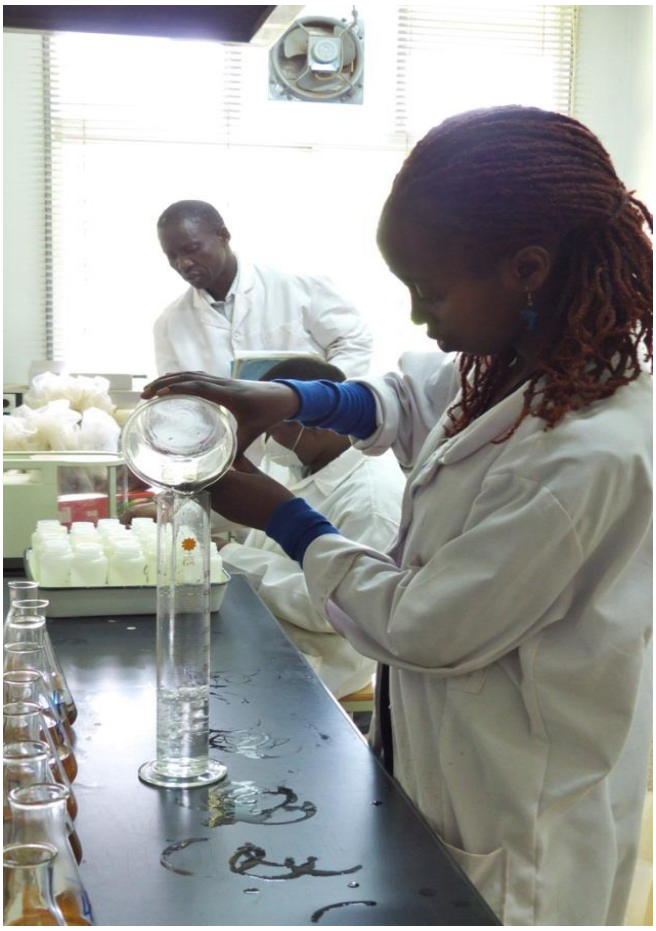
Improving Capacity in Forest Resources Assessment in Kenya (IC-FRA)



Manual for Preparation and Organic Carbon Analyses from Forest Soil and Mangrove Sediment Samples

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Cover caption by: Jukka Alm: Soil analysis at KEFRI

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1 Introduction

Naturally occurring organic carbon (C) forms are derived from the decomposition of plants and animals. In soils and sediments, a wide variety of organic carbon forms are present and range from freshly deposited litter (e.g. Leaves, twigs, branches, and roots) to highly decomposed forms such as humus. Soils may also contain carbon in inorganic form, e.g. as carbonate minerals. Inert forms of elemental carbon from charred organic carbon (black carbon) from forest fires or prescribed burning, coal, graphite, carbides, or soot particles deposited from incompletely combusted fossil fuels may occur in soils.

Forests sequester and store more carbon aboveground than any other terrestrial ecosystem. Part of the carbon store is in the organic humus layer of soil, but also deeper in the soil profile, driven by the infiltrating rain water. Soil organic carbon (SOC) constitutes a significant part of the forest carbon store. Where litter falls in water saturated environments and is deposited as peat, soil organic carbon stores may greatly exceed those in the aboveground biomass. Soil organic matter is also a store of nutrients such as nitrogen (N) and phosphorus (P).

Mangrove forests are among the most productive ecosystems in the world. A major part of the organic carbon stock is found belowground in mangroves. The sediment has high salinity and is largely water saturated, thereby preserving the organic residues of litter, debris, and dead roots. Mangrove sediments have high chloride content as well as high organic content, but also may include significant amounts of carbonates from the underlying coral reefs, which all affect the analytical procedures of TOC.

Total carbon (TC) in a soil sample includes both inorganic and organic constituents. Inorganic carbon can be present from calcareous parent materials and is not subject of this Manual. Total organic carbon (TOC) can be determined from dried and acidified soil samples using dry or wet combustion methods.

There are challenges in preserving the samples, transporting them to laboratory, storing and analyzing them without a systematic error following from these procedures. Another challenge is the presence of inorganic carbon in form of carbonates derived from the parent materials that can severely disturb the determination of TOC, as carbonate minerals dissolve in acidic treatment directly adding to the analysis estimate of TOC. It is thus desirable to have a simple, rapid and accurate method for the analysis of soil organic carbon.

