



## **A Protocol for Modeling, Measurement and Monitoring Soil Carbon Stocks in Agricultural Landscapes**

**World Agroforestry Centre (ICRAF)  
The Earth Institute, Columbia University (EI)**

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## **FOREWORD**

This protocol has been developed over a number of years through various projects and is currently being refined in the context of the Africa Soils Information Service (AfSIS: [www.africasoils.net](http://www.africasoils.net)), funded by the Bill & Melinda Gates Foundation and the Alliance for a Green Revolution in Africa (AGRA), and the Carbon Benefits Project: Modeling, Measurement and Monitoring, funded by the Global Environment Facility (GEF) of the United Nations Environment Program (UNEP).

## Abbreviations and Acronyms

AfSIS	Africa Soil Information Service
CBP	Carbon Benefits Project
CDM	Clean Development Mechanism
cm	Centimeter
FAO	Food and Agriculture Organization
FT-NIR	Fourier Transform Near-Infrared diffuse reflectance spectroscopy
FT-MIR	Fourier Transform Mid-Infrared diffuse reflectance spectroscopy
g	Gram
GEF	Global Environment Fund
GIS	Geographic Information Systems
GPS	Global Positioning System
ha	Hectare
ICRAF	World Agroforestry Centre
IR	Infrared spectroscopy
ISRIC	International Soil Reference Information Service
LDSF	Land Degradation Surveillance Framework
MIR	Mid Infrared Diffuse Reflectance Spectroscopy
MLR	Multiple Linear Regression
MPA	Multipurpose Analyzer
NAMA	Nationally Appropriate Mitigation Action
NIR	Near Infrared diffuse reflectance spectroscopy
PCR	Principal Components Regression
RER	The Ratio Error Range
PLS	Partial Least Squares Regression
RPD	Ratio of Prediction to Standard Deviation
REDD	Reducing Emissions from Deforestation and Forest Degradation
RMSEP	Root Mean Square Error of Prediction
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
SOP	Standard Operating Procedure
SSN	Sample Serial Number
T	Tonne
TXRF	Total X-ray Fluorescence spectroscopy
UNEP	United Nations Environment Program
UNFCCC	United Nations Framework Convention on Climate Change
VNIR	Visible Near Infrared Diffuse Reflectance Spectroscopy
XRD	X-ray Powder Diffraction Spectroscopy

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# 1 INTRODUCTION

## 1.1 Background

Burning of fossil fuels and land-use change, particularly deforestation, have resulted in a steady accumulation of CO<sub>2</sub> and other greenhouse gases in the atmosphere, which is the cause of global warming (IPCC, 2003). The two major strategies to mitigate the potential negative effects of climate change are reducing the emission of greenhouse gases and the capture and storage of CO<sub>2</sub> from the atmosphere. Through the framework of the Clean Development Mechanism (CDM) under the Kyoto protocol, increasing terrestrial sinks through afforestation and reforestation are the two accredited activities. Besides afforestation and reforestation, reducing Emissions from Deforestation and Forest Degradation (REDD) and enhancing carbon stocks through sustainable land management (REDD+) are given due credit in mitigating climate change (Campbell, 2009).

Measuring, reporting and verification (MRV) of such climate mitigation actions through Nationally Appropriate Mitigation Actions (NAMAs) is one major outcome of the Bali convention (United Nations, 2007). MRV gives opportunities to developing countries to claim financial, technical and capacity building supports from developed countries to implement their NAMAs. Understanding these benefits, a growing number of developing countries (e.g. Algeria, China, South Africa, Indonesia, Costa Rica) have drafted, adopted and, in some cases, started implementing national climate action plans (Fransen et al., 2008). However, lack of a robust method of measuring NAMAs and the technical gaps are serious challenges in developing countries (Ellis and Larsen, 2008).

The United Nations Framework Convention on Climate Change (UNFCCC) has recommended a three-tier approach to allow for increasing level of effort and accuracy as appropriate or economically viable when estimating carbon benefits. Soil has much more variability than vegetation and therefore needs more sampling effort, which sometimes may exceed the benefits expected from the increase in stock (IPCC, 2003). Therefore developing locally calibrated models that can use easily collected data can minimize the cost of demonstrating a change in soil organic carbon stock (IPCC, 2003).

The Global Environment Facility (GEF) has called for the development of a system for measuring and monitoring carbon benefits of sustainable land management projects and natural resource management interventions. A number of carbon measurement schemes are emerging for specific applications (Lal et al., 2001). However, there is so far no comprehensive and standardized protocol for measurement and monitoring of carbon in diverse tropical agricultural landscapes that is applicable everywhere. A robust and cost effective method of measuring above- and below-ground carbon stocks would facilitate the MRV of NAMAs. Developments in soil infrared spectroscopy, which is proposed in this protocol for rapid soil carbon measurement, has the potential to improve the cost-effectiveness of measuring and monitoring soil organic carbon (SOC) stocks (Shepherd and Walsh, 2007).

The protocols presented here are a result of a number of years' work by the World Agroforestry Centre and the Earth Institute at Columbia University. They were developed within a broader framework of monitoring land health – the capacity of land to sustain delivery of essential ecosystem services – which is especially critical for food security, livelihoods, poverty alleviation, and safeguarding the environment in tropical developing countries. The methods were developed in response to the need for improved methods for measuring and monitoring the land and soil resource base at different scales, to help target and assess interventions that are designed to enhance productivity and maintain ecosystem functions. There has been a lack of application of scientific and systematic approaches to land monitoring (Young, 2000) to the degree that we do not have reliable data for planning purposes and reliable learning on outcomes.

The overall framework is Land Health Surveillance — an approach to measurement and monitoring of the health of land resource base that draw heavily on scientific principles used in public health surveillance. These principles include use of statistical sampling frames, consistent application of standardized measurement protocols, case definitions based on



population data, screening tests to rapidly diagnose cases, and synthesis through rigorous statistical analysis (e.g., Shepherd and Walsh, 2007). The Land Degradation Surveillance Framework (LDSF) is a field implementation of land health surveillance and now forms the basis for monitoring soil and vegetation condition in the Africa Soil Information Service.

