Chapter 7

Methods for Smallholder Quantification of Soil Carbon Stocks and Stock Changes

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Abstract Smallholder agricultural systems in tropical and subtropical regions may have significantly contributed to greenhouse gas emissions (GHG) over the past number of decades. As a result, these systems currently offer large GHG mitigation potentials (e.g. soil organic carbon (SOC) sequestration), which can be realized through the implementation of good management and sustainable agricultural practices. In this chapter we synthesize current available methodologies designed to assess SOC stocks and stock changes. From this analysis, it becomes apparent that the design and subsequent implementation of any quantification and monitoring scheme envisaged for studies focusing solely on the soil component greatly differs from those developed for whole ecosystem accounting, not just in its approach, but also in the amount of resources needed to implement it within a given degree of accuracy. We provide analyses and recommendations on methods specifically dealing with quantification and assessment of SOC at both the individual farm and the landscape scale in smallholder agricultural systems.

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7.1 Introduction

Agricultural activities are responsible for about one third of the World's GHG emissions and this share is projected to grow, especially in developing countries (IPCC, 2007). Indeed, smallholder agricultural systems are highly dynamic and heterogeneous environments that may have significantly contributed to greenhouse gas (GHG) emissions over the past number of decades (Berry, 2011). Furthermore, these systems traditionally suffer from severe soil organic matter depletion due to intense decomposition following soil ploughing, the removal of most of the aboveground biomass during harvest, and the enhanced soil erosion inherent to those activities. Yet, they may also offer large mitigation potentials through the implementation of good management and sustainable agricultural practices, particularly through improvements in land-use management, as nearly 90% of IPCC-identified technical potential lies in enhancing soil carbon sinks (Lipper et al., 2011).

A number of methodologies are currently available for the quantification of carbon stocks in terrestrial ecosystems, varying widely in terms of accuracy, scale and resources needed for their implementation (e.g. Pearson et al., 2005; Ravindranath and Ostwald, 2008; Hairiah et al., 2010). While nearly all the schemes feature soil as a component of the total carbon pool, the number of methods specifically designed to assess soil organic carbon (SOC) stocks and stock changes are considerably more limited (Table 1). This is despite the wide acknowledgement that many ecosystem services are strongly correlated with SOC levels, and their huge importance for sustaining local livelihoods. The design and implementation of any quantification and monitoring methodology for studies focusing solely on the soil component may greatly differ from those developed for whole ecosystem accounting, not only in approach or the accuracy but also in necessary resources. Therefore, it is justified to develop methods that can effectively deal with soil carbon quantification and monitoring for a given accuracy within the available budget. In the present work we focus on the soil component and provide analyses and recommendations for methods to quantify SOC in smallholder agriculture in tropical environments.

The SOC inventory in a given soil profile is controlled by the complex interaction of many factors, including climate, soil texture, topography, fire frequency, land use and land management (Bird et al., 2001; Saiz et al., 2012). These drivers exert contrasting influences on SOC stocks at different spatial scales. At the local scale, biotic factors and management activities play a fundamental role in affecting the quantity and quality of carbon inputs and decomposition processes, while at larger scales the variation in SOC stocks is mainly controlled by topographic, edaphic and climate related factors (Giardina and Ryan, 2000; Wynn and Bird, 2007; Allen et al., 2010; Saiz et al., 2012).

Given the inherent high spatial variability of SOC, accurate quantification and monitoring of SOC stocks and stock changes is a complex task even in relatively homogeneous ecosystems. This complexity is further exacerbated in smallholder environments by the existence of multiple land use activities occurring at various management intensities. Moreover, sources of uncertainty and suitable levels of precision and accuracy differ when working at the landscape scale as opposed to the farm scale because biogeochemical processes affecting SOC dynamics

operate and interact at different spatial scales (Veldkamp et al., 2001; Milne et al., 2013). Therefore, efficient sampling designs are needed across smallholder agricultural systems to ensure that SOC stocks and stock changes can be detected at various scales for a given accuracy and at minimum costs (Milne et al., 2012; Singh et al, 2013). Chap. 2 and Chap. 4 in this book, provide some critical discussions on sampling designs specific to smallholder contexts. These chapters deal with systems characterization and targeting, and determination greenhouse gas emissions and removals associated with land use and land cover change.

In the present work, we propose an integrated field based approach for smallholder systems that encompasses estimates of SOC stocks and stock changes both at farm and landscape scales over a wide range of land use management intensities.

