4.2. METHODS FOR SAMPLING ABOVE- AND BELOW-GROUND ORGANIC POOLS

By:

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4.2.1. INTRODUCTION

The following research protocol was developed as part of the global ASB (Alternatives to Slash-and-Burn) project, to allow comparison of data collected in Indonesia (Lampung, Jambi), Cameroon, Brazil and Peru. The data collected can be used:

- directly to assess the current C stock in above- and below-ground pools;
- to extrapolate to the 'time-averaged C stock' of a land-use system;
- to initialise the CENTURY (or similar) simulation model for C, N and P dynamics of the various pools of organic matter; and/or
- compare biodiversity and profitability assessments with C stock data to study trade-offs among global environmental benefits and private incentives to the farmer.

The following text is an update from Palm *et al.* (1994). For the soil methods the basics are covered in the TSBF handbook of methods (Anderson and Ingram, 1993). Results of the C stock assessments in Indonesia are summarised in Tomich *et al.* (1998).

4.2.1.1. CHOICE OF SAMPLE SITES

The samples are chosen to represent a certain stage of a land-use pattern, as it develops in a typical cycle. In general, two rectangular plots (5 m x 40 m = 200 m^2) are selected within a plot of at least 1 hectare, avoiding borders of the plot, unless specifically indicated in the sample design. Plot location is stratified if there are marked discontinuities in the vegetation. In other words, be sure that the two plots do not all fall in the area with the densest or least vegetation. Measurement of above-ground biomass in this protocol includes destructive and non-destructive sampling, for the litter and undergrowth layer, and the trees, respectively.

The rectangular plots are chosen, as they tend to include more of the within-plot heterogeneity, and thus be more representatives than square or circular plots of the same area. The larger the total area sampled the more accurate the estimate. Instead of sampling a

large, contiguous area it is better to divide the sampling into several, smaller, areas within the field of study (randomly chosen or based on some a priori stratification).

4.2.1.2. OVERVIEW OF METHODS

Above-ground biomass data collection procedures are summarised in Table 4.1.

Table 4.1. Above-ground parameters and methods used in C-stock measurement.

PARAMETER	METHODS
1. Understorey	Destructive
2. Litter: • Coarse/standing litter • Fine litter • Surface roots	Destructive
3. Charcoal	Destructive
4. Ash	Destructive
5. Living trees	Non-destructive, apply allometric equation
6. Dead standing trees	Non-destructive, apply allometric or cylinder equation (for branched & unbranched remains, respectively)
7. Dead felled trees	Non-destructive, apply cylinder (or allometric) equation
8. Stump (trunk) remains on forest	Non-destructive, apply cylinder equation

4.2.2. BIOMASS AND NECROMASS ESTIMATION BY NON-DESTRUCTIVE SAMPLING

An estimate of the vegetation biomass can provide us with information about the nutrients and carbon stored in the vegetation as a whole, or the amount in specific fractions such as extractable wood. To measure the biomass of vegetation which includes trees is not easy, especially in mixed, uneven-aged stands. It requires considerable labour and it is difficult to obtain an accurate measurement given the variability of tree size distribution. It is hardly ever possible to measure all biomass on a sufficiently sample area by *destructive sampling* and some form of *allometry* (see Section 4.2.1.2) is used to estimate the biomass of individual trees to an easily measured property such as its stem diameter. Allometric equations can be locally developed by destructive sampling, derived from literature for supposedly comparable forest types, or estimated from fractal branching analysis (compare Section 4.3). They normally use the tree diameter at breast height (DBH, measured 1.3 m above the ground) as basis, but for trees with plank roots or trees damaged by tapping

panels or holes, as found in rubber or *damar* trees, adjustments may be necessary. Empirical equations for total biomass can have a polynomial form $Y = a + b.D + c.D^2 + d.D^3$, or follow a power function: $Y = a\ D^b$, with the b parameter typically between 2 and 3. The polynomial equations are clearly restricted to the range of D values used for deriving the model, as for D = 0 they predict a biomass of a, and they have one or more points of inclination. The power function is continuously rising and passes through the origin, so its general shape is more attractive. The parameters of allometric models can be derived directly from empirical data by regression analysis, or via the parameters of a fractal branching model as explained in Section 4.3. For palms, bamboo's and rattans separate equations are needed, as their stem diameter does not increase by secondary thickening and thus does not reflect actual canopy size. If possible, the equations used for estimating biomass should be developed for each location, species, or group of species, and for trees of similar sizes and ages. For example, equations derived from destructive sampling of a virgin forest, where many of the trees have dense wood and are tall, will not be appropriate for estimating biomass of a young secondary forest where there are many soft-wood trees, branching at lower heights.

For the purposes of the Alternatives to Slash and Burn projects, if equations have not been developed at the sites, the equations of Brown *et al.*, 1989 can be used (Table 4.2). The equations developed by Brown and colleagues are based on diameter (D) at breast height (1.3 m); height of tree (H); and the density of the wood (s). Often only diameter measurements are possible to obtain; however the estimates generally improve with more parameters. Separate equations have been developed for tropical forests in different rainfall regimes: dry < 1500 mm rainfall per year; moist 1500-4000 mm; and wet > 4000 mm.

Table 4.2. Allometric relations for estimating biomass from tree diameter (for D > 5 cm) and height (after Brown, 1997).

Life zone *(Rainfall, mm/yr)*	Equation *(Y=tree biomass, kg/tree; D=DBH, cm; H= height, m)*	Range *(cm)*	Number of trees	R^2
Dry (<1500 mm)	$Y = 0.139D^{2.32}$	5 - 40	28	0.89
Moist (1500-4000 mm)	$Y = 42.69 - 12.8D + 1.242D^2$	5 - 148	170	0.84
	$Y = 0.118D^{2.53}$	5 - 148	170	0.97
Alternative	$Y = 0.092D^{2.60}$	5 - 148	170	-
Wet (>4000mm)	$Y = 21.3 - 6.95D + 0.74\ D^2$	4 - 112	169	0.92
	$Y = 0.037D^{1.89}H$	4 - 112	169	0.90

Once an allometric equation has been established for different classes of trees in a vegetation, one only needs to measure DBH (or other parameter used as a basis for the equation) to estimate the biomass of individual trees. The sum of the biomass estimates for all trees within the measurement transect can be converted to a biomass in Mg ha^{-1}. This non-destructive method is rapid and a much larger area and number of trees can be

sampled, reducing the sampling error encountered with the destructive method. Yet, half of the biomass of a natural forest can be in the few trees of the largest diameter class (> 50 cm) and sampling error is still high for a 200 m² transect which can have 0, 1 or 2 large trees included (Table 4.3). Accuracy would be improved if trees with a DBH above say 30 cm would be sampled in a 20 m x 100 m sampling area. After a slash-and-burn event or forest fire, the remaining charred trees, branches and litter can be measured following the same protocol.