Soil health and vegetation productivity are closely related to above- and below-ground carbon stocks. In most systems, without excessive biomass removals, higher vegetation productivity generally leads to greater soil carbon stocks and protects soil against erosion, while greater carbon stocks, especially in agricultural systems, generally promote good soil biological, physical and chemical properties and soil productivity. Indeed, soil organic carbon is one of the most widely used indicators of soil health. Therefore it makes sense to embed carbon measurement within broader land health surveillance schemes. On the other hand, carbon alone does not provide sufficient information to guide wise use of land resources and therefore standalone carbon measurement systems will have limited value, especially given the resources required to take the measurements. Therefore the objective of this protocol is to give guidelines for measurement and monitoring of soil organic carbon stocks within a broader land health surveillance framework. The protocol includes:

- Sampling design
- Field measurements
- Laboratory measurements
- Data analyses
- Field test of the protocol

## 1.2 Land Degradation Surveillance Framework (LDSF)

The Land Degradation Surveillance Framework (LDSF) is designed for sampling entire landscapes in order to provide baselines of land resources (e.g. soil and vegetation) and socio-economic profiles (e.g. household indicators), as well as a framework for monitoring and evaluating project interventions and their impacts on land and people. The framework is flexible and may be adapted to projects of varying size (spatial coverage) and with different objectives such as measuring land cover change, assessing soil carbon stocks and sequestration potentials, and biodiversity assessments. The LDSF is standardized and therefore can be used to compare project baselines and monitoring results over a wide range of ecosystems. This is currently not achievable in most studies and projects due to inconsistencies in measurement procedures. Also, the framework is relatively simple in that the exact same measurement procedures are followed both in baseline measurements and in monitoring and evaluation.

The LDSF uses the concept of sentinel sites, a landscape-scale sampling unit within which nested sampling designs are employed to quantify land soil characteristics at different spatial scales. The baselines are designed to be of help in project implementation by quantifying and locating priority areas; for example areas for reforestation or enrichment planting, or areas with specific biophysical constraints (e.g. soil fertility decline, soil physical degradation, etc.). The baselines can also be used to help assess whether project interventions are socially and economically acceptable or viable. There are numerous other potential applications depending on individual project objectives.

## 2 SAMPLING DESIGN

The first step is to define and bound the target area. The sentinel sites can be replicated at different scales, within projects, watersheds, administrative boundaries, countries, or even continents. For example the Africa Soil Information Service (Africa Soil Information Service, 2010) is sampling 60 sentinel sites in Sub-Saharan Africa, at randomized locations within major climate zones. For project assessment, it is often advisable to additionally sample areas outside the project so that leakage can be assessed and to provide control areas for project impact assessment.

Stratifying the area in terms of factors that influence carbon stocks will normally reduce errors associated with project-scale estimates of carbon stocks. At a continental level, climate tends to explain more variation in soil organic carbon than any other single factor (Wang et al., 2010) but locally historic land use often has a dominant influence, and this may not be well reflected by current land use (e.g. Vagen et al., 2006). Stratifying on too many variables can rapidly become un-manageable in terms of the number of strata produced and in practice it is often adequate to stratify on at most several major ecological zones. The sentinel sites are typically large enough to capture variation in conditions at the landscape scale (e.g. valley bottoms, slopes, ridgetops).

Randomizing sites within the target area and strata is important to provide unbiased estimates of carbon stocks and other land health indicators. Providing unbiased data on the statistical distribution of variables is not only useful for reporting prevalence of land health problems (e.g. low carbon stocks) but also provides a means of setting local reference values (e.g. what is low, moderate or high), which can in turn be conditioned on various factors (e.g. soil texture). A small probability sample generally provides much more useful information than a large biased sample.

The number of sentinel sites to be characterized per strata depends on the level of variability within strata in the target area, the required levels of precision and resource availability. Once an initial set of sites have been characterized the data can be used to establish the gains in precision achievable by additional sampling and to target where additional sampling could most increase precision on carbon stock estimates.

It is wise to first conduct reconnaissance of the sentinel site to plan field operations. Viewing the site on satellite images or using Google Earth can provide information on terrain and vegetation type, road access, population centre, etc. Site visits to establish permissions from local authorities or land owners and to explain the purpose of the survey to local communities are essential. If a sentinel site is inaccessible due to reasons such as insecurity or lack of access permissions, then alternative randomized locations are used.

The LDSF Sentinel sites are 10 x 10 km in size. The basic sampling unit within sites is called a Cluster. A Cluster is a 1-km radius circle within which 10 circular plots of 0.1 ha (1000 m<sup>2</sup>) are randomized (Figure 2.1).