A traditional dry combustion method based on ignition mass loss of oven-dried samples is unselective due to loss of carbonate minerals and is time consuming. Modern dry combustion methods based on detection of CO_2 release from the heated sample can provide automated analysis sequences with help of peak integration software and sample autoloader to save human labour. Unfortunately, the determination of soil carbon content by automated dry combustion methods requires the use of costly and specialized equipment. Wet combustion using dichromate sulphuric acid (Walkley-Black method, originally published by Walkley& Black, 1934) requires traditional manual analytics, applies poisonous and harmful chemicals, but can yield to a reasonable daily throughput of samples with moderate costs and commonly available laboratory equipment.

Carbonate containing soils cause difficulties in TOC determination in dry combustion methods. Elimination of the carbonate effect from TOC is hard, because some organic Carbon (OC) compounds may be lost in acid treatment when carbonate is removed, leading to 10–30% error in TOC estimate in some cases. Even when an accurate TOC result may not be obtained in samples rich of carbonates it is imperative to know of the carbonate effect not to overestimate soil OC. Carbonates do not interfere significantly with the dichromate wet combustion method. Thus Walkley-Black Wet Oxidation method can be a solution for calcareous soils. Walkley-Black is applicable when enough laboratory personnel is available. However, the Walkley-Black results are known to be subject to uncertainties since only the easily combusted OC fractions are oxidized, and the results must be corrected using a proper correction factor. Literature derived correction factors are often used, but better results could be obtained using specific comparisons with other methods for each soil type.

This Manual aims at describing methods for reasonably accurate TOC analyses that are compatible with other results obtained in the region. For above-ground litter and woody debris, the organic carbon content is

estimated as a proportion of dry biomass. Different methods are presented for upland soil samples and mangrove sediment samples. Upland soil organic carbon is determined using Walkley-Black method, while the mangrove sediment carbon content is measured using Loss On Ignition (LOI) method.

In a nationwide forest soil inventory, the number of samples to be analyzed is large. Several laboratories may be sharing the analysis workload. The methods used in this Manual are familiar with the ones used in Kenyan soil laboratories, and can be used to obtain results comparable to earlier work in Kenya. Kenyan laboratories have a tradition of calibrating their methods using cross-laboratory analysis of same samples. Therefore the QA/QC will be planned adopting the routines familiar to all players, with addition of some external control using different dry combustion techniques. The QA/QC procedures are explained in the NFRA plan in more detail.

2 Procedures for upland soil samples, litter and woody debris

It is necessary to follow all the steps related to sample handling, processing and preparation for physical and chemical analysis during the determination of organic carbon in soil.

2.1 Receiving the upland soil samples

After soil sampling in the field, the samples are transported to the laboratory as soon as possible e.g. by means of public transportation. The following samples are taken from each inventory plot;

- Composite litter/woody debris (each stand of the plot, if at least 3 sampling points are available, default 4 sub-samples, but some sub-samples may be void of litter/debris)
- Composite soil samples separately from three depths, 0-10 cm, 10-20 cm, 20-30 cm (each stand of the plot, if at least 3 sampling points are available, default 4 subsamples)

The following sample is taken from one inventory plot of each cluster

- Non-volumetric deep soil sample representing 30-60 cm soil layer, from one soil pit within a plot only

For litter and woody debris, the samples may consist of all, or a representative part of the materials collected from the soil pit locations for each stand of the plot. For soil, the volumetric composite samples represent each of the sampled soil layers, 0-10 cm, 10-20 cm, and 20-30 cm. In cases when soil is too shallow to allow for sampling of all the layers, only the complete surface-most layers are taken. Non-volumetric soil samples from depth of 30-60 cm are taken from one plot of each cluster. The samples need to be accompanied by either the original field forms, or copies of each field form.