Table 1 Comparative analysis of methods for smallholder quantification of SOC stocks and changes with regard to level of accuracy, scale, resources demanded, and land covers considered

Method / Quantification Approach	Accuracy and precision		Scale		Resources demanded				Land covers considered				
	Uncertainty given	Widely tested/used	Landscape	Farm /paddock	Cost	Specialist expertise	Specialist equipment	Landscape stratification for sampling	Cropland	Rangeland/ grazing Land	Grassland	Agroforestry	Woodland
a) Methods specifically designed for SOC quantification													
A protocol for modeling, measurement and monitoring soil carbon stocks in agricultural landscapes. Aynekulu et al. (2011)	x	x	x	a	MH	M	LM		x		x	x	x
VCS Module VMD0021 Estimation of Stocks in the soil carbon pool	x		x	x	H	MH	MH	x	x	x	x	x	x
Sampling, measurement and analytical protocols for carbon estimation in soil, litter and coarse woody debris. McKenzie et al. (2000)	x	x	x	x	M	M	M	x	x	x	x	x	x
Soil testing protocols at the paddock scale for contracts and audits – Market-based instrument for soil carbon. Murphy et al. (2013)	x		x	b	LM	M	M	x		x	x		
b) Methods considering SOC as part of whole ecosystem carbon quantification													
Measuring Carbon Stocks Across Land Use Systems (ICRAF). Hairiah et al. (2010)	x	x	x	x	LMH	LMH	LMH	x	x		x	x	x
Guide to Monitoring Carbon Storage in Forestry and Agroforestry Projects. MacDicken (1997)	x	x	x		LM	LM	LM	x				x	x
GOFC-GOLD (2009)	x		x		MH	MH	MH						x
Forest Carbon Stock Measurement: Guidelines for Measuring Carbon Stocks in Community-Managed Forests. Subedi et al. (2010)	x		x		M	M	M	x				x	x
Small-Holder Agriculture Monitoring and Baseline Assessment (SHAMBA) methodology. Berry et al. (2012)	x		c	x	LM	M	M	d	x			x	
Integrating Carbon Benefits into GEF Projects. Pearson et al. (2005)	x	x	x	x	M	MH	MH	?	x	x	x	x	x
Carbon Inventory Methods. Ravindranath and Ostwald (2008)	x		x		LMH	MH	MH	?	x		x	x	x

Notes: LMH (low, medium, high) [a] If remote sensing available; [b] Specifically designed for paddock (generally an extensive rangeland); [c] Not designed for sites with signs of significant erosion; [d] only areas with climate smart agricultural activities considered.

7.2 Quantification of Soil Carbon Stocks

7.2.1 Sampling Design - Stratification of the project area

While the establishment of a geographical extent for quantification of SOC stocks and stock changes at the farm level can be straightforward, it is not the case for smallholder landscape assessment. The landscape concept may be defined by a geographic or ecological boundary, which often includes a mosaic of land covers and land uses that are managed in several different ways by the multiple stakeholders involved. In this context, Chap. 3 in this book provides recommendations for stratifying the landscape according to its agricultural productivity, economic outputs, potential GHG emissions, and social and cultural values. A SOC quantification scheme could integrate with such a stratification approach at the landscape level.

Herein, we describe the methods specifically dealing with quantification and assessment of SOC at both the individual farm and the landscape scale in smallholder agricultural systems. Once the study boundaries have been defined, assessment of SOC stocks at the landscape scale can be done following a spatially stratified randomized sampling design, as this will allow for a more optimum areal coverage and unbiased assessment of sample mean, variance and estimation variance of the sample mean.

Landscape level At the landscape level, the stratification can be done either through: a) ancillary data, or b) geographic coordinates, which may include the use of a systematic grid over the project area (de Gruijter et al., 2006).

Stratification through *ancillary variables* requires the establishment of discrete strata on which selected factors affecting SOC stocks show some degree of uniformity. Such stratification needs to be performed considering at minimum, available soil classifications, soil texture, landform information, topographic position, land cover, land use, management history, fire records, and obvious soil erosion/deposition processes. The initial stratification should be conducted in a hierarchical order whereby the factor that exerts the strongest influence on SOC stocks is ranked first, and other factors with less influence on SOC are subsequently assigned (e.g. a classical ranking approach might be climate, soil texture, land cover and management, etc.). The VCS module (VMD0018) provides detailed methodology on how to implement and adapt the stratification to the needs of the sampling process.