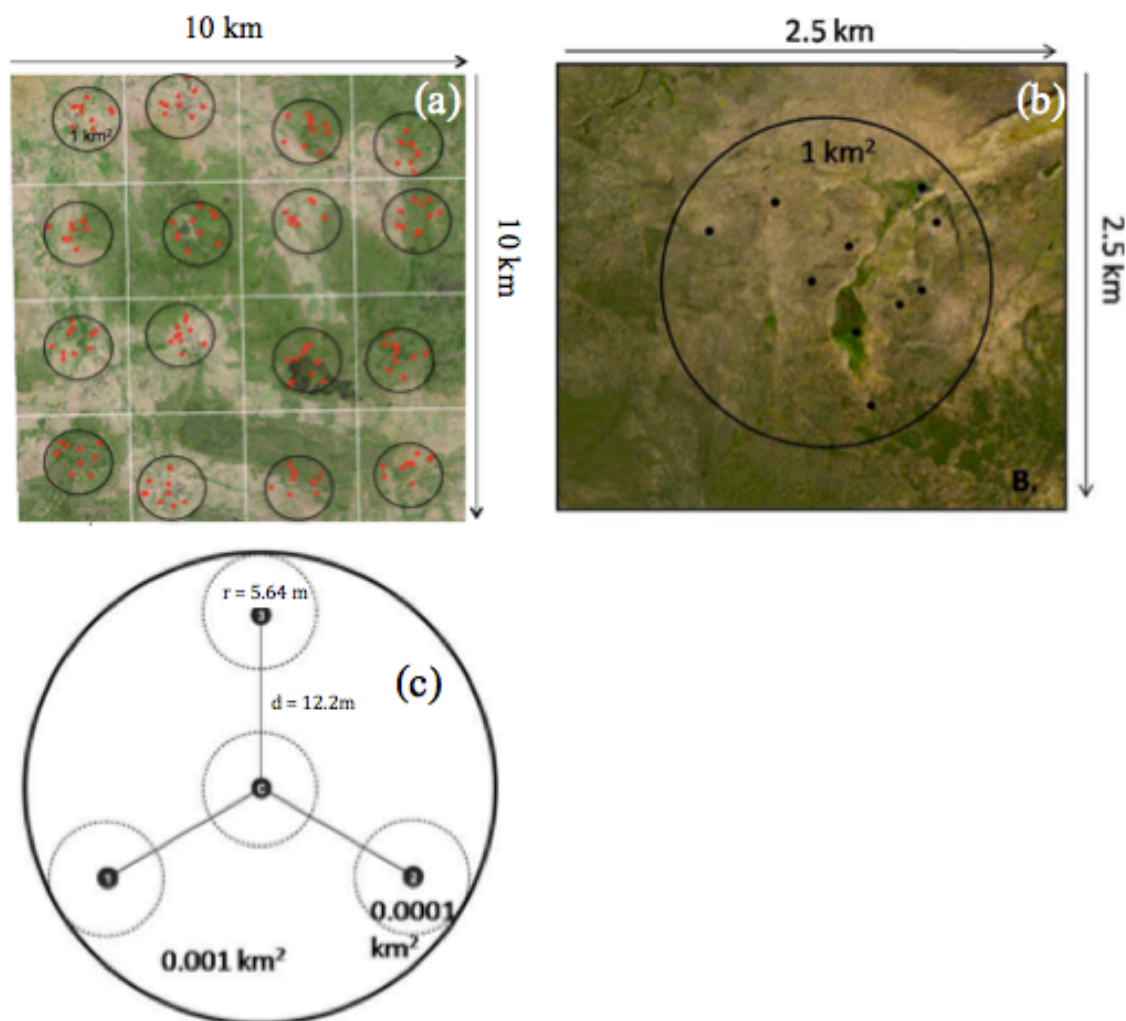


Figure 2.1 LDSF hierarchical sampling design. (a) Represents the 100 km<sup>2</sup> grid divided into 16 clusters, (b) illustrates the 1 km<sup>2</sup> clusters with 10- 0.001 km<sup>2</sup> plots, (c) illustrates how the plots are load out with 4- subplots. The background is a fine resolution, QuickBird satellite image.

The center-point of each cluster in LDSF is randomly placed within each 2.5 x 2.5 km tile in each Sentinel site and the sampling plots are randomized around each cluster center-point, resulting in a spatially stratified, randomized sampling design. Both the number of plots per cluster and the cluster size may be adjusted depending on the specific purpose of the survey being conducted. For example, 1 km<sup>2</sup> clusters are useful for large-area reconnaissance surveys; whereas, 0.1 km<sup>2</sup> (10 ha) clusters may be more appropriate for more detailed project-level surveys. There is in other words a high degree of flexibility as long as randomization is maintained and samples are collected using a nested design (i.e. Plot within Cluster within Site). Randomizing the plots in the cluster is extremely important to minimize any local biases that may arise from convenience sampling. The randomization procedures are done using customized programs or scripts ([www.africasoils.net](http://www.africasoils.net)), but may also be done in any common spreadsheet program and can be downloaded to a Global Positioning System (GPS) so that field crews can navigate to the sampling points. A consistent projection system should be used to enable plots to be revisited.

On each plot, detailed observations and measurements describing land and vegetation cover and soil condition are recorded, following the guidelines provided in the LDSF guide to field sampling and measurement procedures (Africa Soil Information Service, 2010).

## 2.1 Sample size determination and sample allocation

### 2.1.1 Sample size determination

The number of plots required to estimate SOC stocks depends on the desired precision. Optimal size does not necessarily guarantee the desired precision of carbon estimate unless it is complemented with a proper unbiased sampling design. The number of plots required to measure carbon stocks is often within a precision level of  $\pm 10\%$  of the mean SOC stocks at 95% confidence level (Boscolo et al., 2000). The number of samples needed for a given area can be calculated using Equation 1 (Pearson et al., 2005).

$$n = \frac{(N \times S)^2}{\frac{N^2 \times E^2}{t^2} + (N \times S^2)} \quad (\text{Eq. 1})$$

Where:  $n$  = number of plots

$E$  = allowable error. Calculated by multiplying the mean carbon stocks by the desired precision (that is, mean carbon stock  $\times 0.1$ , for 10% precision)

$t$  = the sample statistic from the  $t$ -distribution for the 95% confidence interval;  $t$  is usually set at 2 as sample size is unknown

$N$  = number of sampling units in the population

$S$  = standard deviation of stratum

#### Example

Taking the mean and standard deviation of SOC stocks of the five sentinel sites in western Kenya (see Section 8), the number of plots needed to report SOC stocks with a precision level of  $\pm 10\%$  of the mean at 95% confidence level is calculated as follows.

Area of sentinel site	= 10,000 ha
Plot size	= 0.1 ha
Mean SOC stock	= 21.53 t C ha <sup>-1</sup>
Standard deviation	= 13.62
Precision	= 10%
$N$	= 10,000/0.1 = 100,000
$E$	= 21.53 $\times$ 0.1 = 2.15
$t$	= 2

$$n = \frac{(100,000 \times 13.62)^2}{\frac{100,000^2 \times 2.15^2}{2^2} + (100,000 \times 13.62^2)}$$

= 160 plots

### 2.1.2 Sample allocation

If we have a fixed number of plots they can be allocated to each stratum based on the principle of optimum allocation (Eq. 2). In this case, observations are allocated to the strata so as to give the smallest standard error possible with a total of  $n$ - observations Freese (1984).

$$n_h = \frac{N_h \times S_h}{\sum_{h=1}^L N_h S_h} \times n \quad (\text{Eq. 2})$$

Where:  $N_h$  = size of stratum  $h$   
 $s_h$  = standard deviation in stratum  $h$   
 $L$  = number of stratum

**Example**

The number of plots required for stratum 1 (Table 2.1) is calculated as follows:

$$n_{\text{stratum 1}} = \frac{50 \times 12.64}{(50 \times 12.64) + (30 \times 18.91) + (20 \times 24.97)} \times 160$$

$$n_{\text{stratum 1}} = 60$$

Similarly, the number of plots required for the other strata are calculated and summarized in the following table

Table 2.1 Example data for sample allocation

Site	Area (km <sup>2</sup> )	Standard deviation for SOC stock	Number of plots
Stratum 1	50	12.64	60
Stratum 2	30	18.91	53
Stratum 3	20	24.97	47
<b>Total</b>	<b>100</b>		<b>160</b>

### 3 FIELD MEASUREMENTS

The following field measurement guide is extracted from the Land Degradation Surveillance Framework (LDSF) field guide, which we recommend using for the field measurements. The LDSF field guide is available at [www.africasoils.net](http://www.africasoils.net). The LDSF includes measurement of tree and shrub densities, vegetation structure, visible erosion, and infiltration capacity among other environmental variables, in addition to collecting soil samples. While this protocol just describes soil sampling, it is recommended to collect multiple environmental variables at the same point in time.