Box 4.1. Sampling protocol for live tree biomass

Equipment:

1. *Line for center of transect, 40 m long*
2. *Sticks to measure width, 2.5 m long*
3. *Wooden sticks of 1.3 m length*
4. *Measurement tape (linear or special ones for tree diameter, which include the factor π)*
5. *Knife*
6. *Tree height measurement device (e.g. 'Hagameter', optional)*

Procedure:

Set out two 200 m² quadrats (5 m × 40 m), by running a 40 m line through the area and then sampling the trees > 5 cm diameter that are within 2.5 meter of each side of the tape, by checking their distance to the central line. For each tree the diameter is measured at 1.3 m above the soil surface, except where trunk irregularities at that height occur (plank woods, tapping or other wounds) and necessitate measurement at a greater height. If trees branch below the measurement height, all branches > 5 cm are measured at 1.3 m above the ground and an equivalent diameter is defined as $SQRT(\Sigma D^2)$ on the basis of all D values. Further tree information, e.g. botanical species or local name is optional but can help in getting improved estimates of wood density.

If trees > 50 cm diameter are present in the sampling plot, whether or not they are included in the transect, an additional sample of 20 m × 100 m is needed where all trees with a diameter > 30 cm are measured.

Calculations:

Calculate the tree biomass in kg/tree for each tree using an appropriate allometric equation (see Table 4.2 if no site or tree specific equations is available). Palms, bamboo's and lianas need a separately established equation.

Box 4.2. Sampling protocol for tree necromass

Sum the tree biomass for each quadrat and divide by the sampling area in m^2. If a large plot for big trees is used, exclude trees > 30 cm from the biomass calculations for the smaller plots.

Procedure

Within the plot of 200 m^2 (5 m x 40 m) all trunks (unburned part), dead standing trees, dead trees on the ground and stumps are sampled that have a diameter >5 cm and a length of > 0.5 m. Their height (length) is recorded within the 5 m wide transect (see Figure 4.4) and diameter (halfway the length included), as well as notes identifying the type of wood for estimating specific density.

Specific gravity (wood density) of dead wood (optional):

In advanced stages of decomposition standard rings normally used for measuring soil bulk density can be driven into the wood and recovered for drying and weighing. Otherwise drills should be used to obtain a 'plug' of known volume.

Calculations

For the **branched** *structures an allometric equation is used, as for live trees. For* **unbranched** *cylindrical structures, an equation is based on cylinder volume:*

$$Biomass = \pi.D^2.h.s/40$$

where, biomass is expressed in kg, h = length (m), D = tree diameter (cm) and s = specific gravity (g cm^{-3}) of wood. The latter is estimated as 0.4 g cm^{-3} as default value, but can be around 0.7 for dense hardwoods, around 0.2 for very light species, and generally decreases during decomposition of dead wood laying on the soil surface.

Table 4.3. Expected number of trees in sample plots of different size.

Diameter (cm)	Average number per ha	Expected number per plot	
		2 x (5 x 40 m^2)	20 x 100 m^2
5-10	400	16	-
10-30	200	8	-
30-50	50	2	10
50-70	10	0.4	2
>70	4	0.1	1

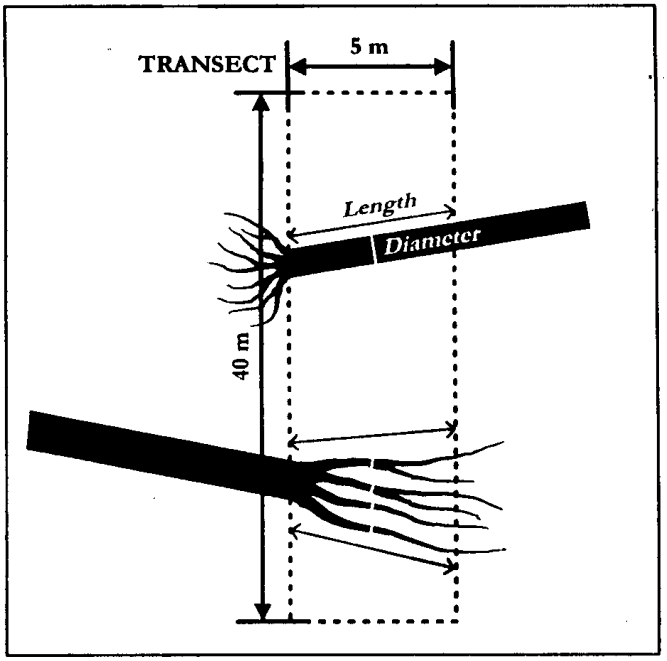

Figure 4.4. Measuring length and diameter to estimate biomass of fallen or felled trees in a transect after slashing and burning.

4.2.3. DESTRUCTIVE SAMPLING OF UNDERSTOREY AND LITTER LAYER

In destructive sampling, the vegetation in a given area is cut and weighed (fresh weight), and subsamples of parts of the vegetation (understorey biomass, coarse litter, unburned branches (< 5 cm diameter or < 50 cm length), flowers and fruits) are taken, weighed fresh in the field, subsampled and weighed again after oven-drying.