Ideally, the number of samples to be measured in each stratum should be determined as a proportion of the area and the variance observed for that particular stratum. For this, a pilot soil sampling can be conducted that would serve a double purpose: to obtain an initial estimate of the variance for each stratum and serve as a training exercise for technicians who will be involved in subsequent sampling (MacDicken, 1997). Nonetheless, it is likely that in smallholder systems, a stratum defined by biophysical factors may still be made up of land parcels managed in highly contrasting ways. Indeed, land management could account for more variation in SOC stocks at the landscape or regional level than either soil types or land use. Under such circumstances, there may be a need to stratify into a greater number of land use categories to account for land use management practices between farm tenancies (Bell and Worrall 2009). Consequently, the number of samples needed to account for spatial patterns and uncertainty in a highly heterogeneous environment can quickly become impractical due to the cost and time associated to sample collection, preparation and analyses. To avoid this, *spatially stratified systematic sampling*

approaches such as the one employed by the Land Degradation Surveillance Framewok (LDSF; Aynekulu et al., 2011; Vagen et al., 2012) are easier to establish and monitor, and therefore may be a cost effective alternative to provide a representative landscape estimate of SOC stocks and their changes. Moreover, the resulting sampling locations are spatially dispersed across the study area, but the range of variation in SOC stocks is not as effectively covered as with the stratification by ancillary variables. Therefore, the user should make his/her own choice depending on the available resources and the degree of accuracy required. We advocate the stratification by ancillary variables. However, in the case of very large heterogeneous regions, we recommend the implementation of a spatially stratified systematic sampling.

The number of plots required to estimate SOC stocks in each stratum depends on the desired precision, often set at ±10% of the mean at 90 or 95% confidence level. The number of plots per stratum can be ascertained through the relationship described by Snedecor and Cochran (1967); *See specifics in the detailed methodology section.*

Farm level While, both stratification approaches (spatial and using ancillary variables) can yield relatively accurate information about SOC stocks at the landscape level, they lack proper accounting at the farm scale unless specific sampling strategies within a given household are further implemented. Intensive work conducted over the past decade in smallholder agricultural systems in sub-Saharan Africa has demonstrated the existence of within-farm variability of soil fertility and related soil properties (Prudencio, 1993; Carsky et al., 1998; Tittonell et al., 2005a; 2005b; 2013). A common feature of these farming systems is the existence of strong gradients of decreasing soil fertility with increasing distance from the homestead, which mainly occur as a result of differential resource allocation driven by the farmer. This spatial gradient must be taken into account when designing SOC sampling strategies in these agricultural systems, and more so considering that previous work has also identified strong correlations between yields, soil quality indicators, land use management and the distance from the homestead (Tittonell et al., 2005b, 2013). On the other hand, the presence of either annual or perennial vegetation on a given land use will have a strong impact on SOC stocks, as they will significantly determine both the quantity and quality of organic matter inputs into the soil (Guo and Gifford, 2002; Saiz et al., 2012). Therefore, distance from the homestead and land use classified by presence of annual or perennial vegetation, are the main criteria to use in order to categorize field types for the purpose of soil sampling. Accordingly, fields are classified into Home gardens, close-distance, mid-distance, and remote fields following a similar procedure as in Tittonell et al. (2005b). These areas may contain several land uses, and as it may not be feasible to sample all of them, priority should be given to the actual representativeness of the land uses being considered. Therefore, sampling should be preferentially done in the largest fields provided that management activities with potentially heavy impact on SOC stocks, such as manure additions or recurrent burning of stubble, are roughly comparable between the different land uses. However, this assumption may not hold quite true in these farming systems, and thus it is worth noting that if land use management needs to be adequately quantified, then the sampling effort may need to be increased quite considerably. Nonetheless we hypothesize that, on the whole, soil sampling across a spatial gradient may partially account for the effect of land management intensities along the farm, given that such activities are also likely to occur along the same gradient.

The level of precision required for a SOC inventory will undoubtedly influence the number of plots to be sampled, which will have necessarily a very strong impact on the cost associated with

fieldwork and soil processing. Indeed, the largest component of the total cost incurred in SOC surveys corresponds to soil sampling and preparation (Aynekulu et al., 2011). Except for the case of surveys in which extremely large numbers of samples are collected (>2000), the actual cost of soil analyses is relatively low compared to the total expenditure of collecting and preparing samples. With all, and in order to minimize the number of samples to be analysed, an extensively applied method is the bulking (pooling) of samples collected within a plot at the same depth interval. This procedure has been shown to be a cost-effective technique for smoothing out local heterogeneity and for achieving robust local and regional estimates of SOC inventories (Bird et al., 2004; Wynn et al., 2006; Saiz et al., 2012).

The specific objectives of the study shall ultimately dictate the sampling priorities, which combined with the available resources, will determine the methodology and sampling intensity to apply.