#### 3.1 Preparation for a field work

It is important to consider the following points before commencing fieldwork:

- Proper preparation before going to the field is critical to ensure a successful field sampling campaign, and for the safety and well-being of the field team. Prior to any field campaign, it is important to have a good understanding of the area to be surveyed, including its topography, climate and vegetation characteristics, accessibility, and its security situation
- Collate existing information about the area to be surveyed including: maps (topographical, geological, soils and/or vegetation), satellite images and/or historical aerial photographs, long-term weather station data, government statistics, census data etc.
- Load coordinates of sampling locations into the GPS units before going to the field. If possible, load local maps into the unit to aid in navigation in the field
- Do a thorough equipment check before leaving for the field
- When conducting field campaigns in new countries it is generally recommended that a reconnaissance survey is conducted where local contacts are established and agreements made.
- Obtain permission from the land owner(s) to sample a given area, and make sure that he/she understands what you are doing. Inform local government officers and community leaders about your activities.

The following equipment and supplies are recommended for field crews:

- GPS and extra batteries
- Clinometer
- Notebook computer
- Digital camera
- Sheet holder
- Paint or ribbon for marking plot center
- A soil auger
- Buckets
- The metal sampling plate
- Sample tags, bags, and a permanent marker
- Measuring tape
- Builder's sand
- 2-mm sieve
- Graduated cylinder
- Mixing trowel
- First aid kit

### 3.2 Laying out the plot

The LDSF field measurement protocol is implemented at the level of the 1000 m<sup>2</sup> plot.

- Using a measuring tape or a pre-marked chain, measure out the distance (12.2 m) from the plot centre-point (Figure 3.1a) to the centre of the up-slope sub-plot. Distance should be corrected when plots fall on steep terrain (see Section 3.3.2).
- Mark this sub-plot (2) centre point.
- Sub-plots 3 and 4 should be offset 120 and 240 degrees from the up-slope point, respectively. The angles can be measured using a compass or the sampling plate can be marked and used to locate Sub-plots 3 and 4 (Figure 3.1b).

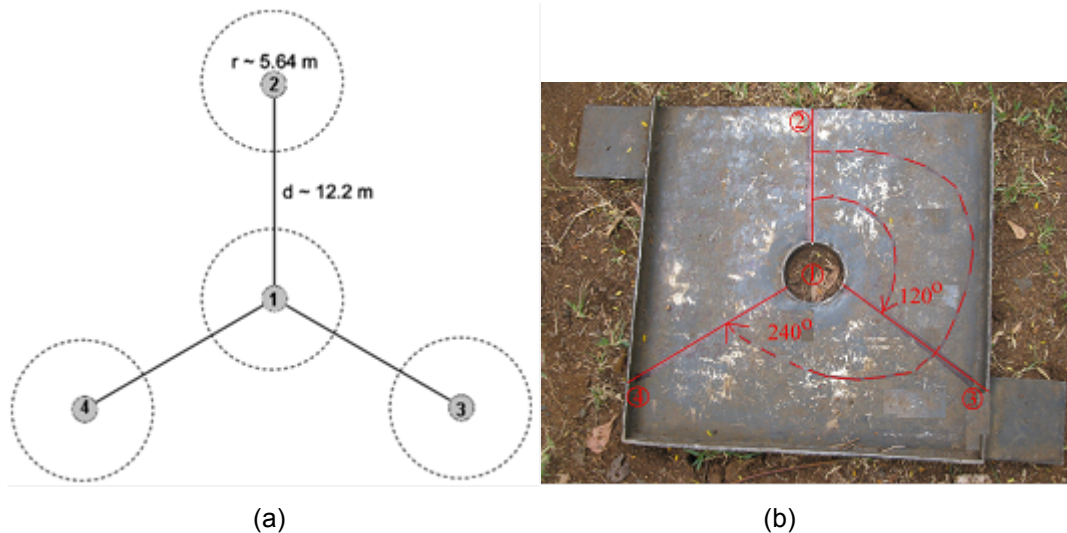


Figure 3.1 (a) Sampling plot layout, with the four subplots (dotted circles). Sub-plots have a radius of 5.64 m (area = 100 m<sup>2</sup>), and the distance along the radial arms between subplot centres is 12.2 m. The whole plot has a radius of 17.84 m (area= 1000 m<sup>2</sup>). (b) Cumulative mass soil sampling plate showing the angles at which to locate the sub-plots. The angles can be measured using a compass or the sampling plate can be marked and used to locate sub-plots 3 and 4.

### 3.3 Plot level measurements

#### 3.3.1 Georeferencing

At the plot level, basic site characteristics are described and recorded. Initially, georeference the centre of the plot by letting the GPS average the position for at least 5 minutes. Store this as a waypoint in the GPS, and record the easting (longitude), northing (latitude), elevation and position error on the field-recording sheet.

#### 3.3.2 Slope

Stand in the centre of the plot and take an up-slope sighting along the steepest part to a point on the up-slope plot boundary. Use a clinometer to measure the slope in degrees. Repeat the process in the downslope direction. Ensure that you sight to a location that is at the same height as the observer's eye-level.

In steep terrain (slope > 10%), use the following formula to calculate the distance from the centre-point to the other sub-plots:

$$Ls = \frac{L}{\cos(S)} \quad (\text{Eq. 3})$$

Where: *Ls* = slope distance  
*L* = horizontal distance  
*S* = slope angle in degrees

### 3.3.3 Soil sampling

Two types of soil samples are collected at each plot: composite soil samples and cumulative mass soil samples. Composite soil samples are considered a representative sample of the plot and used for analysis of carbon. Cumulative mass soil samples are collected to estimate bulk density of the soil.

#### 3.3.3.1 Composite soil sampling

Top- and subsoil samples are collected from the center of each subplot at 0-20 cm and 20-50 cm depth increments, respectively. Top soil subplot samples are pooled (composited) into one sample for each plot, the same is done with subsoil samples.

Field sampling procedure is as follows:

- Collect topsoil (0-20 cm) and subsoil (20-50 cm) samples from the center of each subplot using an auger. Place each sample into a separate bucket.
- Pool (composite) topsoil samples from each subplot into one bucket. Mix soil in the bucket thoroughly. The same is done for the subsoil
- Take a representative ~700 g subsample of the topsoil and place it in a plastic bag. Label the bag. The same is done for the subsoil.
- Auger depth restrictions are recorded at each sub-plot (in cm), if present.

