabilistic pattern provided sufficient number of samples are taken to encompass the full variability of the properties. If insufficient samples are taken from a

heterogeneous population, there could be considerable bias in the average determined (see ISO 18400-104:2018, Annex I).

Ideally 20-30 sampling points should be taken to provide a good estimate of the mean concentration (i.e. the 95 % confidence limits are close to the mean) whilst fewer than 10 samples will provide only a poor estimate (i.e. there is a large difference between the sample mean and the 95 % confidence limits), see ISO 18400-104:2018, Annex I, I.7.2.

A systematic sampling pattern (e.g. square grid) should be used in preference to other predefined sampling patterns because they are more practical to use in the field and because some patterns (e.g. circular) inherently yield biased results.

Consideration should be given to taking duplicate samples to provide information on sample variability (see ISO 18400-104:2018, 5.8 and Annex C).

NOTE 1 Guidance on the calculation of mean values and associated statistical parameters (e.g. confidence limits) is provided in ISO 18400-104:2018, A.5.4 and Annex G.

NOTE 2 Composite samples are subject to bias if insufficient sampling points contribute to the composite. This major weakness of a composite sample is even more problematic since the bias is concealed.

NOTE 3 The different levels of information on variability that can be obtained when point samples rather than composite samples are taken are illustrated in ISO 18400-104:2018, Annex E, E.3.

NOTE 4 Guidance on sampling patterns is provided in ISO 18400-104.

5.2.3.4 Special sites

Special sampling approaches could be required for areas with woody perennial vegetation, such as orchards, vineyards or woodlots, or for areas with widely separated vegetation such as row crops. In such cases, sampling should be carried out in accordance with the relevant guidance in ISO 18400-205.

5.2.3.5 Size of samples

The following amounts are recommended for investigations covered by this document, but should be adjusted to meet the needs of the investigation as necessary:

- in the case of mineral soils: roughly 1,0 kg;
- in the case of organic horizons from forest or woodland (arboreal) soils: up to 0,75 kg (or 0,5 kg of fine soil).
- in the case of soils with increasing fractions of gravel, cobbles and stones, greater masses are required (see ISO 18400-104:2018, 6.6).
- additional soil material is needed for preparation of replicate samples.

NOTE 1 There could be occasions when it is easier to specify required sample sizes in terms of a volume.

NOTE 2 The mass of an organic layer collected as a single sample is likely to be smaller than that recommended for mineral soils, as the volume of organic material required to attain the same mass of dry matter is 5 to 15 times greater.

5.2.3.6 Conditions of sampling

In some circumstances, it can be necessary to restrict sampling to specific periods of the year. For example, if the characteristic or substance to be determined is likely to be affected by seasonal factors or human activities (weather, soil conditioning/fertilization, use of plant-protective agents).

The soil sampling should be planned for a time when:

- the soil is trafficable;

- disruption of the vegetation community can be minimized;
- soil samples can be collected without alteration of soil bulk density.

NOTE 1 For medium- and fine-textured soils, compaction often occurs during sampling at moisture contents close to or above field capacity, conversely when moisture content approaches the wilting point, soils can become impenetrable.

NOTE 2 Depending on the soil types and sampling device used, there could be additional restrictions on when samples can be collected. For example, to avoid soil compaction due to large mechanically driven soil coring systems lower soil water contents could be needed.

5.2.3.7 Sampling depth

The sampling depth should, at minimum, span the soil layers where meaningful changes in soil carbon could occur due to both natural and anthropogenic processes. Field reconnaissance (and study of soil survey reports) is useful to determine the appropriate sampling depth and to plan subdivision of soil profiles into sampling layers. This will therefore require knowledge of the processes impacting the soil and expert judgment. Indicators could include plant community (e.g. annual vs perennial), active rooting, tillage disturbance, soil colour changes indicating active organic matter accumulation or leaching, or textural changes resulting from mobilization of fine fractions. As a general rule, if the soil is less than 1m deep, it is recommended to sample the full profile.