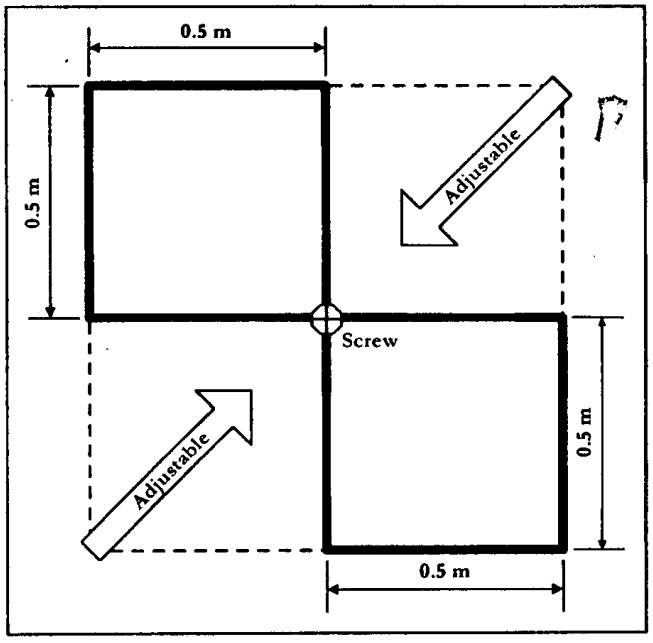

Figure 4.5. Design of a sampling frame, which can be used for 1 m x 1 m samples, or for two adjacent 0.5 m x 0.5 m samples.

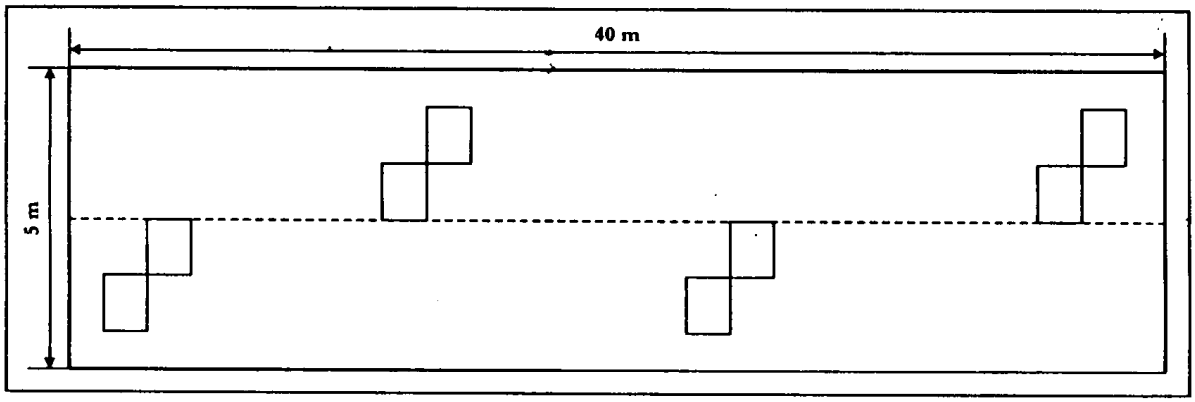

Figure 4.6. Position of understorey sampling within a 40 m x 5 m vegetation transect.

Box 4.3. Field sampling protocol for destructive sampling of understorey biomass and litter layer

Equipment:

1. *Quadrat of 1 m x 1 m and 0.5 m x 0.5 m (Figure 4.5)*
2. *Knives and/or scissors*
3. *Scales: one allowing weights up to 10 kg (with a precision of 10 g) for fresh samples and one with a 0.1 g precision for subsamples*
4. *Marker pens, plastic and paper bags*
5. *Sieves with a 2 mm mesh size*
6. *Trays*

Field procedure

Locate sampling frames within the 40 m x 5 m transect, as indicated in Fig. 4.6, placing it once (randomly) in each quarter of the length of the central rope for 4 x (1 m^2) or 8 x (0.25 m^2) samples.

Understorey biomass: *All vegetation less than 5 cm DBH is harvested within the 1 m x 1 m quadrat. Weigh the total fresh sample (g m^{-2}), mix well and immediately take and weigh a composite fresh sub sample (~300 g), for subsequent oven drying.*

Litter is sampled within the same frames in two steps:

- **Coarse litter:** *(any tree necromass < 5 cm diameter and/or < 50 cm length, undecomposed plant materials or crop residues, all unburned leaves and branches) is collected in 0.50 m x 0.50 m quadrats (0.25 m^2), on a randomly chosen location within the understorey sample. All undecomposed (green or brown) material is collected to a sample handling location.*

Box 4.3. Field sampling protocol for destructive sampling of understorey biomass and litter layer (contd.)

- ***Fine litter:*** *Subsequently collect the 0-5 cm soil layer in the same quadrats (including all woody roots) and dry-sieve the roots and partly decomposed, dark litter. If time allows, the sieving can be done on-site, but it may be more convenient to collect bags of the topsoil and process elsewhere.*

Box 4.4. Sample handling for destructive biomass and litter samples

- ***Biomass:*** *Dry the subsample at 80°C for conversion to dry weight and for analysis of C, N, and its quality (lignin and polyphenolic concentration which influence the decomposition rate of organic material); if oven capacity is limited, samples can be sun dried (in a ventilated plastic shelve system) and only sub-subsamples processed in the oven.*
- ***Coarse litter:*** *To minimize contamination with mineral soil, the samples should be soaked and washed in water; the floating litter is collected , sun dried and weighed, the rest is sieved on a 2 mm mesh sieve and added to the fine litter fraction. Depending on the total amount, a subsample can be taken at this stage for obtaining an 'oven-dry' correction (oven at 80°C). As alternative to the washing procedure, samples can also be ashed (at 650°C) to correct for mineral soil contamination.*
- ***Fine litter and roots:*** *The litter (incl. dead roots) and (live) root material collected on the 2 mm sieve (by dry sieving) is washed and dried. The soil passing through this sieve is collected as 0-5 cm sample for C_{org} or C fraction analysis (see below).*

Calculations:

$$\text{Total dry weight (kg m}^{-2}) = \frac{\text{Total fresh weight (kg)} \times \text{Subsample dry weight (g)}}{\text{Subsample fresh weight (g)} \times \text{Sample area (m}^2)}$$

Take the average of the 8 samples to record the understorey and litter biomass for the transect replicate.