Sample labeling: Site Name, Cluster, Plot, Depth

**Note that there should be one bag of the topsoil and one bag of the subsoil for each plot.**

#### 3.3.3.2 Cumulative mass soil sampling

To estimate the soil carbon stock, bulk density should be measured for each depth and plot (IPCC, 2003). Bulk density is the mass of oven-dry material per unit volume of soil in its natural undisturbed states. Its value mostly ranges from 1.0 to 1.8 g cm<sup>-3</sup> for mineral soils (Dewis and Freitas, 1970). Bulk density is usually estimated by taking undisturbed soil samples using a core sampler. However, in landscape level studies this method is often impractical due to the tediousness of this method, limiting repeatability across landscapes. Undisturbed samples are further complicated if stones are present, if stones are present, when sampling 2:1 clay soils that have shrink-swell characteristics, and lack of cohesion in sandy soils. It is also impractical to attempt to use different methods in different soil types. In this protocol bulk density is estimated by recovering soil from augered samples and determining the soil mass per unit volume augered. Since bulk density is generally less variable than carbon concentration, it can be determined at the centre subplot only. The cumulative mass soil samples require different processing than the regular (composite) samples. The total air-dry weight of the sample is determined as well as the weight of the coarse fraction (> 2 mm) and the oven-dry moisture



content is determined on a subsample. Laboratory processing steps are given in Section 4.1.2 and calculation procedures in Section 5.2.

Depending on soil texture, a clay, combination or sand auger can be used, but use the same auger for the entire depth (do not switch augers as this may change the volume of soil collected). A sampling plate (Fig. 3.1b) is used as an auger guide, to prevent collapse of the hole near the surface, and to aid full recovery of the soil sample.

Field sampling procedure is as follows:

- Press the sampling plate firmly onto the soil, so the sheet is flush with the soil surface. Stand on either side of the plate to further secure it.
- Place the auger in the center of the hole and begin to auger straight down, using the same auger for all depths (Figure 3.2a). If your augering becomes crooked, please stop and start a new hole, as this will give an inaccurate measurement of the depth.
- Auger down to 20 cm depth and transfer all of the soil from the auger and any soil that fell onto the sampling plate into the bucket.
- Transfer all of the soil to a labeled plastic bag.
- The next sample is from 20-50 cm and 50-80 cm.
- Auger depth restrictions are recorded, if present.

Sample labelling: Site Name, Cluster, Plot, CM, Depth (e.g. 0 – 20 cm) (CM is meant to indicate a cumulative mass soil sample).

If the soil is very dry, it may be difficult to auger and collect all of the soil from the depth increment, in which case pre-wetting the soil before augering each increment may be helpful. If you wet the soil be sure to double bag the sample and do not place the sample label tag in the bag with the wet soil, as the tag will stick to the soil and make it difficult for processing in the lab. Sampling points should be 1 m distance from tree stems and should avoid disturbances like animal holes, trails.

During soil sampling the mass of soil was determined for each depth and the volume of the auger hole can be calculated using auger diameter and soil depth (see Section 5.2). The volume of the auger hole can be calibrated using the sand back-filling method (Figure 3.2b). With this method, the volume of the auger hole is checked by back filling with sand. First you sieve a quantity of dry sand through a 2 mm sieve, and then fill it into a graduated cylinder (Figure 3.2b). Then you pour the sand into the auger hole until flush with the top of the soil surface and record the volume of sand required to do so. Sample processing methods used are described in the laboratory methods section.



(a)



(b)

Figure 3.2 (a) Field soil sample collection and (b) determining auger-hole volume using sand filling method.

## 4 LABORATORY MEASUREMENTS

### 4.1 Soil sample processing

All soil processing procedures are described in the AfSIS standard operating procedure for soil processing ([www.africasoils.net](http://www.africasoils.net)) and are only summarized here.

#### 4.1.1 Composite soil samples

##### Drying and sieving

- Air-dry composite soil samples (collected from the four sub-plots per plot) by spreading a sample out as a thin layer into a shallow tray. Drying can be done in large room, a custom-made solar dryer, or a forced-air oven at 40° C.
- Break up clods as far as possible to aid drying. It is important to ensure that no material from a sample is lost or discarded as weights of soil fractions are to be recorded on processing. Contamination from dust, plaster or other potential contaminants should be avoided. Drying time depends on the samples and ambient conditions, but the samples should be thoroughly dry (i.e. constant weight)

##### Weighing and sieving

- Weigh the whole dried soil sample to 0.1 g using a calibrated top-pan balance and record the weight.
- Using a wooden rolling pin, gently crush the sample to pass through a 2 mm mesh size sieve. While crushing, remove any plant materials (e.g. roots) and any possible pieces of gravel (making sure they are gravel and not soil aggregates) and place in a separate pile (the coarse fraction).
- Pass the crushed sample through the 2 mm sieve. DO NOT use the sieve as a grinder; i.e. do not rub or mash the soil on the sieve, but shake the sieve gently to allow the soil to pass through.
- Once the entire sample has been sieved, weigh and record the coarse fragments (> 2 mm).

Note: The whole sample should be processed and no material should be discarded. You will remain with two fractions:

- The coarse fraction (>2 mm), which cannot pass through the sieve.
- The soil fines (<2 mm), which have passed through the sieve.
- The weight of the fine fraction is calculated by subtracting the weight of the coarse fragments from the total air-dried soil sample.

##### Subsampling of fine fractions

- If the weight of the soil fines is much greater than 350 g, subsample the soil fines using coning and quartering (see below) or a sample divider (riffle box) to give about (not less than) 350 g of soil.

**Coning and quartering procedure**

Use a large cleaned surface or heavy-duty plastic sheeting. Thoroughly mix the soil sample and spread the sample into a conical pile. Further mix the soil by circumventing the cone symmetrically, repeatedly taking a spatula-full of soil from the base and transferring the soil to the apex of the cone. Ensure the spatula is large enough to reach to center of the cone. Circumvent the cone twice.

Flatten the cone to a height of about 1 cm. Use a flat spatula or ruler, divide the pile into quarters along two lines intersecting 90° to each other. Select one pair of opposite quarters as the sample to be retained. If the sample is still too large then repeat the procedure from the beginning.