Land use and management practices strongly influence the vertical distribution of soil C and N content as well as the bulk density. As an example, tillage will dilute soil organic matter generally within the first 30 cm whereas no-tillage will concentrate it in the first few centimetres. Thus, the minimum depth of sampling should be adapted according to the present, but also past and anticipated future management practices.

NOTE 1 Sampling to a minimum depth of 30 cm is needed for climate change reporting. However, it is now recognized that measuring carbon contained in deeper soil layers (i.e., at depths greater than 30 cm) might also be important to consider. This is particularly the case at locations where: there has been change, or the potential for change between annual and perennial crops; where crops have subterranean structures (e.g. beet roots, potato tubers); or, where crops are deep rooted (e.g., alfalfa). Despite the fact that errors tend to accumulate as the depth of sampling increases, sampling at deeper, more appropriate depths is critical to consider especially as such samples could prove valuable for carbon stock comparisons after long time intervals.

Any surface vegetation cover, moss-covered litter layer, visible roots, large pieces of plant or woody plant litter and visible soil fauna should be removed to minimize the addition of fresh organic carbon to the soil. For forest soils, it is sometimes difficult to define precisely the limit between the organic layer and the upper bound of the mineral soil. The inclusion of litter material in the soil surface samples can significantly affect the determination of the soil carbon stock and changes to this over time. It is therefore important to collect the organic layer separately and to document how it was sampled, as well as measuring its thickness and determining its bulk density.

5.2.3.8 Selection of sampling tools

Sampling techniques typically comprise:

- a means of accessing the material to be sampled;
- a means of taking the sample.

The sampling technique(s) to be used should be selected following the guidance in ISO 18400-102 having regard in particular to:

- health and safety;
- environmental protection;
- whether they can provide the samples required;

— availability of equipment at the place where the work is to be carried out.

Sampling from vertical cores obtained using hand operated equipment designed to recover undisturbed cores or machine-driven coring tools is favoured (see Table 2). Cores can be rapidly taken from depths of 1 metre or more and can be transported off-site for sampling obviating the need for samples to be taken from the core under what could be difficult weather conditions. When sampling using soil cores, cores should be minimally disturbed as they are withdrawn from the soil, and inter-mixing of soil should be avoided^[12]. During the process of subdividing the soil core sections into appropriate depth increments, the soil sample can crumble and become disturbed. This is not a concern as long as the original volume and (stone-free) mass of the entire core is determined following soil collection.

Table 2 — Examples of equipment and tooling for sampling mineral soils (adapted from ISO 18400-102)

	Type of material	Advantages	Disadvantages
Hand operated	Non-rotating and non-augering soil core samplers	Portable and useful for locations with poor access Moderate compaction Limited operating expense	Time consuming Only limited depths can be achieved if obstructions present, e.g. stones. Ease of use very dependent on soil type.
Machine driven	Non-rotating and non-augering soil core samplers (pneumatic or percussion hammering devices, hydraulic down pressure devices)	Fast sampling is possible. Can achieve greater depths than hand operated core samplers.	May compact soil (depends on soil type), More expensive Depending on equipment the footprint may be quite large The equipment may be difficult to transport across challenging terrain

In stony soils that preclude use of core samplers, sampling can be done from hand or machine dug pits (excavation technique). This technique is potentially highly disruptive if the site must be resampled a number of times in future years and subsequent samples should be collected distant (5 to 10m) from the original soil pit.

Organic layers on mineral soils are sampled using a sharpened and rigid template (square, rectangular or circular area). The thickness of the layer is measured, and all organic material is collected.

5.2.4 Sample handling, storage and transport in the field

Sample processing and preparation for subsequent analysis must be carefully controlled and closely integrated with collection from the field. Samples shall be then handled, stored and transported using cool boxes in accordance with ISO 18400-105.

Carefully labelled samples are collected in trays or bags. The size and form of the sample containers to be used should be agreed with the laboratory that is to carry out the analysis.

If the laboratory agrees that plastic bags may be used, they should be of a type that will not be easily damaged during transport.

5.2.5 Sample handling and storage in the laboratory

Samples shall be handled and stored in the laboratory in accordance with ISO 18400-206 as appropriate.