7.2.2 Sample Collection *(see also the Simplified Protocol for this purpose in Appendix B)*

Collection of samples should be routinely conducted at roughly the same time of the year, and in between relevant agricultural practices (i.e. harvesting, fertilization, etc.). We propose to take four soil samples in each selected plot, which will correspond to a given field and land use. The initial sampling location will roughly be allocated at the centre of the field, with three replicates laid out according to a pattern of three axes separated 120 degrees with respect to an initial axis pointing north. The replicates will be selected along these axes at approximately mid distance between the centre of the field and its boundaries. The final sampling locations will be geo-referenced using a GPS, and notes should be taken about the sampling location with regard to the proximity of perennial vegetation (i.e. shrubs, trees, etc.), and any other relevant information such as presence of rock outcrops. Unless very intensive sampling is required in a given particular field type, then the low sampling intensity proposed at the field scale (4 samples) does not allow for proper inter-comparison of small-scale intercropping, nor for comparison between furrows and ridges. Therefore, sampling should be systematically allocated at the same ploughing feature (e.g. furrow).

Previous to any sampling surface litter will be removed by hand. Soil samples will then be collected at 0-5, 5-20 and 20-30 cm depth intervals making use of a steel corer. This procedure will allow for determinations through the retrieval of a single soil core of both OC abundance and accurate soil bulk density (SBD) at each depth interval. Accurate determination of SBD in the topsoil layers is particularly critical given that it is at these shallow locations where SBD shows the largest variability and significantly large quantities of OC are stored. Nevertheless, it is important to note that while the use of a steel corer may be a feasible procedure in many arable lands as a result of both soil being regularly disturbed and stones being progressively removed over the years, the use of a soil auger may be necessary to collect samples in stony or very hard soils. Indeed, deep soil augering needs to be carried out at three of these locations, with samples being taken at 30-50 and 50-100 cm (impenetrable layers permitting). In the cases where soil augering is implemented, the determination of SBD is achieved through cumulative mass soil sampling, which consists on collection and quantification of all the soil mass augered in a given depth interval, which may then be followed by determination of the auger-hole volume through sand filling. This is however a time consuming as well as demanding task, and hence it should be limited to cases in which coring is not possible. Furthermore, since sampling by mass avoids potential biases derived

from varying bulk density caused by land use change or agricultural practices, it is often regarded as the method of choice for SOC monitoring over time (*see McKenzie et al. (2000) for detailed guidance on the method*).

7.2.3 Sample preparation and analytical methods *(see also the Simplified Protocol for this purpose in Appendix B)*

Once in the laboratory, samples are weighed in their sealed bags, clumps broken by hand and then oven dried at 40°C to constant weight. Thereafter, an aliquot of each sample will be oven dried at 105°C for 4 h which will allow for the calculation of soil bulk density (SBD), while the remainder of the samples will then be dry sieved to 2 mm and gravel and root content >2 mm determined by weight.

Standard methods of soil carbon analysis such as dry combustion or wet oxidation are extensively used in SOC studies as they provide optimum quality results. Moreover, elemental (dry) combustion appliances can be coupled to mass spectrometers to provide stable isotopic carbon signatures of SOM, which broadens the possibilities for better assessing soil carbon dynamics (Bird et al., 2004). However, the elemental combustion technique is resource-demanding and may be impractical or too expensive for large sets of samples and for continuous monitoring (Aynekulu et al., 2011; Batjes, 2011). Nonetheless, the amount of time required to estimate SOC stocks and the sampling and analytical costs can be greatly reduced by employing emerging techniques for in situ estimation of SOC. Among such techniques the one that has been most widely used, and thus tested, is the Infra-Red Reflectance Spectroscopy, either at the Near or Mid infrared reflectance spectroscopy (NIRS or MIRS), which once calibrated, can provide rapid accurate SOC estimates (Shepherd and Walsh, 2002, 2007; Aynekulu et al., 2011). Despite its usefulness and versatility, it is still necessary that a significant proportion of samples (i.e. 20%) covering the projected range of SOC values for a given inventory are analyzed using standard SOC analytical procedures. This will in turn provide the necessary calibration set to confidently apply either MIRS or NIRS to the total set of samples.

7.2.4 Quantification of SOC Stocks

The average SOC stock for a given depth interval (d) is calculated according to the following formula:

$\mu_d = BD_d \times OC_d \times D \times (1 - gr) \,/\, 10$; where:

μ_d is SOC stock (Mg OC ha^{-1})
BD_d is soil bulk density (g cm^{-3})
OC_d is the concentration of OC in soil (< 2 mm; mg OC g^{-1}soil)
D is soil depth interval (cm)
gr is fractional gravel content, the soil fraction > 2mm.