- Continue the coning and quartering technique on all samples to obtain a representative 20 g subsample for laboratory analysis.
- Place the remaining 350 g sample of soil fines into a strong Size 5 khaki paper bag and send it to soil laboratory. Excess soil fines should be stored in a labeled bag in case further analyses are done later.

**4.1.2 Cumulative mass soil samples**

The cumulative mass samples are processed the exact same as the composite soil samples. In addition, the gravimetric moisture content on a subsample is determined in order to calculate the actual oven dried mass of each sample, and is described below.

- Air-dry cumulative mass soil
- Weigh the entire dried soil sample to 0.1 g and record the weight
- Determine gravimetric moisture content (Eq. 4).
  - Weigh a labeled sample **tin** for taking oven-dry moisture content and record weight
  - Take an approximately 350 g representative subsample of the original sample and place it into the weight sample tin and record weight of **tin + air-dried soil**
  - Place tin+air-dried soil into oven at 105 °C until a constant weight is obtained ~48 hrs.
  - Once soil is dry, weigh the **tin + oven-dried soil** (record the weight).
- Gently crush the entire sample to pass through a 2 mm diameter
- Pass the crashed sample through the 2 mm sieve
- Weigh the coarse fragments (> 2 mm)
- The weight of the fine fraction is calculated by subtracting the weight of the coarse fragments from the total air-dried soil sample.

These data will be used to calculate the oven-dried weight of the original cumulative mass soil

$$\theta_g = \frac{\text{mass of water}}{\text{mass of oven dried soil}} \quad (\text{Eq.4})$$

**Example**

A soil sample taken from top 20 cm weighed 1091.3 g (Table 5.1: Stratum 1, plot 1) and representative soil sample weighed 350 g (including tin) was taken to determine soil moisture content. The weight of the tin was 90 g. After drying the soil at 105 °C to a constant weight, the soil and tin weighed 320 g. The gravimetric moisture content and the oven-dry mass of the cumulative soil mass are calculated as follows:

Mass of air-dried soil sample = 350 – 90 = 260 g

Mass of oven dried soil sample = 320 – 90 = 230 g

$$\theta_g = \frac{260 - 230}{230} \times 100$$

$$= 13\%$$

13% of the original soil sample is moisture and the remaining 87% is oven-dry soil mass. The oven-dry soil mass of the original sample is calculated as follows:

$$1091.3 \times (1 - 0.13) = 948.9 \text{ g}$$

**4.2 Soil carbon analysis**

Total carbon and organic carbon concentration ( $\text{g kg}^{-1}$ ) are determined on soil reference samples only, by thermal oxidation (Skjemstad and Baldock, 2008) using a carbon analyzer according to Standard ISO 10694: Soil quality - Determination of organic and total carbon after dry combustion (elementary analysis). Soil carbon is determined on total and acidified samples, i.e. fumigated with hydrochloric acid to remove inorganic carbon (carbonate) (Harris et al., 2001). Inorganic carbon is estimated as the difference between unacidified and acidified carbon.

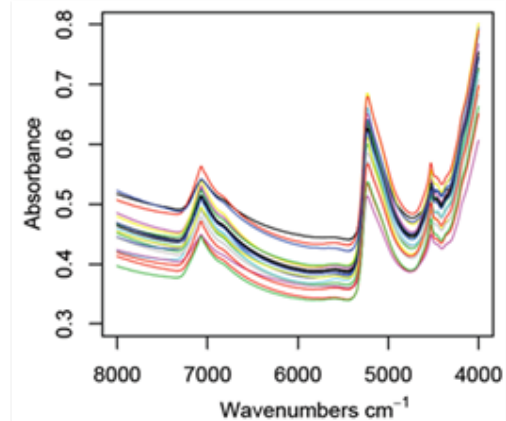
The World Agroforestry Centre's standard operating procedure for carbon analysis in soils and plants uses the Thermal Scientific FlashEA 1112. It is based on the flash dynamic combustion method, which produces complete combustion of the sample within a high temperature reactor, followed by an accurate and precise determination of the elemental gases produced using a thermal conductivity detector. A complete standard operating procedures for carbon analysis in soils and plants using the Thermal Scientific FlashEA 1112 can be found at [www.africasoils.net](http://www.africasoils.net).

**4.3 Soil infrared spectroscopy**

Diffuse reflectance infrared spectroscopy (IR) is an established technology for rapid, non-destructive characterization of the composition of materials based on the interaction of electromagnetic energy with matter (Figure 4.1). IR is now routinely used for analyses of a wide range of materials in laboratory and process control applications in agriculture, food and feed technology, geology and biomedicine (Shepherd and Walsh, 2007). Both the visible near infrared (VNIR, 0.35-2.5  $\mu\text{m}$ ) and mid infrared (MIR, 2.5-25  $\mu\text{m}$ ) wavelength regions have been investigated for non-destructive analyses of soils and can potentially be usefully applied to predict a number of important soil properties, including: soil colour, mineral composition, organic matter and water content (hydration, hygroscopic, and free pore water), iron form and amount, carbonates, soluble salts, and aggregate and particle size distribution (Shepherd and Walsh, 2004). Importantly, these properties also largely determine the capacity of soils to perform various production, environmental and engineering functions. Infrared spectroscopy enables soil sampling density (samples per unit area) to be greatly increased with little increase in analytical cost.



(a)



(b)

Figure 4.1 Soil spectral analyses: (a) the use of FT-NIR and (b) representative absorbance soil spectra.

To guard against prediction failure we recommend a two-phase sampling procedure whereby all sampled soils are scanned using IR and a subset of samples (e.g. 10%) is selected for reference analysis using conventional laboratory procedures. For IR prediction of soil organic carbon MIR generally outperforms NIR by more than 10% increase in prediction accuracy, and can be used with small sample sizes, however a further fine-grinding step is required for MIR.

At the present time we recommend use of laboratory-based infrared spectrometers that have in-built standards and validation software to ensure stability in measurements over time. Consistency in sample preparation and presentation is also important for achieving reproducible results. Full standard operating procedures for VNIR, FT-NIR and FT-MIR are available at [www.africasoils.net](http://www.africasoils.net).