If soil samples must be stored prior to air-drying, the storage conditions should minimise mineralization of carbon and nitrogen by microorganisms i.e. in the dark and cool (e.g. 4 °C). Storage duration should be as short as possible, up to a maximum of 28 days after collection^[13].

Field-moist samples shall be weighed (field-moist soil weight), mixed and a representative sub-sample taken to determine gravimetric field soil moisture content, transferred to foil drying trays (clean, dry, oil-free) and preserved by air-drying (≤ 45 °C) as soon as possible. Soils shall be air-dried to ≤ 6 % dry weight basis for fine textured mineral soils and to ≤ 3 % for medium and coarse textured ones. In humid locations, the humidity of the drying room may need to be lower to attain adequate dryness within 10 days. After the soil is air dried, soil samples shall be stored in a dark, cool and dry room.

NOTE ISO 18512 *Soil quality – Guidance on long and short term storage of soil samples* provides guidance on how to store and preserve soil samples for laboratory determinations and how to prepare them for analysis after storage. Special emphasis is given to storage times as a function of storage conditions including the condition of the sample, e.g. moist, air-dried or oven dried. ISO 18400-206 *Soil quality — Sampling — Collection, handling and storage of soil under aerobic conditions for the assessment of microbiological processes, biomass and diversity in the laboratory*. It describes how to minimize the effects of differences in temperature, water content, and availability of oxygen on aerobic processes as well as the fractionation of soil particles to facilitate reproducible laboratory determinations. It includes guidance on long term storage of samples.

5.2.6 Safety

Sampling, including the selection and application of sampling techniques, should be carried out with proper regard to the safety of those carrying out the work and the general public. Guidance on safety during sampling, including on agricultural and similar sites is provided in ISO 18400-103.

Anthropogenic soil materials can sometimes be contaminated with substances or organisms (e.g. bacteria, fungi, virus, etc.) that present a hazard to those handling the material. Underground electricity, gas or liquid fuel lines can be encountered. Appropriate risk assessments and investigations should be carried out prior to any sampling followed by appropriate sampling and testing of the materials of interest. This could include using an under-ground utility locating service and employing personal protective equipment (steel-toed footwear; gloves, etc).

5.2.7 Environmental Protection

Sampling including the selection and application of sampling techniques should be carried out with due regard to the environment (e.g. disturbance to wildlife, damage to vegetation, risk of soil compaction).

5.2.8 Quality assurance during sampling

A quality assurance programme should be established in accordance with ISO 18400-106.

5.2.9 Sampling report

A sampling report should be prepared in accordance with ISO 18400-107.

5.3 Determination of the dry mass and the volume of the soil sampled

Bulk density can be estimated from the dry mass and the volume of a soil sample following ISO 11272. Soil bulk density is calculated as the stone-free dry soil mass divided by the volume sampled in the field. The bulk density should be determined for the very same soil sample collected for analysis of soil organic carbon and total nitrogen concentrations.

The volume of the soil sample is known (core method) or must be determined (excavation method):

- The core method is applicable to stoneless and slightly stony soils (<5 %). Core samples of known volume are taken with a metal sampling tool (either manual or mechanically driven).
- In stony soils that preclude use of soil cores, bulk density can be determined by excavating a quantity of soil, and determining the volume of the excavation by filling it with sand. The sand can be replaced by water in an impermeable membrane (e.g. polyethylene, rubber) that closely conforms to the irregular shape of the pit excavated. The disadvantages of using the excavation approach is that sand is heavy, difficult to recover and alters the site, and water is heavy, prone to leakage and, if the impervious membrane does not closely conform to pit walls, the volume will be under-estimated.

NOTE 1 Portable, digital volume scanners can be used to estimate soil volume. Such devices range in cost and include (but are not limited to): sensors using multispectral cameras integrated or used with handheld cellular devices (i.e., smart phones or tablets); stand-alone multispectral scanners; and, terrestrial Light Detection and Ranging (LiDAR) portable laser equipment.

A sub-sample is oven-dried at 105 °C to estimate gravimetric water content and therefore the dry mass of the whole soil sample. This subsample then is discarded.