7.2.5 Scaling SOC stocks to landscape and whole farms

There is a lack of standardized methodologies to scale up SOC stocks from a point source (pedon) to regional (landscape) and larger spatial scales. In this work, the scaling up of SOC stocks at the *landscape* scale is achieved through the proposed spatially stratified randomized sampling

design. Accordingly, the average SOC stock for a given stratum is calculated as follows:

$\mu_{st} = \frac{1}{n} \sum_{i=1}^{n} y_i$; where:

μ_{st} is the mean SOC stock for stratum (st)

y_i represents each calculated SOC stock in that stratum

n is the number of observations in that stratum (see appendix A for detailed calculations on the number of samples required in each stratum).

The *variance* in SOC stocks for a given stratum is calculated according to the following formula:

$\sigma_{st}^2 = \frac{1}{n-1} \sum_{i=1}^{n} (y_i - \mu_{st})^2$; where:

σ is the SOC stocks variance

y_i represents each calculated SOC stock in that stratum

μ_{st} is the mean SOC stock for stratum *st*

μ_{st} is mean SOC stock associated with the stratum *st*

n is the number of observations in that stratum.

The *average* SOC stock for the area of study (landscape) is calculated considering both the mean SOC stock obtained for each stratum and the area occupied by each stratum. Therefore, the calculation is as follows:

$\mu = \frac{\sum_{h=1}^{H} a_h \times \mu_h}{A}$; where:

μ is the mean SOC stock

a_h is the area of the stratum *h*

μ_h is mean SOC stock associated with the stratum *h*

A is the total area of the study.

The *average* standard error in SOC stocks for the area of study (landscape) is calculated according to the following formula:

$SE = \sqrt{\sum_{h=1}^{H} \left(\frac{a_h}{A}\right)^2 \times \frac{{S_h}^2}{a_h}}$; where:

SE is the standard error for the entire population

a_h is the area of the stratum *h*

S_h *is the variance of stratum h*

A is the total area of the study.

Scaling SOC stocks from a few point source measurements (fields) to the whole farm necessarily requires a series of assumptions unless all fields within the farm get sampled (which may be highly unpractical). Here, it is assumed that the center and perimeter of each field gets georeferenced so that its surface area can be determined. In the proposed scheme, samples within a given farm should be taken along the previously described land use intensity gradient (i.e. Home gardens, Close-distance, Mid-distance, and Remote fields) at their most spatially representative fields. If for a given section (I.e. Close-distance fields), there is an occurrence of individual fields with annual and perennial vegetation (crops or trees), and the area of the smaller field is at least half the size of bigger field, then sampling should be conducted at both fields. The *average* SOC stock for the selected farm is then calculated considering both the mean SOC stock obtained for each section and the area occupied by each section. The calculation procedure is similar to the one described for the landscape scale, and it simply replaces strata by sections.

7.3 Quantification of Soil Carbon Stock Changes

The determination of the sampling intensity required to demonstrate a minimum detectable difference in SOC stocks over time has been the subject of numerous studies (Garten and Wullschleger, 1999; Conen et al., 2003; Smith et al., 2004). The actual number of samples to detect SOC differences for different degrees of statistical confidence will be directly dependent on the background level that the study requires (i.e. the detectable difference in SOC relative to the stock baseline estimated in the first inventory). Moreover, considering the inherent natural variability of soil properties, the demonstration of small changes in SOC stocks will require the collection of an impractically large number of samples (Garten and Wullschleger, 1999), whose costs may quickly overrun any benefit derived from a potential increase in SOC levels. As an illustrative case, Smith et al. (2001) indicate that between 10 and 20 samples should be collected to detect a 15% change in SOC stocks in a relatively homogeneous system (<25% coefficient of variation). Further information about the number of samples required is given in the detailed methodology section.

While IPCC (2003) and UNFCC (2006) recommend a five-year and 10-20 year monitoring intervals respectively, a relevant sampling interval suited to site-specific conditions can be ascertained by using models of SOC dynamics to plan both the frequency and intensity of subsequent surveys determining SOC stock changes (Smith et al., 2004). However, modelling of highly heterogeneous environments such small household agricultural systems is a challenging task, which is unlikely to provide a single answer with regard to when and how intensively different sites should be measured to detect significant changes in SOC stocks. Therefore, we recommend adopting the strategy similar to the one pointed out by Lark (2009), which suggests sampling only a proportion of the initial baseline sites in any one stratum. This strategy purposely focuses efforts in those locations likely to show the larger differences in SOC stocks over a fixed term (i.e. 10-year period). Thereafter, the strata that show a large change could then be sampled more intensively. Alternatively, estimation of changes in SOC over shorter periods could be achieved through the measurement of changes in particular soil carbon fractions (e.g. particulate organic matter) given that these are more sensitive to changes than total carbon in the bulk soil (Six et al., 2002). However, the implementation of a SOM fractionation procedure requires specific laboratory

equipment (i.e. sonicator) and access to relatively expensive consumables (i.e. heavy liquid; Wurster et al., 2010).