Special Concerns

Burned (partly burned) litter, charcoal and ash in sampling sites directly after burning

The burned and unburned woody litter, charcoal, and ash are collected and separated from eight 0.5 m x 0.5 m quadrats as described for coarse litter, but no sample washing is necessary.

Establishing site-specific allometric equations

All trees over 5 cm DBH are sampled in a rectangle sampling area 40 m x 5 m (200 m^2). Each tree is cut. Height and DBH are recorded (and can be used later for producing site specific allometric equations). The tree is separated into leaves, small branches (less than 2.5 cm diameter), large branches (greater than 2.5 cm diameter), and trunk (which includes the largest branch to 2.5 cm diameter). Each fraction is weighed fresh in the field. Fresh subsamples are taken and weighed, then dried (80°C) to correct for water content. Subsamples can also be used to determine nutrient contents. If the branching points are described as explained in Section 4.3, the validity of the fractal branching model can be tested. Additional samples of specific gravity (g cm^{-3}) of the woody fractions, as well as the specific leaf area (m^2 g^{-1}) and average area per leaf are necessary for a full implementation of the FBA model (see Section 4.3).

4.2.4. SOIL SAMPLING PROCEDURES

Two types of soil samples: disturbed soil samples for chemical analysis (where the results will be expressed per unit dry weight of soil) and undisturbed soil samples for physical analysis, especially the 'bulk density' (specific gravity) of the soil, which is essential to convert the soil dry weights into soil volume.

Box 4.5. Procedure for taking soil samples for chemical analysis

Field procedure

1. *Continue after removing the 0-5 cm (usually organic) layer (see above), and take samples of the 5-10 cm, 10-20 cm and 20-30 cm soil depth. Approximately 1 kg of fresh soil is sufficient, combining soil from three patches within the 0.5 m x 0.5 m sample grid, to obtain 24 subsamples per 5 m x 40 m transect per layer.*
2. *Soil samples from the same depth taken in the replicate sampling grids within a single transect can be combined directly in the field, or subsequently mixed in the sample processing site.*

Sample processing

3. *Mix the composite sample thoroughly, and divide into 3 bags: 1 kg of fresh soil for LUDOX fractionation, 0.5 kg for chemical analysis and another 0.5 kg of soil for archiving; the remainder can be discarded*
4. *Air dry the soil of all three subsamples by placing them in a shallow tray in a well ventilated, dust and wind free area. Break up any clay clods, and crush the soil lumps so that gravel, roots and large organic residues can be removed*
5. *Sieve the soil samples intended for chemical analysis through a 2 mm sieve, and grind them in a mortar in order to pass through a 60 mesh screen.*
6. *Sieve the soil samples intended for SOM fractionation (without grinding). For further treatment see procedure SOM fractionation in Section ?.*
7. *Write clear labels for each sample using a water proof marker pen of each sample, and wrap into a second plastic bag to prevent it from physical damage during transportation. Send it to laboratory for chemical analysis (Table 4.4).*

The sampling depths for soil analysis may have to depend on the data comparisons you want to make, and the costs for analysing them in the laboratory. The 0-5 cm layer is the most sensitive to land-use change. Around 20 cm maybe a natural transition in many upland forest soils. The scheme recommended here is coordinated with the soil macro-fauna protocol (see Section 4.5). For some purposes, sampling down to 1 m is required.

Table 4.4. Soil chemical analysis.

SOIL PARAMETERS	METHODS
pH_{H2O}	1:1 H_2O
pH_{KCl}	1:1 1 M KCl
C-org, %	Wet oxidation, Walkley and Black
Total N, %	Kjehldahl
P-Bray2, mg kg^{-1}	Molybdate blue, spectrophotometer
K-exch, $cmol_c$ kg^{-1}	1 M NH_4OAc pH 7, Flamephotometer
Na-exch, $cmol_c$ kg^{-1}	1 M NH_4OAc pH 7, Flamephotometer
Ca-exch, $cmol_c$ kg^{-1}	1 M NH_4OAc pH 7, Flamephotometer
Mg-exch, $cmol_c$ kg^{-1}	1 M NH_4OAc pH 7,Flamefotometer
Al-exch, $cmol_c$ kg^{-1}	1 M KCl, Titration method
H-exch, $cmol_c$ kg^{-1}	1 M KCl, Titration method
ECEC, $cmol_c$ kg^{-1}	K+Na+Ca+Mg +Al-exch + H-exch
Al-saturation, %	(Al-exch / ECEC) x 100%
Sand, %	Pipette
Loam, %	Pipette
Clay, %	Pipette
LUDOX fractions (light, intermediate and heavy), g kg^{-1} soil	Size and particle density fractionation – see Section 4.2.5

Box 4.6. Undisturbed soil sample for soil bulk density measurement

REMEMBER:
Data quality of this property are scarce and potential land-use impacts large).

Equipment:

1. *Ring samples (stainless steel) with a sharp edge and of known volume and 100-200 cm3, for example 5 cm diameter and height*
2. *External ring to push ring samples gently into the soil*
3. *Soil knife to remove the ring and any excess soil adhering to it*
4. *Plastic bags, rubber bands and marker pen*

Procedure:

1. *Sample close to the sample sites for destructive samples, but avoid any place with possible soil compaction due to other sampling activities*
2. *Remove the coarse litter layer and insert the first ring gently directly from the soil surface, to sample the 0-5 cm depth layer; if the sample could not be inserted smoothly (e.g. due to woody roots or stones), try again nearby*
3. *Excavate the soil from around the ring and cut the soil beneath the ring bottom*
4. *Remove excess soil from above the ring using a knife: first remove excess soil on top of the sample, then place a cover on top of the ring and turn it upside down to remove soil adhering to the ring and cut a smooth surface at the bottom of the ring*
5. *Either transport the cleaned ring to the laboratory, or remove all soil from the ring to a plastic bag, which is closed immediately*
6. *On a nearby site, remove the top 5 cm of soil and insert a ring for sampling the 5-10 cm depth layer in a similar way. Repeat for the 10-20 and 20-30 cm depth layer, taking samples around 15 and 25 cm depth*
7. *One set of ring samples per sample quadrant will give you 8 (16) per 5 x 40 m^2 transect and 16 per land-use sample*

Sample processing:

Weigh the samples fresh (W1), dry at 105 °C for 2 days, and weigh again (W2):

- *Bulk density = W_2 / V, (g cm^{-3})*
- *Volumetric soil water content (Theta) = $(W_1 - W_2)/V$, (cm^3 cm^{-3})*

4.2.5. BELOW-GROUND BIOMASS

4.2.5.1. INTRODUCTION

Roots as carbon stock or organic inputs in tropical agriculture have often been neglected due to difficulties in measurement. Two techniques for measuring root biomass are a semi-quantitative and a quantitative one, based on root mapping and monolith (pinboard) sampling, respectively. Both techniques involve destructive plot. These methods are explained here in more detail than in Anderson and Ingram (1993).