To weigh the coarse mineral fragments and stones, the air-dried soil should be sieved using a screen with 2 mm openings. At this time, it often is convenient to collect a representative sub-sample (preferably using a rotating tube sample divider fed by a uniformly flowing stream of soil) for subsequent fine-grinding (e.g. to pass a 100 mesh sieve with 150 µm openings) that often is required for elemental microanalysis. The sub-sampling procedure should be considered carefully to prevent the introduction of bias, which may be easily accessed by collecting multiple sub-samples to assess uniformity.

NOTE 2 Specifications for the equipment to be used are provided in ISO 11272.

NOTE 3 Where composite core samples were taken, the volume of all cores collected can be calculated as $\pi \times r^2 \times L \times n_{\text{core}}$ where r is the core radius and L is the length of each individual cores, and n_{core} is the number of cores composited within the sampling area. Since the cores are combined in the field, the composited cores subsequently will be handled as a single soil sample. Bulk density is then taken as the stone-free oven dry mass of the soil sample divided by the composite volume.

5.4 Chemical analysis

5.4.1 Sample processing for chemical analysis

Sample processing encompasses all the steps between sample collection in the field and weighing for chemical analysis.

ISO-11464:2006 describes the operating modes to obtain the samples that will be sent for chemical analysis. All equipment used should be clean and dry and work surfaces cleaned between samples to avoid cross-contamination. Every sample should be clearly and precisely identified at each step of the sample processing.

The procedure includes the determination of the mass of stones and coarse mineral fragments (>2 mm) as described in 5.3.

5.4.2 Chemical analysis

Carbon content should be determined by dry combustion following ISO 10694, or by infrared spectrometry following ISO 17184.

If the soils are known to be carbonate-rich, acidification will be required to remove inorganic carbon before dry combustion can begin. ISO 10694 should be followed if acidification is needed. The acidification procedure for calcareous soils must avoid the influence of salts when acids reacts with carbonates. These salts often add dry mass to the sample and biased (downwards) estimates of soil OC and TN concentrations. The error may be circumvented by small-scale acidification using HCl where the original pre-acidified soil mass is used to calculate soil OC concentration.

Nitrogen content should be determined in accordance with ISO 13878. Samples processed for chemical analysis of C and N must not be exposed to temperatures above 60 °C.

NOTE 1 Dry combustion is the reference method. Infrared spectroscopy allows rapid measurements but is less precise than dry combustion. Spectroscopic techniques can be used when technical capacities for adequate chemometric calibration for C and N are available.

NOTE 2 ISO 10694 assumes “natural” or “near natural” soil. If the soil is contaminated (e.g. with hydrocarbons, residual carbon in coal ash, coal fragments) or contains significant amount of oxidisable or volatile material the results might be potentially misleading.

NOTE 3 C/N concentration ratios can be useful to expose analytical errors. Typically, the ratios are expected to be in range of 6 to 18, and often cluster around 10. Ratios >18 often indicate errant contributions of carbonate C to allegedly soil organic C, but in surface layers may simply reflect the presence of N-poor plant litter. Ratios <5 are atypical of most soil organic substrates but could be attributable to inorganic N pollution.

NOTE 4 Thermal techniques may be used as well to quantify the organic carbon content in soils, following for example the methods described in DIN-19539 or Reference [14]. With adapted analytical methods and appropriate calibration, thermal techniques provide organic carbon content values that are as accurate as those from dry combustion method (see [14] to [18]), with fast analysis. For carbonate-rich soils, isolation of the organic matter from the mineral part is not required and both organic carbon and mineral carbon contents can be quantified in a single analysis. The risk of overestimation or underestimation of organic carbon content due to incomplete removal of carbonates or loss of organic matter during acidification can then be avoided (see [18] to [20]). Moreover, some information on thermal stability of the soil organic matter can be gathered and used to estimate persistent organic carbon content in soil, assuming that thermal stability is a proxy for biogeochemical stability (see [21] to [24]).