The following mandatory information accompanying each sample is checked when receiving the sample in the laboratory:

- Check sample visual condition: Broken plastic bag, part of the sample possibly lost, etc.
- Check from the copy of the Soil Field Form
    - Name of crew leader and name of the sampling region are given.
    - Sampling date is given.
    - Complete sampling code (*Cluster number*, *Plot number, Stand number,* and for soil samples also *Layer*) are marked on the sample
    - Number of sub-samples included in the composite sample. It is very important that the number has been correctly given and is the same for both the litter/woody debris samples and for the soil samples.
        - In some cases there may be no litter found within the sampling ring, but such a spot must be added in the number of sampling spots for obtaining *a correct average* of the litter/debris mass.
    - The total mass of litter/debris was given in field. If only a sub-sample of litter/debris is sent to laboratory, the fresh masses of the respective sub-samples are given.
    - The fresh mass of each depth layer composite sample is given.
    - Soil is characterized for colour, texture

After checking above information, laboratory will assign its routine laboratory codes for the samples and start processing the samples according to the following procedure.

2.2 Processing of litter and woody debris samples

Litter and woody debris total fresh mass should be measured in the field. If in doubt of the field result, the fresh mass may be re-weighed in laboratory. The litter and woody debris samples for each stand are oven-dried in aluminium containers until constant weight at 105°C, and immediately weighed with accuracy of at least 1 g (preferably 0.1 g). Use of analytical balance is not needed. Since the sample and its container are hot, the scale needs to be protected by a light, effective heat insulating plate tared to zero prior to weighing the sample. The mass of the empty container needs to be known and is weighed as empty (can be done once or repeatedly, and marked using a permanent marker).

2.3 Processing of upland soil samples

The samples should be handled using plastic gloves at all times. The samples are immediately poured from plastic bags to ca. 400 cm^3 aluminium containers and dried in order to stabilize the soil against decomposition of organic matter by microorganisms. If immediate drying is not possible, the samples should be kept in refrigerator at a temperature of +4°C until the next day or freeze at –20°C over longer periods of time.

If the oven capacity allows, the samples are immediately moved into 400 cm^3 aluminium containers with the paper tag showing the sample's origin and oven-dried at 105°C to constant weight.

If there is no oven capacity when the samples arrive, the samples are first preserved by air-drying in 800 cm^3 aluminium containers. All the samples from the field in plastic bags must be preserved and later oven-dried, because dry bulk density corresponding to the volume taken in field is only obtained from oven-dried samples.

A low oven temperature is preferred so that organic matter is not lost during heating. Because reaching a constant weight for a sample with high moisture may take long, all samples can be air-dried prior to the final oven-drying. Air dried samples are restored in a clean, dry plastic bags with the paper tags describing the sample origin, and are carefully air-tightly closed and stored in a dark, preferably cool place to wait for oven-drying. But when oven capacity allows, the air-dried samples are oven-dried to constant weight at 105°C.

Immediately after oven-drying, the samples are weighed for dry mass. The samples can be weighed in their aluminium containers, and the mass of the container must be known. The containers can be pre-weighed empty and their specific mass marked on the container using a marker that persists washing and heating of the container.

After weighing, the oven-dry samples are sieved using a 2 mm sieve, and roots and coarse soil fraction are separated. The gravel and small stones are weighed in order to obtain the proportion of soil where no OC occurs. Roots can be discarded, because the soil sampling procedure in the field may have on purpose avoided the plant roots. The root content in the sample volume may thereby be biased. In the final reporting of the FRA inventory results, the contribution of tree roots to SOC is estimated during the soil OC stock calculation with help of biomass models.

In some cases dust covers the gravel and pebbles of the coarse fraction. In that case the material needs to be washed clean from dust and dried again prior to weighing. It is worth to notice that if washing becomes a dominant routine for all soil samples, it would be easy to determine the total volume of the coarse fraction in addition to is mass. That could be done by pouring the coarse fraction sample into a beaker with volume scale, and by adding an accurately known volume of water in the beaker. The mass and volume of the coarse fraction could be used to accurately determine the density of the stones (=dry mass of stones, g, divided by their volume, ml [cm^3]). The volume of the coarse fraction is the difference of total volume minus the added

water volume. The excel calculation application currently uses an assumption of 2,560 g cm^{-3}, and the volume estimate is not needed in the present formula. However, changing of the calculation method is easy.