Finally, it is important to note that sampling by mass instead of volume avoids potential biases derived from varying bulk density caused by land use change or agricultural practices. The adoption of this method may improve our ability to make comparative measurements across time, treatments, locations, and equipment (McKenzie et al., 2000; Gifford and Roderick, 2003; Wuest, 2009).

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Appendix A: Detailed methodology for quantification of soil carbon stocks and carbon stock changes

Number of samples required

The number of plots required to estimate SOC stocks in each defined stratum depends on the desired precision, often set at ±10% of the mean at 90 or 95% confidence level. In the case of strata defined by ancillary variables, the number of plots per stratum can be ascertained through the relationship described by Snedecor and Cochran (1967);

$\mathrm{n} = \left(\frac{\mathrm{t}_{\alpha}\ \mathrm{S}}{\mathrm{D}}\right)^2$; where t_α is Student's t with degrees of freedom at either 0.95 or 0.90 probability level, *S* and *D* are the standard deviation and the specified error limit respectively for values obtained from an initial assessment of the stratum. On the other hand, and for the case of a given area stratified by geographical coordinates, the number of samples required could be determined using a slightly modified relationship (Pearson et al., 2005; Aynekulu et al., 2011);

$\mathrm{n} = \frac{(N \times S)^2}{\frac{N^2 \times D^2}{\mathrm{t}_{\alpha}{}^2} + (N \times S^2)}$; where t_α, *S* and *D* are as above and derive from values obtained from an initial assessment of the area considered, *N* is the number of sample units in the population, that is the total area divided by plot size. The resultant number of plots can be further allocated into a number of defined strata by using:

$\mathrm{n}_h = \frac{N_h \times S_h}{\sum_{h=n}^{L} N_h \times S_h} \times n$; where N_h, S_h, *L* and *n* are the area of the stratum *h*, standard deviation of stratum *h*, number of strata, and number of total plots respectively.

In the cases where the confidence interval exceeds +/- 10% with 90% confidence, the user may undertake one of three actions (VCS module VMD0018): a) re-stratify according to any significant correlation observed between the sample variance and geographic or other factors, b) Increase the number of plots, and c) set lower confidence intervals, increasing thus the estimates uncertainty. The determination of the number of plots to be sampled in each stratum as a proportion of both its area and the observed variance may certainly be an efficient approach. Adding to this efficiency, it can also be expected that the number of plots required for determination of SOC stocks for a given stratum defined by ancillary variables may be significantly small compared to the ones needed in the less homogeneous strata defined by geographical coordinates.

With regard to the number of samples required to demonstrate a given minimum detectable difference in SOC stocks over time the reader is referred to Garten and Wullschleger (1999); Conen et al., (2003) and Smith et al., (2004) for sound descriptions of the methods and equations used.

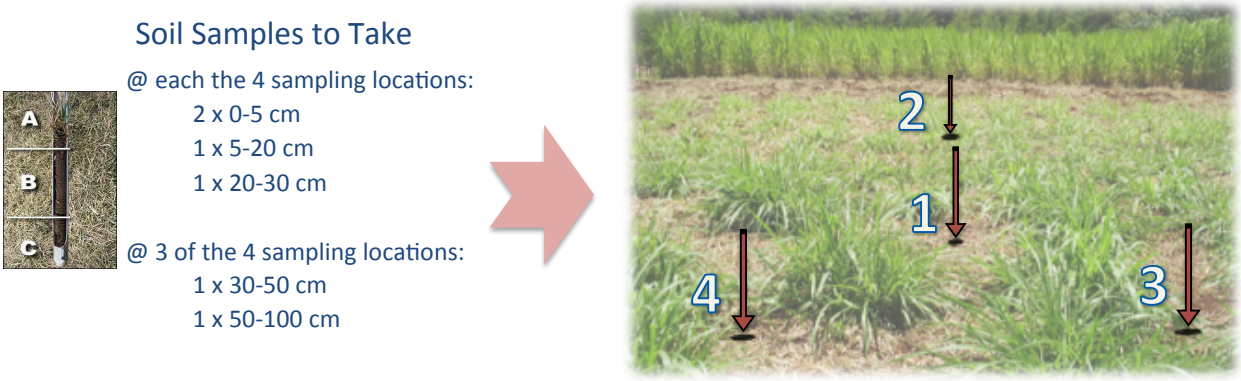
Appendix B: Simplified protocol for taking and processing soil samples

This protocol covers both the soil sampling procedure and sampling processing and assumes the plots/subplots to be sampled have already been pre-selected

Soil sampling

Soil samples are collected in four different locations within the plot of choice to account for the inherent heterogeneity of SOC. At each of these four locations, surface litter (when present) is removed by hand and the following depths intervals are sampled according to the following procedure:

Start roughly at the centre of the plot/subplot (replicate 1) and establish the other 3 replicates laid out according to a pattern of three axes separated 120 degrees with respect to an initial axis pointing north. Make sure the other three replicates are set up at a prudent distance from the edges of the plot/subplot (+5 m if possible) to avoid any boundary effects, but do try to cover ground. The final sampling locations will be geo-referenced using a GPS.