4.2.5.2. ROOT MAPPING ON PROFILE WALLS

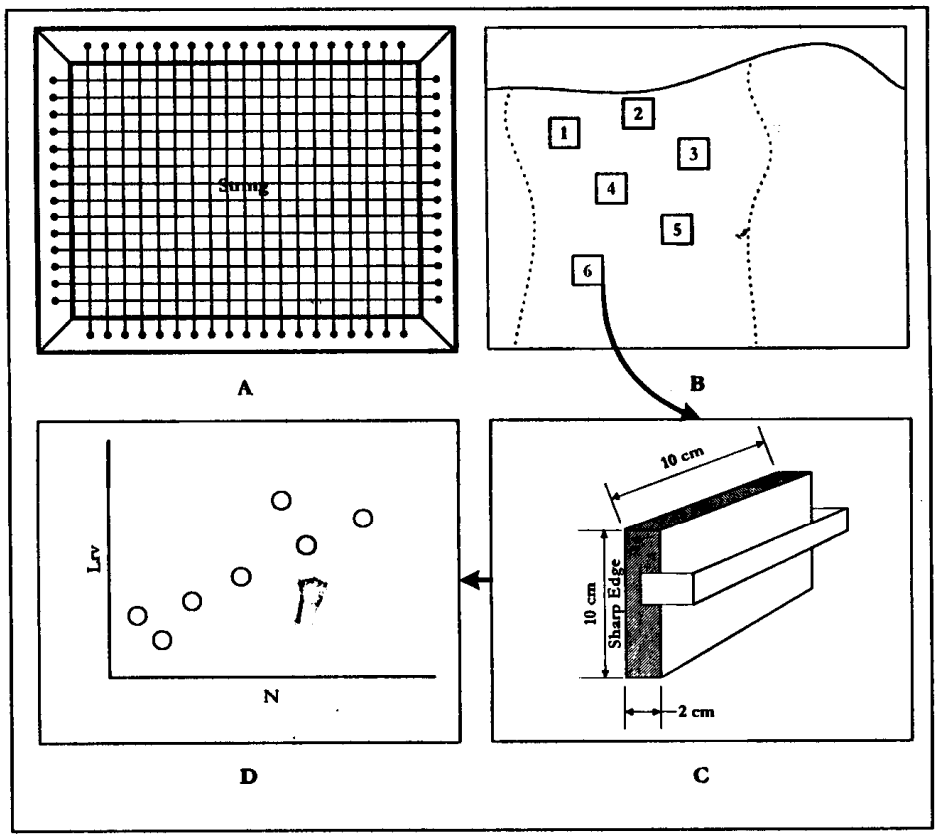

Figure 4.7. Root mapping. A. Frame with 10 x 10 cm grid of strings, B. Possible position of subsamples on the profile wall, C. Metal box for taking subsamples for washing fine roots and measuring their length, D. Calibration line of the number of intersections per unit area and root length density per unit soil volume for the subsamples.

Box 4.7. Root observations on profile walls

Equipment:

1. *Quadrat (60 cm x 100 cm) grid (10 cm x 10 cm) net*
2. *Knapsack sprayer*
3. *Soil knife*
4. *Spade*
5. *Needle*
6. *Scissors*
7. *Transparent Polytene (PVC) sheets*
8. *Marker pen*
9. *Filter paper*
10. *Metal Box (10 cm x 10 cm x 2 cm)*

Field procedure

1. *Dig a soil pit close to the plant (about 10 cm) selected for study.*
2. *Identify some main roots, and carefully follow their course in the profile (abandon them when they disappear too far beyond the plane of observation) and observe root distribution and rooting depth; then smoothen the profile wall.*
3. *Cut roots which stick out of profile wall and clean the soil profile with a sharp knife.*
4. *Spray the profile wall with some water to remove about 2 mm of soil to expose roots. (For clay soil gently brushing the profile wall may help).*
5. *Place a clear PVC sheet on the profile wall and carefully place the grid wooden frame on it.*
6. *Mark major features in soil structure (e.g. soil crack, termite holes etc), and also horizon boundaries.*
7. *Mark all roots with dots on sheet, differently coloured pens can be used for different size classes or plant species. Branch roots outside the observations plane can be neglected. Use the grid to work systematically and pay equal attention to all grids.*
8. *Calibration Line (optional): Take about 12 small block samples (circa 20 cm x 10 cm x 2 cm) from various layers (Figure 4.7); map the position of each sample on the map and store the sample in a plastic bag with a label referring to the number of the root map and the sample number.*
9. *When the map is complete, use the upper right corner to write the date, location, map number and persons mapping the roots. Then take the map off the profile, dry it and store between filter paper (to prevent 'printing' additional roots).*

Data analysis

Root maps: For analyzing data, cover the map with a 10x10 cm grid and count the number of interceptions per cell. Express results as (N, number of dots cm^{-2}) per soil

horizon (or depth interval) and as a function of distance to the plant. More advanced methods of map analysis can quantify spatial correlation of roots and other map features (cracks, termite holes, roots of another species), but these need some form of computer image analysis tools.