The fine fraction extracted by sieving trough the 2 mm sieve is first homogenized by thorough mixing. Then about 100 g of the fine fraction is ground using a mortar. A representative sample for TOC analysis of ca. 1.000 g is taken and weighed using analytical scale of accuracy 0.001 g. The rest of the ground fine fraction is stored in air-tightly closed plastic containers and archived with the codes uniquely describing the sampling site and time, i.e. *Cluster number, Plot number, Stand number,* and *Depth layer,* and *Date of sampling*.

If the humus content in soil is high such as in natural forests, the size of the sample taken for OC analysis sometimes has to be smaller than 1 g because of the limitations in titration. The appropriate mass of soil required for analysis can be roughly estimated from the formula;

W =0.5/% N (Normality)

For example, sample sizes of 0.500 g and even 0.250 g can be used. However, the smaller the sample, the less sensitive the result is for any un-homogeneities in the sample.

2.4 Procedures for upland soil TOC analysis

Organic carbon content in the soil is determined using the Walkley-Black Wet Oxidation method. The detailed procedures are outlined as below:

A known mass (typically ca. 1.000 g) of soil is treated with an excess volume of standard potassium dichromate solution in the presence of concentrated sulphuric acid. The soil is slowly digested at the low temperature by the heat of dilution of sulphuric acid and the organic carbon in the soil is, thus oxidized to carbon dioxide. The highest temperature attained by the heat of dilution reaction, produced on the addition of sulphuric acid is approximately 120°C, which is sufficient to oxidize the active forms of the soil organic carbon, but not the mere inert form of carbon, that may be present.
The excess of potassium dichromate not reduced by the organic carbon is titrated back against a standard solution of ferrous ammonium sulphate, in the presence of 85% ortho-phosphoric acid using 5 % barium chloride as indicator. This method only oxidizes 77 % of organic carbon present in the soil.

Apparatus

1. Burette, 50 ml capacity
2. Pipette, 10 ml and 20 ml capacity
3. Measuring cylinder/Acid dispenser
4. Conical flask, 500 ml capacity
5. Volumetric flask, 1 litre capacity
6. Analytical balance
7. Sieves (2 mm, 0.5 mm)
8. Grinder (ceramic mortar)

Reagents

- Potassium dichromate ($K_2Cr_2O_7$), AR grade
- Ferrous ammonium sulphate ($FeSO_4(NH_4)_2SO_46H_2O$; FAS; Mohr's salt)
- Sulphuric acid (H_2SO_4), concentrated
- Ortho-phosphoric acid (H_3PO_4),85%
- Barium chloride ($BaCl_2$), 5%
- Diphenylamine sulfonate, 0.1%

Preparation of reagents

1. *To make 1N potassium dichromate solution:*
   Dissolve 49.04 g of AR grade potassium dichromate (previously dried at 105^{o}C for 2 hours) in distilled water and make up the volume to exactly 1 litre.

2. *To make ca. 0.5N ferrous ammonium sulphate (FAS; Mohr's Salt):*
   Dissolve 196 g of ferrous ammonium sulfatein 800 ml distilled water and add 5 ml concentrated sulphuric acid and make up the volume with distilled water to exactly 1 litre. The exact normality (N_{FAS}) of this solution is found by titration against the standard dichromate solution.

2.5 Determination of TOC%

The appropriate mass of soil needed in grams (W) required for analysis is roughly calculated from formula:

W = 0.15 ÷N (normality)

Weigh accurately W g of soil in a 500 ml conical flask which was passed through 0.5 mm sieve.

Add 10 ml of 1N potassium dichromate solution and shake to mix.

Then add 20 ml of concentrated sulphuric acid and swirl the flask 2 or 3 times.

Allow the flask to stand for 30 minutes.

After half an hour, add about 200 ml of distilled water, 1 ml (30 drops) of diphenylamine indicator and 0.5 g sodium fluoride.

Back-titrate the solution with ferrous ammonium sulphate solution. At the end point, the colour becomes brilliant green.

Note the volume of the ferrous ammonium sulphate solution consumed.

Carry out a blank titration (without soil) in a similar way.