## 5 DATA ANALYSIS

### 5.1 Spectral libraries

Shepherd and Walsh (2002) proposed a scheme for the use of spectral libraries as a tool for building risk-based models for soil evaluation (Figure 5.1). This approach is intended to safeguard against prediction failures, and generalize results of soil assessments that are conducted at a limited number of sites to a wider population of samples. At the heart of this process is a classic two-phase or double sampling strategy as follows: sampling the Independent (Spectral) Phase: the variability of soils in a given study area is initially sampled thoroughly. A large sample of size  $m$  is drawn from a population of size  $M$ . This is potentially the most critical step in building a soil reflectance library, as it determines how well the library will represent the target soil population. In the absence of additional information from soil maps, digital terrain models and/or remote sensing data, spatially stratified random sampling can often be fairly efficient in this regard (see, Webster and Burgess, 1984). For this initial sample only the spectral measurements  $x_{ij}$  ( $i = 1 \dots \text{no. wavelengths}; j = 1 \dots m$ ) are obtained. Sampling the Dependent Phase: once the spectral variation of a target population has been thoroughly sampled, the more time consuming and/or expensive soil properties (e.g. soil carbon) are measured on a subset of soils ( $n < m, k = 1 \dots n$ ). Depending on the specific application, a variety of sub-sampling schemes may be used here, ranging from equal probability to stratified- or design-based random sampling approaches. The second stage sample provides estimates of the parameters for the dependent phase including the mean and variance of the reference soil property under consideration.

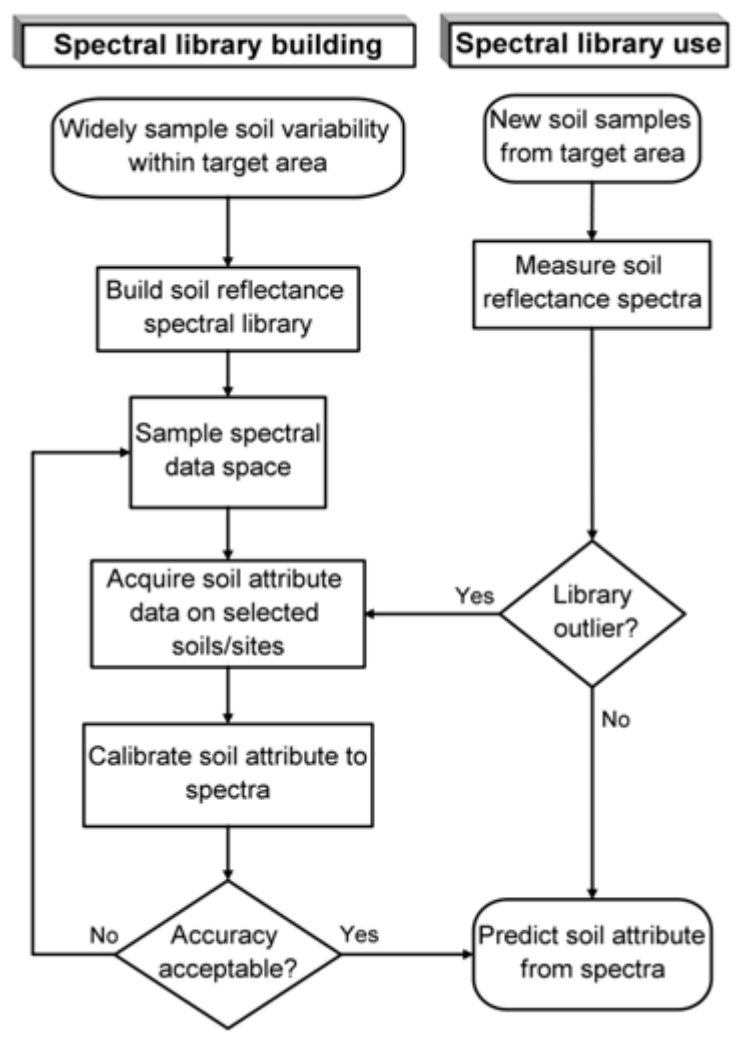


Figure 5.1 Logical scheme for use of reflectance spectral libraries in a risk-based approach to prediction of soil functional attributes. Source: Shepherd & Walsh (2002).

For selection of calibration samples when using the LDSF sampling scheme, we recommend selecting topsoil and subsoil samples of Plot 1 from each cluster within a site to give a spatially-stratified random sample (see soil processing SOP at [www.africasoils.net](http://www.africasoils.net)). Selecting both topsoil and subsoil samples for carbon and other reference measurements provides for fitting a function of concentration with depth and calibrating the parameters of the concentration-depth function to IR spectra.

### 5.1.1 Data pre-treatment

Various signal processing or spectral data pretreatments, such as smoothing and filtering, transformation, standardization, and numerical treatments are used to improve signal-to-noise-ratio, correct for light scattering, convert data into more physically meaningful form, and extract meaningful or useful information before calibration. First derivative processing and smoothing have found to be generally optimal for calibration of many soil properties. Wavelet transforms have shown promise as a way to simultaneously optimize soil spectral information, reduce data volume and solve multicollinearity problems (e.g. Ge et al., 2007; Viscarra Rossel and Lark, 2009).

Transformation of the y-variable is usually also necessary to obtain normally distributed data in order to satisfy the assumptions of parametric methods and to help minimize non-linearity in calibrations. Soil element concentrations are typically highly skewed due to a low frequency of large values. Performance statistics are calculated on the back-transformed values.

### 5.1.2 Spectral calibrations

Sampling of the dependent phase is followed by a calibration step, which describes the relationship between the reference property ( $y$ ) and the multivariate spectral signal ( $x_k$ ), for example in linear form (Eq. 5):

$$y = b_0 + \sum_{k=1}^k b_k \times x_k + f \quad (\text{Eq. 5})$$

Where:  $b_0$  &  $b$  = regression coefficients  
 $k$  = the number of x-variables  
 $f$  = the y-residual

Commonly used calibration methods include multiple linear regression (MLR), principal components regression (PCR) and partial least squares regression (PLS) (Martens and Martens, 2001; Naes et al., 2002). PLS and PCR are similar in that both employ orthogonal linear combinations of wavelengths to overcome the problem of high-dimensional, correlated predictors (multicollinearity) (Martens and Naes, 1989). PLS, the most widely used calibration method in infrared spectroscopy, orientates the components to the y variable.

Guidelines on treatment of calibration outliers are given by Naes et al. (2002). Only influential outliers are normally of concern, i.e. those with large leverage (distance in x-space) and large y-residuals.