It is assumed that a stainless steel corer, a soil auger and a spade will be used for retrieving the samples. All samples will be placed in **labeled** zip-lock bags. It is very important that the bags are clearly labeled with permanent marker. Always a good idea to label them immediately after you take the sample otherwise they may get mixed up (if a marker is not around, write it in a paper and put it inside of each bag). A good labeling should mention at the very least:

- Plot/subplot name or number (e.g. DCR)
- Replicate number (e.g. 3 for replicate 3)- Depth i.e. (5-20)

Then in the same bag and line in big clear letters following the example given it should say: *DCR-3 (5-20)*

Detailed Sampling Procedure

- **0-5cm**

In the case of the interval 0-5 cm, 2 samples within 1 metre radius will be put in the same bag. This is the only depth interval 2 samples are put together in the same bag, and it is done to smooth out local heterogeneity, which is particularly pronounced at this shallow depth.

Remove vegetation and surface litter.
Push short corer (steel cylinder) into soil until the 5 cm mark is reached. Retrieve it gently by carefully shaking it back and forth sideways to compact a bit the surrounding soil (this will get subsequent sampling at depth much easier and will avoid soil crumbling into the hole).
Pull the corer out rotating carefully (always clockwise as this will be very relevant when using the other soil sampling gear at depth)
Place the soil into plastic bag, trying not to touch it with the hands. Starting with the topside (loose crumbly soil gets out first), and then turn the cylinder upside-down.
To help the soil come out, use the rubber mallet to impact the cylinder walls while it gets turned around. The soil will come out eventually. Get all the soil out of the tube.

- **5-20 cm and 20-30 cm**

  Hammer the next sampling cylinder into the soil until the depth markings. You may be using a regular cylinder or the one with a detachable cutting edge (preferably the latter as it is more robust). If using the latter, then you will have to carefully detach this cutter and scrap the sample out onto the bag. This can be done by a second person, thus improving sampling speed. Regardless, beware of what you are using as the diameters (crucial for bulk density determination) change for each choice.
  Shake it back and forth carefully sideways (to compact surrounding soil).
  Rotate clockwise, pull out and extract soil sample (using the sample extruder if using cylinders without detachable cutting edge).
  Again: put the soil into a labeled plastic bag avoiding contact with the hands.

- **30-50 cm and 50-100 cm**

  If the soil is relatively soft and free of stones, use the cylinders (as bulk density can still be used). If that is not the case, then use the soil auger or spade.
  If using the soil auger you will have to discard the top sand lateral soil that comes out with the auger. You can do this with the scraper before you put it in the bag (this is to avoid cross contamination).
  If using a spade to reach the required depth, the sample will be obtained by scratching the soil out of the walls. Prior to obtaining any sample the walls of this hole (pit) need to be cleaned (scratched) to avoid contamination. Start scratching/ sampling from the bottom once the hole has been finished. Take roughly the same amount of soil material along the targeted depth interval, as you do not want to take most of your sample at a concentrated point. It would be good to have a graduated ruler or stick with depth marks.

In general, also consider the following:

- Take notes that may help you to interpret results later on (GPS, land use history, farmers' comments on management, type of soil, current vegetation, evidence of erosion, fire, etc…).
- A sample that comes broken in the first 30 cm (as a result of coarse stones/roots) cannot be used. Sampling has to be done again in another location nearby.
- Always take note of what corer you are using (because of diameters!). It may be that you are exchanging between cylinders with different sizes for whatever logistical reason (e.g. cylinder with detachable cutting hoe vs normal cylinder. These two have different diameters and will definitely affect bulk density calculations). This is very important, take notes.
- Be careful that the sampling hole does not get contaminated while taking samples, e.g. do not step on the hole, do not let litter or surface soil fall in, etc.
- After sampling a plot, the cylinders and scraper need to be thoroughly cleaned (have wet cloths with you).
- Fill up or cover sampling holes (good practice).
- From the outside of the plastic bag, crumble by hand big clumps of soil into smaller parts, which will be critical for easy soil processing later on.
- Closure of bags: rolling them up releasing air from the bag and then close it, so that it contains as little air as possible.
- Take several pictures of the plot/subplot.