Calculation formula for OC%

$$\% \text{ Organic Carbon} = \frac{(B-S) \times N_{FAS} \times 0.003 \times 1.3 \times 100}{W}$$

Where,
B = Volume of FAS consumed for blank titration in mL
S = Volume of FAS consumed for sample in mL
N = Normality of FAS from blank titration
W = Dry mass of soil sample in g

In the analysis 1 ml of 1 N Dichromate solution is equivalent to 3 mg of carbon.

As this method is considered to have a 77 % recovery rate of organic carbon, a correction factor of 100/77 = 1.3is applied.

It is traditionally considered that the ratio of organic carbon in soil organic matter (SOM) is 58%, giving a conversion factor of SOC x 1.724 = SOM (Warington and Peake 1880). However, the proportion of carbon is not invariable, and a recent review by Pribyl (2010) shows that the conversion factor 1.724 is too low.

We therefore adopt a conversion factor of 2.0, i.e. SOM = 2.0 x SOC, and conversely, SOC = SOM/2 (or 50%). Similarly, we apply the same conversion factor, 50%, between organic C and dry biomass and use it in calculation of OC bound in the samples of (partially degraded) litter and woody debris.

Note:

- Sample preparation techniques should be carried out in a well-ventilated area and protective clothing must be worn when handling concentrated acids.
- Indicator diphenylamine is a carcinogenic, so it should be handled with extreme caution (wear gloves).
- Special masks should be used during machine grinding and sample preparation.
- Where the chloride content of the soil is high, some interference will occur. This can be suppressed by the addition of silver sulphate to the concentrated sulphuric acid at the rate of 25 g/L.
- Ferrous ammonium sulphate (FAS) solution should be prepared daily.

2.6 Computation of results for upland soil, litter and woody debris OC stock

The data gathered in the field and the results of laboratory analysis are ultimately combined in an Open Source Forest Information System (OSFIS). Thus the soil-related parameters can be combined with those describing the tree stands. As a first step, an excel workbook application has been created for help in calculation of the results obtained in laboratory. The sampling location of litter, woody debris, and soil are identified in the OSFIS using the codes for plot and forest stand within an inventory cluster, *Cluster number* (identifying the cluster as stored in the field team's GPS), *Plot* and *Stand* (identifying the exact part of the inventory plot where the samples were taken), and *Layer* (identifying the sample layer of litter (and woody debris), 0-10. 10-20, 20-30, or 30-60 cm). In addition to the strictly defined key location codes, free form laboratory codes are needed for the internal book-keeping of the laboratory.

The excel application (workbook) is divided into separate worksheets as follows.

Worksheet name	*Functions*	*When to fill the worksheet*	*Obs.*
Field data	Stores the obligatory location codes, background information for the inventory mission and each sample	Can be filled after inspection of the samples and the field forms. Fill this worksheet *first*	Background data becomes automatically available in other worksheets of the workbook
Titration	Stores titrant properties and consumption Automatically calculates OC%	After titration of the sample(s)	
Mass, BD, C store	Stores weighing results from the field and laboratory Automatically calculates soil C stores per each soil layer	After weighing the soil samples	The soil C stores are calculated only after the titration worksheet is also filled
Litter and debris	Stores weighing results of Litter and Woody debris from the field and laboratory Automatically calculates C stores in litter and woody debris	After weighing the litter and debris samples	
Summary		Is automatically filled using the data in other worksheets	Copy-paste values when combining with results from other workbooks

A blank workbook allows for 200 data rows (individual samples) to be stored, i.e. the formulas are copied on the first 200 rows the sheet. They can be copied further downwards if desired. While it is possible to expand the formulas by copying the cells containing functions further down, a separate workbook for a particular region (inventory mission) could be established for convenience or parallel laboratory work of separate sets of samples. This is by no means necessary. If the results are kept in separate excel workbooks, the Summary sheets may need to be combined for statistical use with other inventory data.

Work flow for laboratory measurements and analyses and calculation of the results is possible to make so that the different worksheets are updated as soon as the information becomes available. The data input work can even be divided between separate persons. It is useful to keep the conventional laboratory record in paper form as well.