Calibration line: Wash the sample on a fine sieve (0.3 mm mesh), determine total root length by counting intersections with a grid (Anderson and Ingram, 1993) and calculate root length density, L_{rv} (cm cm^{-3}). Dry the subsample, weigh and express as root weight density D_{rv} (mg cm^{-3}). For each subsample also count the number of intersections (N, cm^{-2}) with the map and make a calibration line of L_{rv} versus N and one for D_{rv} versus N. If roots have no preferential orientation and all roots are mapped correctly, the calibration line should be approximately $L_{rv} = 2$ N. Total root biomass per unit area can now be calculated from root counts N for the whole map and the calibration line.

Potential problems with this method:

a) Roots of different plants may be hard to distinguish (it helps to trace some of them to the stem base to be sure of their identity)
b) Distinction of live and dead roots is not easy.
c) A considerable fraction of fine roots may be overlooked, especially in the topsoil; an 'operator bias' is likely to remain and comparisons of maps made by different persons are less reliable (check with the calibration lines)
d) Difficulties of observing plants roots due to condensation behind the PVC (it helps to build a small shelter and avoid direct sunlight on the profile wall).

4.2.5.3. PINBOARD MONOLITH SAMPLING

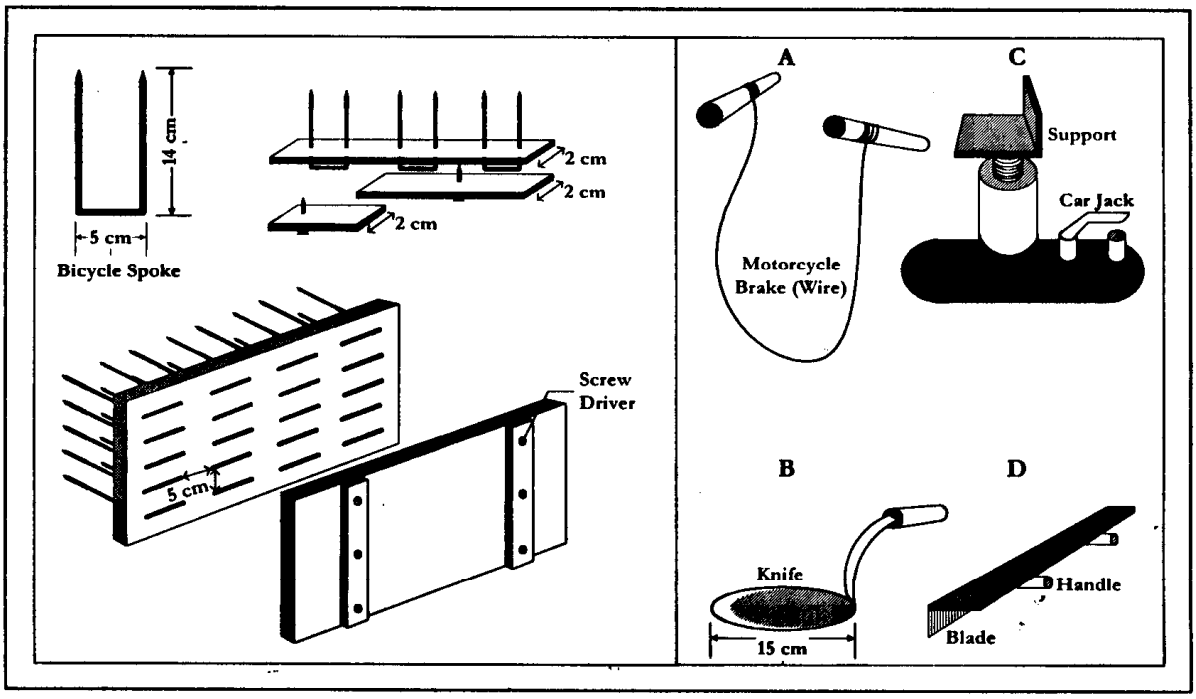

Figure 4.8. Left: Pinboard design. U-shaped pins are inserted into a board in order to hold the roots in place when soil is washed from a monolith. Right: Auxiliary equipment for taking root samples with pinboards: A. cutting wire, B. Knife, C. Jack to support the pinboard, D. Blade to smoothen a profile wall.

Box 4.8. Root sampling by pinboard

Equipment

1. *Pinboard ('fakir beds') are made by inserting U-shaped stainless steel pins into a piece of plywood or board with holes every 5 cm (Figure 4.8). These pins can be made from bicycle (in Indonesia:* becak*) spokes bent into a U-shape, with a 5 cm base and upright length of about 14 cm (if the plywood or board is 2 cm thick, this gives an effective sampling length of 12 cm). The tops of the pins are sharpened, but take care as it becomes a dangerous piece of equipment. After inserting the pins, a back cover is screwed on to the board. The size of the pinboard is determined by rooting depth of plants and practical considerations. The pinboard can be stored and transported in pairs, or in a disassembled state.*
2. *Coarse mesh screen, slightly larger than the pinboard*
3. *Spade*
4. *Blade*

Box 4.8. Root sampling by pinboard (contd.)

5. Rubber hammer
6. Car jack (Figure 4.8)
7. Knives
8. String (motorcycle brake) or a steel cable with diameter 2 mm
9. Old sacks (to pack the sample for transporting it)
10. Forceps
11. Thymol (bactericide, used to temporarily store wet root samples)

Procedure

1. Select a representative crop stand and note any weed growth surrounding it. Put the mesh screen on the pins and pull it down till reach the bottom of board.
2. Dig a soil pit next to the area to be sampled. The length and depth of the soil pit are determined by the root distribution and rooting depth of the plant to be observed. When plants are in the row, the pit should be dug perpendicular to the crop row. A width of about 0.5 m is required for working. Keep separate heaps for topsoil and subsoil in order to reduce long term site disturbance.
3. Smooth the profile wall where the sample is to be taken with a blade; the wall should be made straight.
4. Describe the soil profile; all relevant information are should be noted e.g. soil horizon, crack, termites hole, or old tree root channel and some soil physical parameters (Up to this stage the method can be combined with root mapping).
5. Place the pinboard vertically with the pins against the profile face, adjust so that the top row of pins is at ground level, and push the pinboard into the soil by hammering the back of the pinboard.
6. Remove about 15 cm (a few centimetre beyond the tips of the pins) of soil underneath the pinboard with a knife.
7. Support the pinboard with a car jack.
8. Cut away soil profile on both sides of the board, also a few centimeters further than the tips of the pins.
9. Put the steel cable along the bottom and up the other sides of board and have two persons draw it up in a sawing movement, so that the monolith is cut away from the soil mass. In the mean time, one person should stand in the soil pit and hold the sample on the car jack when it is cut free (take care when the steel cable emerges form the soil).
10. Pull the board backwards, and support it against the opposite wall of the soil pit; cut away soil until the level of the pins and any additional soil from the bottom and side of the sample.
11. Carefully lift the monolith out of the soil pit (now you'll notice why you should not make the pinboards too large).
12. Label the sample and wrap it in old sacks for transport to the laboratory.