Soil bulk density (SBD) determinations

In all cases, calculation of SBD should include fractions >2 mm. So before any sieving takes place the following should be done:

As soon as possible, and certainly before 2 days after collection from the field, let the samples air-dry (after opening and rolling down bags) in a rain-protected location. It is always a good idea to progressively (each day) break the soil clumps with your fingers while the bags are being dried (but be gentle or you may break the bag). A bit everyday is the best, otherwise you will find handling of samples much harder in the coming days, and will have to use a hammer. Also, avoid cross-contamination between samples by doing it from the outside of the bag (gently squeezing it with your fingers). When an oven becomes available, put the bags inside at 40 °C. After a number of days, when samples are seemingly dry (5-7 days will be safe- but of course it all depends on initial moisture content), take them out of the oven and **weigh each sample** (including the plastic bag) but wait about half an hour after the samples have been taken out to do this weighing.

After this weighing, take an aliquot of each sample and place them in labeled paper bags (about ~ ¼, of the total sample, but **weigh how much exactly before you put them inside the oven**). Dry them at 105 °C for 24 h. As before, **weigh all the samples after about half an hour after they were taken out of the 105 degrees oven**. Once the weights of these aliquots have been recorded you can throw this material away.

In total you should have 3 weighs for each sample (i.e. total soil weight, sample before oven dried at 105°C, sample after oven dried at 105°C). This will allow for proper calculation of soil bulk density (SBD).

In general, also consider the following:

- Make sure you always take weighs knowing which bag you are using as each different type of bag will have different weight (both plastic and paper).
- Get an average weight of 5 bags of each type you use, so that can be deducted from the calculations later on.
- Let the samples dry by air (open plastic bag) and roll them down
- Always check that the oven works well.
- Let the samples cool down at room temperature for at least 30 min, unless there was a desiccator that could be used for storing samples prior to weighing. In such case, then the weighing should occur immediately after extracting the samples from the desiccator.
- Weigh the soil with its bag. Very Important!
- Balance/scale should be precise up to 0.1g.

Sample processing

Sieving

The remaining of each sample dried at 40 degrees (most of it) needs to be weighed again and sieved to 2 mm. Gravel and root content >2 mm will be weighed separately. Therefore we will get the fractions of coarse roots and gravel. But first remove carefully all large clumps with a rolling pin (bakery). Put a cloth or something similar on top and the bottom of the table to avoid breaking the bag while rolling it over. If you need to use new bags because these are deteriorated, do so with the same labeling. Then sieve the samples. Again, **in total you should have 3 weighs for each sample (bag**) (i.e. total soil weight, roots>2 mm, and gravel (>2 mm)).

Pooling/bulking

There are numerous ways of pooling, and the final choice depends on the purpose and load of work that can be undertaken. The methods explained below are just two ways that lead to fewer analyses to be undertaken and cover two different purposes:

1) If the aim is to get bulk soil samples to undertake just a single analyses at each plot/subplot bulk by depth interval (e.g. all samples from the same plot/subplot collected at 5-20 cm), then do as follows:

- Use the same weigh for all the replicates (20 or 30 g), and put them together in a bowl or tray. Do not use the entire sample from each bag! Keep them as back ups.
- Mix them a bit always with clean, dry hands (10 sec should be alright).

- Put the mixture in a new bag with the same code as before but indicating 'Bulk" at the end.

2) If the aim is to also get a **'Master soil sample' 0-30 cm** for subsequent analyses (texture, mineralogy, organic matter fractionation, ECEC, etc) then from the previous bulked bags put together the following weights in a separate bag.

- 0-5 cm: 10 g
- 5-20 cm: 30 g
- 20-30 cm: 20 g

This bag is to be called Master with same code as before and indicating (0-30) at the end of the labeling.

Sometimes it may be necessary to have an extra bag with about 20 g of Master soil (0-30) that will be used for soil textural analyses. Take about 20 g from this bag and put them into a small bag with the same coding indicating that is for **Texture**.

Powdering

If powdering is needed, then proceed as follows:

- Take about 3 g of your sieved, pooled/bulked sample.
- Powder the sample with the aid of a mortar-pestle or micro-mill device.
- Put the sample into a small plastic bag with the code on it.
- Be real careful that all instruments used for powdering get properly cleaned (if using water then it is very important that everything is absolutely dry again – or the analyses will be bad) or even better, use acetone or methanol. But do use gloves with the latter ones.

Finally, about 50 g of sample per bag should be stored for any further potential analyses.