When a new workbook image is created

- Open the Blank Workbook that is kept in safe both in another folder of the hard disk and in a safe copy device such as CD or memory stick.
- Save the empty workbook with a new name, e.g. "KienaFarmForest_2013"

Data input

- Go to worksheet "Field data" and start to input the new samples background and location data
- When the samples have been weighed ad/or titrated for TOC, fill in the columns marked in red colour. The function cells automatically calculate the results, when the necessary data is input.
    - Note that the worksheets try to observe if there is a value in any input cell that does not seem logical with respect to other values, e.g. those measured in the field. The misfit is indicated by displaying "Check data" in the function cell affected by the problem.
    - When "Check data" appears, the measured values have to be checked for correctness. If the problem can be identified to the field measurements that cannot be re-made, the observation may become invalid and the respective fields left blank.

The function cells (that automatically calculate the results) of the workbook are protected against any accidental changes by typing over etc. There is no password, so the worksheets can be easily unprotected if the functions must be edited. However, changing of the function cells must be done with a clear purpose and the changes must be applied in all cases where the change applies, even in other workbooks. After modifying the functions, it is advised that the worksheets are protected again.

Note that when there are non-volumetric soil samples representing the depth layer 30-60 cm, the soil carbon store figures cannot be normally computed. The rough evaluation of deep soil TOC stores has to be done using other methods. However, the excel workbook allows for calculation of the OC% according to titration results.

3 Procedures for mangrove sediment analyses

3.1 Receiving and preparation of mangrove sediment samples

Mangrove sediments are often water-saturated and highly saline due to their origin in tidal wetlands. *In situ* they are also anoxic or hypoxic, the condition which reduces decomposition rate of organic matter. When the samples are taken, they are exposed to oxic conditions. The procedures of sampling attempt to ensure that the sediment samples are packed as air-tightly as possible. In order to preserve the samples optimally, they should be kept in cold, dark container and transported to laboratory as soon as possible. A cooler box with ice could provide such conditions, but when the samples are transported from coast to upcountry laboratories, the capacity of the cooler box may not be adequate. Therefore, the samples may be exposed to high temperatures over varying periods of time after they have been extracted from the mangrove sites.

When the samples arrive, they should be immediately identified, their ID and location information entered in the specific mangrove excel workbook, and without delay prepared for oven drying. If it is not possible start the drying immediately, the samples can be kept refrigerated for a short period of time (1-2 days), or they can be stored in a freezer at –20ºC for longer periods of time.

3.2 Processing of mangrove sediment samples

Oven drying
Aluminium foil is cut and folded in a shape so that it adequately holds the about 1.9 dl sediment sample. A cup-like form is preferable, because that will optimize the oven capacity. The dry aluminium "cup" is first weighed and its mass marked on the cup.

The sediment sample is taken carefully from its plastic bag and quantitatively transferred to the aluminium cup. The sample ID information from the masking tape tag on the plastic bag has to follow the sample during its processing. Make sure no material is lost during the transfer and all materials from the plastic bag are taken. Place the samples directly to the oven at temperature of –60ºC. The drying process may take several days until all removable water has evaporated, and the samples have reached constant mass.

When the sample has been dried to constant mass in the cup of known mass, the samples will be weighed for determining of bulk density using a laboratory table-top scale (not analytical scale). Then the mass of the aluminium cup is subtracted from the total mass. Because the samples tend to adsorb a considerable amount of water from air humidity as soon as they are taken from the oven, it is advised that the samples are weighed at immediate vicinity of the oven, and taken one by one from the oven directly to the scale. Since the samples are hot, the scale has to be protected using a heat-insulating plate on the scale. The mass of the plate has to be subtracted (tare weight) so that the scale reading is zero with the plate on the scale) prior to putting the sample on the heat-resistant plate. The weighing results can now be recorded in the mangrove excel application.

3.3 Homogenization of mangrove sediment samples

The dried samples are carefully homogenized using a mortar. Any root can be picked out and discarded. The homogenised samples are sieved using a 2 mm sieve to remove roots and other large particles, after which they can further be sieved using a 0.5 mm for improved homogeneity. Protective mask and cloves should be used when handling the samples.

3.4 Procedures for mangrove sediment TOC analysis

It may be possible to analyze the organic carbon content using the Walkley-Black methodology. However, as the sediment sample has high salinity and thereby probably a high chloride concentration, that should be taken into account in the Walkley-Black procedure as indicated for upland soil analyses earlier in this manual. The organic carbon content in mangrove sediments may be very high, even 30-40 %, and it can be expected that the Walkley-Black procedure does not recover the OC% as accurately as in lower concentrations. Commonly applied method for OC% analysis from high organic matter materials such soils, manure, compost, or organic rich sediments is the Loss On Ignition (LOI) method. In that method, a sample with accurately known dry mass is ignited slowly to a temperature where organic matter is removed by burning.