Removing soil and root washing

1) Soak the monolith sample overnight in water.
2) Spray with water gently, start from the bottom and gradually go up to the surface layer; gradually lift the mesh screen so that water can pass underneath.
3) Remove debris and roots of unobserved crops out from the board using a forceps (for total biomass determine their weight).
4) Lift the mesh screen further, so that the root system can be taken out from the pinboard and take a photograph (use on a black cloth as background).
5) Cut the root systems according to thickness of soil horizon and to distance to the plant.
6) Store the root samples in plastic bags filled with water and thymol (a bactericide).
7) Store samples in the refrigerator if available, for further handling.
8) Take the root samples out of the plastic bags, and put into a clear box (25 cm x 15 cm x 7 cm) filled with water.
9) Remove all remaining debris and soil, and determine root length (Anderson and Ingram, 1993), root diameter (if needed) and root dry weight (dry in oven at 80°C for 2 nights). Root length density and root diameter measurements are important parameters for study nutrient uptake only.
10) Estimate total biomass per plant by integrating root weight density per zone and depth over the relevant volume of soil.

Disadvantages

1) It takes much time (Labour), especially for washing and cleaning the subsamples; the method is a lot faster, though, than methods based on soil cores.
2) Some roots might be broken and lost during washing.
3) The soil pits disturb the land in long-term experiments.

Advantages

1) Quantitative assessment of root biomass with less effort than by coring.
2) Distinction between roots of different plants and between live and dead roots is possible.

4.2.5.4. ESTIMATING TREE ROOT BIOMASS FROM ALLOMETRIC RELATIONS

Similar to the approach of above-ground biomass via allometric relations based on stem diameter, the below-ground biomass can be estimated from the proximal roots at the stem base. The theoretical basis for this relation is found in the fractal branching properties (see Section 4.3) of root systems.

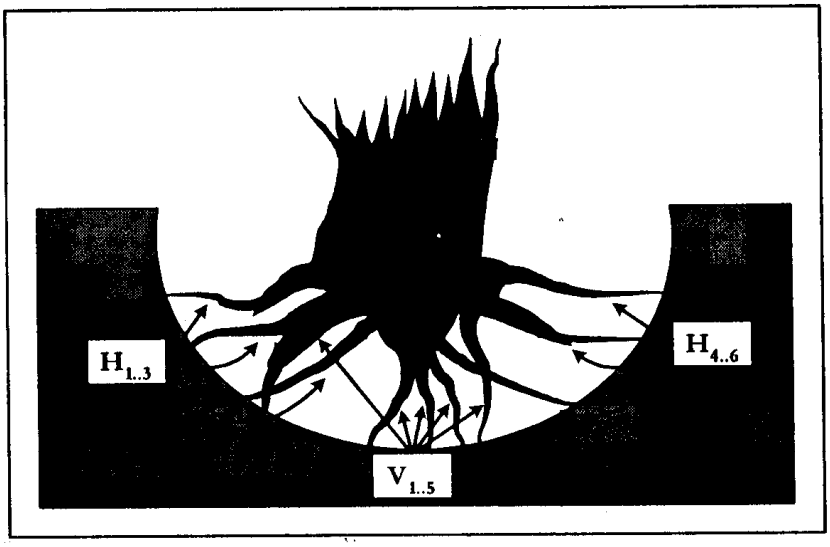

Figure 4.9. Exposing the proximal roots at the base of a tree stem and measuring root diameters of horizontal (H) and vertically (V) oriented roots, as well as that of the tree stem, can be used in FBA to estimate the overall shoot: root dry weight ratio, if the fractal branching parameters for stem and roots are known for the tree species

The fractal branching rules apply for root systems as well as above-ground stems, but so far no relation between the parameters describing the above- and those describing the below-ground patterns in a given species have been established. The FBA program can predict the total size of each root starting at the stem base on the basis of the 'proximal' diameter at the stem base, and we can thus obtain the root system of the whole tree by summation.

$$\textit{Below-ground tree biomass} = \Sigma_i\, a\, D_i^b = \textit{Above-ground Biomass}/\textit{SRratio}$$

where a and b are parameters for a root allometric equation, as derived in FBA, and the D_i refer to all proximal root diameters, measured at the stem base

Default values for the shoot: root ratio (SRratio) are 4 for humid tropical forest on normal upland soils, up to 10 on continuously wet sites, and around 1 at very low soil fertility.

4.2.6. SOIL CARBON FRACTIONS

4.2.6.1. INTRODUCTION

Mineralisation of SOM content is a major source of plant nutrients, but the stock can run out quickly, unless sufficient organic inputs are used. Total soil organic matter content is not a very sensitive indicator as it changes relatively slowly under different management regimes, and often has a high spatial variability, linked to variability of soil texture (Hassink *et al.*,1995).

For studies of soil C-dynamic parameters sensitive to changes of soil management are needed. Physical fractionation of soil organic matter may be more sensitive than chemical fractionation and lead to biologically meaningful fractions of soil organic matter that show a rapid response to soil management. 'Validation' of SOM fractionation procedures should indicate whether the fractions are (a) consistently measurable (small lab error), (b) different in turnover and dynamic behaviour, (c) correlated with different soil functions.

The CENTURY model (Parton *et al.*, 1987) defined five fractions of soil organic matter, covering the biochemical continuum from cellular fractions of higher plants and of microbial origin to humus compounds. Table 4.5 shows these soil organic matter functional pools and their turnover times (Woomer *et al.*, 1994; see Section 3.3).