Both PCR and PLS are now available in most standard statistical packages (e.g. Genstat, S-Plus, SAS, R) as well as in more specialized “chemometric” software packages, such as The Unscrambler® (Camo Inc), Matlab (The Mathworks™), PLS-Toolbox (Eigenvector Research Inc), and ParLes (Viscarra Rossel, 2008). Additionally, non-linear regression methods (e.g. generalized additive models and regression splines, local PLS), and non-parametric classification and regression methods (e.g. classification and regression trees, neural networks, support vector machines, genetic algorithms) have also been successfully used in past soil reflectance studies.

### 5.1.3 Validation

Regardless of the specific technique employed, the most important aspect in developing robust predictive models is to ensure that model validation matches the intended model use. Model validation in this context simply means checking how well the model will perform in predicting new data. The simplest measure of the uncertainty on future predictions is the root mean square error of prediction (RMSEP). This value expresses the average uncertainty that can be expected when predicting the response-values for new samples (see Naes et al., 2002). RMSEP is valid, provided that the new samples represent an independent sample of the population under consideration; otherwise, the actual prediction errors might be much higher. In this case, the term “independent” refers to the notion that knowing something about the validation samples would not be helpful in predicting the response-values of the calibration samples. Soil samples taken in close proximity to one another or at different depths in the same soil profile are typically not independent of one another and their inclusion in both calibration and validation sets can lead to over-optimistic validation performance.

Other metrics for evaluating prediction performance commonly used include the ratio of prediction to standard deviation (RPD) and the ratio error range (RER). These are calculated as (a) the standard deviation of the reference measurements in the validation set, or (b) the



range of the reference measurements in the validation set, divided by the standard error of prediction, respectively. Guidelines on interpretation are given by Malley et al. (2004). Standards for multivariate calibration are given in Standard Practices for Infrared Multivariate Quantitative Analysis (ASTM E1655-05) and Standard Practice for Validation of Empirically Derived Multivariate Calibrations (ASTM E2617-08a).

Cross-validation is commonly used to evaluate calibration model performance and prevent over-fitting, however, cross-validation does not substitute for use of independent validation sets in evaluating model performance. Statistical re-sampling or ensemble techniques such as bootstrap aggregation (or bagging) can also been employed to improve prevent over-fitting, stabilize models and improve prediction accuracy (e.g. Brown et al., 2006; Viscarra Rossel, 1997).

#### **5.1.4 Spectral data handling**

Standard operating procedures for management and storage of spectral data are specified in the Standard Operating Procedures for Spectral Data Management ([www.africasoils.net](http://www.africasoils.net)). Spectra names contain the unique sample identifier used during sample logging so that they can be matched with reference measurement data and field data in the relational database.

The R statistical language and environment (R Development Core Team, 2008) is used to enable easy access to spectral processing and statistical analysis routines R scripts are under continuous development but at the time of writing scripts are available in the R package “soil.spec” on the CRAN server (<http://cran.r-project.org/web/packages/soil.spec/index.html>) for:

- Importing spe-format files
- Principal Component Analysis (PCA)
- Sample selection using the Kennard-Stone algorithm
- Spectral transformation
- Comparison of regression models

### **5.2 Calculation of soil organic carbon stocks**

Soil organic carbon stock can be expressed on an equal mass or equal volume basis. To express changes in soil carbon stocks on an equal mass basis requires that the change in the soil bulk density. Estimates of soil carbon stocks to a fixed depth using single depth bulk density are mostly biased due to the spatial and temporal variability in bulk density (Lee et al., 2009). Despite the high carbon concentration in the top soil, (20 cm) the carbon density is often less than in the sub-soil due to lower soil mass (bulk density) in the top soil than in deeper soil layers. The variability in bulk density with depth can be addressed by establishing relationship between cumulative soil mass and volume (see 8.2). It is likely that projects designed to enhance soil organic carbon (e.g. afforestation) will also cause the soil bulk density to decrease. If it is expected that the soil bulk density will change significantly during the course of the project, it is recommended to assess the impact of expressing the changes in soil carbon on an equal mass or equal volume basis on the total projected change in soil carbon stocks (IPCC, 2003).

To calculate soil organic carbon stocks on an equal mass basis, three types of variables must be measured: concentration of soil organic carbon, bulk density, and soil depth.

#### **Soil organic carbon concentration**

Data on soil carbon concentration ( $\text{g kg}^{-1}$ ) can be obtained from laboratory measurements. It can be also estimated using IR spectroscopy and remote sensing data sources.

#### **Bulk Density**

Bulk density is calculated from oven-dry weight of soil from a known volume of sampled soil (Eq. 6).

$$\rho = \frac{M}{V} \quad (\text{Eq. 6})$$

Where:  $\rho$  = bulk density ( $\text{g cm}^{-3}$ )  
 $M$  = oven-dry weight of soil (g)  
 $V$  = volume of soil ( $\text{cm}^3$ )

**Example**

Assume a dry soil sample taken from a 20 cm depth weights 901.1 g and soil volume of 907  $\text{cm}^3$  (Table 5.1: stratum 1, plot 1), the bulk density of the sample is calculated as follows:

$$\begin{aligned} \rho &= \frac{901.1}{907} \\ &= 0.99 \text{ g cm}^{-3} \end{aligned}$$

Note: Auger diameter of 7.6 cm (radius 3.8 cm) is used to calculate soil volume  
 $(V = \pi \times 3.8^2 \times 20 = 907)$

Soil carbon stock for a given soil layer is calculated by multiplying the carbon concentration in soil fines with bulk density and soil depth (Eq. 7).

$$SOC = \frac{C}{100} \times \rho \times D \times (1 - frag) \times 100 \quad (\text{Eq. 7})$$

Where: SOC = soil organic carbon stock ( $\text{t C ha}^{-1}$ )  
 $C$  = soil organic carbon concentration of soil fines (fraction < 2 mm) determined in the laboratory (%  $\text{g kg}^{-1}$ )  
 $\rho$  = soil bulk density ( $\text{g cm}^{-3}$ )  
 $D$  = depth of the sampled soil layer (cm)  
 $frag$  = % volume of coarse fragments/100  
 100 is used to convert the unit to convert unit to  $\text{t C ha}^{-1}$

Note: SOC is determined on the fine soil fraction (< 2 mm) and the bulk density should be corrected for the proportion of the soil volume occupied by coarse fragments (> 2 mm)

**Example**

Soil carbon stock for stratum 1, plot 1 (Table 5.1) is calculated as follows.

$$\begin{aligned} SOC &= \frac{1.14}{100} \times 1.05 \times 20 \times \left(1 - \frac{5}{100}\right) \times 100 \\ &= 22.67 \text{ t C ha}^{-1} \end{aligned}$$