6 Calculations of the organic C and N stocks

Soil organic carbon or nitrogen stocks can be estimated using [Formula \(1\)](#):

$$OC_{\text{stock}} \text{ or } N_{\text{stock}} = \sum_{i=1}^n OC_i \text{ or } N_i \times FFS_i \quad (1)$$

$$FFS_i = \frac{m_{\text{fsf}}}{V_{\text{s},i}} \times e_i$$

$$m_{\text{sf},i} = m_{\text{tds},i} - m_{\text{dcf},i}$$

where

$OC_{\text{stock}} \text{ or } N_{\text{stock}}$	is the soil organic carbon or nitrogen stock in the soil profile considered (in Mg.ha ⁻¹);
FFS_i	is the fine soil stock of the soil horizon or layer i (in Mg.ha ⁻¹);
m_{sfs}	is the mass of fine soil fraction;
m_{sf}	is the mass of soil fraction;
m_{tds}	is the mass of total dry soil;
m_{dcf}	is the mass of dry coarse fragments;
$V_{\text{s},i}$	is the total volume of the soil sample (in cm ³);
e_i	is the thickness of soil horizon or layer i (in cm);
$OC_i \text{ or } N_i$	is the soil organic carbon or nitrogen concentration in the fine soil fraction (< 2mm) of soil horizon or layer i (in g 100g ⁻¹ of fine soil);
n	is the number of soil layers.

All masses are dry masses (dried at 105 °C until constant weight) and expressed in g. Coarse fragments are larger than 2 mm.

NOTE 1 To eliminate the need for measurements of stone volume and density, the soil BD is taken as the stone-free dry soil mass per volume sampled, rather than the stone-free dry soil mass per stone-free volume.

NOTE 2 Calculations typically are made on a layer-by-layer basis and summed to some fixed volume.

NOTE 3 Since soil bulk density varies with land use, agronomic management, sampling conditions, etc., element stocks within a fixed soil volume will increase with bulk density. To comply with an assumption of soil mass conservation, it often is advantageous to adjust estimates of soil OC and TN stocks so that comparisons among stocks is based on an equivalent soil mass with slightly different thicknesses (see 7.2).

7 Measuring the temporal variations of soil organic carbon and nitrogen

7.1 General

Measuring temporal changes in soil OC and TN stocks will require at least two sampling campaigns separated by 5 to 10 years where soil OC and TN stocks were measured according to this standard (see previous clauses).

To accurately measure temporal changes in carbon or nitrogen stocks, a sampling strategy should result in the collection of samples that represent the population of interest and estimate both the mean and variability surrounding the mean. Effort should be directed toward minimizing the influence of spatial variation. Careful pairing of sampling points from different sampling times can help to lessen the influence of spatial variations and improve resolution of temporal changes [26].

Depending on the changes to be monitored (e.g. in land use, in vegetation, in cultivation practices) the accuracy needed to demonstrate a variation of the soil OC stocks could be different. Generally, land use changes (e.g. from pasture to cropland) affect more quickly and intensely carbon and nitrogen stocks than changes in cultivation practices (e.g. reduction of tillage). This will have an impact on the number of samples to be collected and the monitoring interval.

To be able to interpret any difference in the results from the two sampling times (see 7.2), relevant information should be gathered (7.4) having regard to possible sources of error (7.3).

NOTE 1 Assuming possible rate of changes (mean and standard deviation), minimum detectable difference calculations can be used to estimate the number of sampling points needed to detect the expected stock change (see Annex A).

NOTE 2 Due to the high spatial variability of carbon and nitrogen stocks in soils, it is almost impossible to detect a variation of less than 5 %.

7.2 Calculation of soil OC stock changes and uncertainties

Once results are obtained for each campaign, changes in soil OC stocks can be calculated as the difference between the stocks measured at each sampling time [Formula (1)]. The uncertainty of the two measurement should be taken into account when drawing conclusions about any change together with other sources of error that might occur (7.2).

$$\Delta OC_{\text{stock}} \text{ or } \Delta N_{\text{stock}} = OC_{\text{stock},n} \text{ or } N_{\text{stock},n} - OC_{\text{stock},n-1} \text{ or } N_{\text{stock},n-1}$$

where $OC_{\text{stock},n}$ or $N_{\text{stock},n}$ is the last measurement of the stocks and $OC_{\text{stock},n-1}$ or $N_{\text{stock},n-1}$ is the previous one.