The KEFRI Soil Science Laboratory Manual for analysis methods, Chapter 22 gives methods to analyze such materials. This method can be followed with an exception that the temperature in muffle furnace should not exceed 450°C. The reason for the upper limit of the temperature is that mangrove sediments may contain significant amounts of carbonates from the underlying coral reefs. The procedure for determining total organic carbon stocks in mangrove sediments is given below.

Apparatus. Muffle furnace (450°C capacity).

Procedure

1. Weigh 10±0.1 g of oven dry sediment, homogenized as explained before, in a dry porcelain or nickel crucible.
2. Heat slowly in a furnace (raising the temperature setting in steps of 100, 200 and 450°C). The final temperature setting of 450°C should be maintained for 8 hours.
3. Remove the crucible containing a greyish white ash. Cool in desiccator and weigh.

3.5 Computation of results for mangrove sediment TOC storage

The percentage of ash and organic matter are calculated (using the mangrove Excel application) by the differences in weight of the crucibles before and after combustion as follows:

$$\text{Ash (\%)} = [(W3 - W1)/(W2 - W1)] * 100 \text{ and}$$

$$\text{Organic matter (\%)} = 100 - \text{Ash (\%)}$$

where W1 = the weight of the empty, dry crucible; W2 = the weight of the dry crucible containing the sample; and W3 = the weight of the dry crucible containing the sample following ignition. Note that the weight of the ash = W3 – W1.

Remarks. It may not be possible correct between air dry and oven dry sample masses, because the mangrove sediment samples are not air dried and the respective moisture content ratio is not known. The samples and crucibles need to oven dried if they have been exposed to free, humid air.

Carbon content. The OC (%) of the sample can be calculated (using the mangrove Excel application) from the Organic matter (%) as follows:

OC (%) = Organic matter (%) * 0.50 *see Pribyl (2010) for the coefficient between OM and OC*

Carbon stocks (t ha^{-1} in 100 cm deep layer of the sediment). The carbon stock in the 0–100 cm sediment layer is calculated using LOI results and the mangrove excel application. In short, the sediment samples, taken from the center of the core sections of 0–15, 15–30, 30–50, and 50–100 cm, are considered to represent the OC% and bulk density of the respective layers in the sediment. Using those assumptions, the TOC stocks of each layer, and the total TOC stock of the 100 cm deep sediment layer can be calculated.

The field measurements include systematic probing of the sediment depth within the CCSP, or forest stand within the CCSP, giving the average depth of the sediment if it is less than 100 cm. The average depth is used for correcting the TOC stock in areas with shallow sediment depth. If the sediment is deeper or equals to 100 cm, no correction is taken, and the results represent the TOC stock in the top 100 cm layer of the sediment.

TOC, t $ha^{-1}$for the 0–15 cm sediment layer is calculated as follows:

$$TOC_{0–15\ cm} = BD_{0–15\ cm} * Volume_{0–15\ cm} * OC\ (\%)_{0–15\ cm}/100 * 10^{-6} * 10^{4},$$

where BD is dry bulk density (g cm^{-3}) of the layer; Volume(cm^3), e.g. volume of a 0–15 cm layer is 150 000 cm^3; OC (%) is the proportion of organic carbon (%) in the layer; 10^{-6} is the conversion factor from g to t; and $10^4$is the conversion factor from m^2 to ha, respectively.

The TOC stock for the whole, cored0–100 cm sediment layer is calculated as a sum of the layers analyzed and computed separately:

$$TOC_{0–100\ cm} = TOC_{0–15\ cm} + TOC_{15–30\ cm} + TOC_{30–50\ cm} + TOC_{50–100\ cm}$$

In the mangrove excel application the sediment layer results are kept separate and they will be combined before the data set is transferred to the OSFIS (Open Source Forest Inventory System). Keeping the layers separate provides greater accuracy and versatility in research use of the data.

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