Soil carbon stocks are expressed commonly as the amount of carbon in a fixed-depth layer of soil per unit area of land (e.g. Mg C/ha to a specified depth). A fixed-depth approach is subjected to bias when comparing carbon stocks temporally (or spatially) if bulk density changes. Unless bulk density is very uniform, an "equivalent soil mass" sampling approach should be used to quantify temporal changes in soil carbon stocks or where there is interest in comparing soil carbon stocks under different land uses or management practices. The objective of such an approach is to enable estimation of carbon in a constant mass of soil during the assessment period. The approach involves sampling successive layers of soil and, using measured bulk density values, estimating the depth at which target soil mass is attained (the target mass will normally be the mass of soil to a specified depth at the beginning of the observation period, $t = 0$). The "equivalent soil mass" should include soil in all layers that are likely to be

affected by the treatment or management practice of interest^[5]. The “equivalent soil mass” calculation should remain consistent across all sampling times (see [Annex B](#)).

7.3 Possible source of errors

Errors could come from the sampling (i.e. in field) and/or the analytical procedures (i.e. at the laboratory)^[25]. Since assessing temporal changes in soil organic carbon and nitrogen is a monitoring process, sampling and analytical methods should be well-documented and consistent through time. If possible, it is recommended to store the samples of each campaign (see ISO 16133) and re-analyse part from the older samples to check any deviation due to the analytical method.

Errors due to the field component of a project are the most difficult to control and might include spatial variability, inconsistencies in sampling between samples and operators, adverse weather or soil conditions, etc. In many settings, the distinction between plant C (or N) and soil C (or N) is unclear, as it is not clear where root/undecomposed litter layers and the soil ‘proper’ begins, which can lead to errors in measurement of soil C and N stocks. Hence, large coarse fragments of organic matter may be finely ground and thoroughly mixed with the mineral soil, or they may be isolated and measured as a separate fraction, but they must not be discarded.

Errors in the laboratory are easier to document and control. Possible control measures include insuring appropriate and consistent sample storage times and conditions and use of a certified reference material (i.e. a geostandard with a matrix relevant to soil). Careful attention to sample division (e.g. rotating tube sample divider fed by a uniform sample stream) is required to minimize sub-sampling errors and subsequent analytical errors due to non-homogeneous sub samples.

7.4 Information needed

The interpretation of data will require information on land use and soil management during the monitored period. This information should be collected not only during the first sampling campaign but also at least in the decade prior as previous land uses or practices might still influence current conditions. Examples of agricultural or forestry practices that are known to impact soil carbon and nitrogen stocks include: intensity of tillage, organic fertilization and soil amendment, cover crops, crop types and rotation, residue removal and irrigation. Any change in land use should be documented as it is usually the main driver of change. Information about unusual events such as fires, floods, severe droughts, storms should also be collected as they could change the carbon and nitrogen dynamics or alter soil layers and bulk density.

8 Reporting

8.1 Reporting for soil organic carbon and nitrogen stocks

ISO 15903 provides guidelines on how to report information regarding site and soil description, sampling, on-site sample analyses and laboratory analyses of soil samples. If it is intended to resample the site (e.g. for monitoring purposes or measuring changes in soil organic carbon stocks) the report should clearly state the number of samples collected, their position (e.g. GPS positioning) and depth as such information is critical to the design of the next campaign.

Furthermore, any relevant information used that could help the next sampler should be stated (e.g. compacted areas, saturated soils). Methods to measure either carbon, nitrogen and bulk density should also be reported (especially if deviations have been performed) as well as the method of long-term storage of soil samples (if it was decided to keep part or all of the samples, see ISO 18512).

The report should also contain historical information about the site such as the land use, the cultivation practices (if any) and any event that could change the soil carbon and/or nitrogen stocks (e.g. fire or flood).

8.2 Additional reporting for variation of soil organic carbon and nitrogen stocks

The report should describe the way the sampling campaigns and the calculations were conducted, and the possible source of errors.

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