Table 4.5. Soil organic matter pools, their turnover times based on estimation of CENTURY model (Parton et al.,1987) and their composition (Woomer et al.,1994).

Functional Pool	Turnover Time (years)	Composition	Alternative Name
Metabolic litter	0.1 - 0.5	Cellular contents , cellulose	Plant and animal residues
Structural litter	0.3 - 2.1	Cell walls with lignin and polyphenolics	Plant residue
Active pool	0.2 - 1.4	Microbial biomass, soluble carbohydrates, exocellular enzymes	Labile fraction
Slow pool	8 – 50	Particulate organic matter (50 µm -2.0 mm)	Labile fraction
Passive pool	400 – 2200	Humic and fulvic acids, organo-mineral complexes	Humic substances

In the context with ASB activity phase I, two physical SOM fractionation were compared for different land-use types in Lampung and Jambi (a) one based on particle size only and (b) one based on particle density within the sand-sized fraction. A first step in both procedures is the separation by particle size. The *'particulate organic matter'* fraction recommended by TSBF (Anderson and Ingram, 1993) covers the 53 - 2000 mm particle size range. Further physical fractionation procedures based on density allow the distinction of pools with different degrees of organo-mineral linkage and *'physical protection'* from decomposers. During decomposition plant litter, with an initial physical density around 1.0 g cm^{-3} becomes more intimately associated with mineral particles with a physical density of around 2.5 g cm^{-3}. A fractionation procedure on the basis of colloidal silica suspensions (LUDOX) for the 150 - 2000 mm fraction was developed by Meijboom *et al.* (1995) for temperate area and tested in the tropics by Barrios *et al.* (1996). Results for Sumatra showed that the Ludox fractionation method gives a more sensitive indicator for studying carbon dynamics than total soil C, especially when the 0-5 cm depth layer is studied.

4.2.6.2. FRACTIONATION OF SOIL ORGANIC MATTER BASED ON PARTICLE SIZE: WET SIEVING

A simple technique for fractionation of soil organic matter based on particle size (wet sieving technique) was described by Okalebo *et. al.*, 1993). Using a wet sieving technique, particulate soil organic matter (POM) is defined as the fraction with diameters between 50-250 µm. The assumption is that this POM fraction is the most readily available soil organic matter fraction and determines N mineralization rates, along with fresh organic inputs. With this technique, however, contamination of light fraction with soil mineral components or humified products of the same size is unavoidable and the dry weight of the fraction carries little information. Conventional C_{org} and N_{tot} methods are needed to characterize the POM fraction to calculate POM_N and POM_C.

4.2.6.3. DENSITY FRACTIONATION OF SOIL ORGANIC MATTER USING SILICA SUSPENSIONS (LUDOX)

Differences on soil texture and soil structure may effect the decomposition and mineralisation of organic matter fractions and microbial turnover. In fine textured soils (clay) a larger part of the organic matter may be physically protected due to its location in small pores and on the surface of clays or organic complexes than in coarse texture soils (Hassink, 1992). In clay soils a higher proportion of the microbes is physically protected against predation than in sandy soil, by its location in small pores, where their predators can not reach them (Hassink *et al.*, 1993). If we separate soil material, of a specific size, by its physical density, the light fraction will contain purely organic material, while the heavier fractions contain organic material more closely associated with mineral particles. It seems likely that these heavier fractions represent soil carbon in more stabilised and/or physically protected pools. The fractions with a rapid turnover (active fractions) are assumed to play an important role in soil nutrient dynamics.

A fractionation procedure on the basis of colloidal silica suspension (LUDOX) for the 150 -2000 µm fraction was developed by Meijboom *et al.* (1995) for temperate area and tested in the tropics by Barrios *et al.*(1996) and Hairiah *et al.*(1996). The light fraction appears to be a more sensitive parameter than total soil organic matter, reflecting differences in management and quality of the organic matter input. Based on this measurement soil organic matter will be divided into three density fractions:

a) Light fraction, which has particle density < 1.13 g cm^{-3}, and consisting of recognizable plant residues,
b) Intermediate fraction, which has particle density 1.13 - 1.3 g cm^{-3} and partly is humified material
c) Heavy fraction has particle density > 1.3 g cm^{-3} and consisting of undefined (amorphous) organic material.

This fractionation is performed in the sand-size organic matter (macro-organic matter; >150 µm), as that organic-C is more labile than organic-C in the clay and silt size fractions (Tiesen *et al.*, 1984).

Box 4.9. Soil organic matter fractionation by the LUDOX method

Material and methods

1. *Sieves:*
   - *Top sieve : mesh sieves 2 mm*
   - *Middle sieve : mesh sieves 250 µm*
   - *Bottom sieve mesh sieves 150 µm*
2. *Tray with a mesh screen 150 µm*
3. *Boxes + 'sieve-spoon'*
4. *Tissue paper*
5. *Paper bags*
6. *LUDOX is an aqueous colloidal dispersion of silica particles produced by Du Pont TM 50, it has a maximum particle density of about 1.4 g cm^{-3}.*

Particle Densities (PD) of suspensions needed are 1.13 and 1.3 g cm^{-3}. To make the required suspension density, add tap water, stir well and calibrate (see below)

Procedure

This technique basically has 2 steps (Figure 4.9) i.e. (1) Recovery of macro-organic matter and (2) Density fractionation in silica suspension.

Box 4.9. Soil organic matter fractionation by the LUDOX method (contd.)

<u>First step:</u> Sampling and washing of the samples to obtain macro-organic matter

1. *The sample should be previously sieved (< 2 mm) and homogenized, with roots, stones and other bigger debris removed. If samples have been stored in dry state, they should be rewetted for 24 hr before the fractionation starts.*
2. *Take a small subsample (about 5 g) for determining soil moisture content (weigh fresh, dry in an oven at 105°C and weigh again) and expressing results to a soil dry weight basis*
3. *Assemble a wet sieving apparatus with mesh sizes 250 μm (upper sieve) and 150 μm (lower sieve)*
4. *Weigh 0.5 - 1 kg (fresh weight) of soil, and wet-sieve over the two layers of sieves, using a reasonable pressure of tap water.*
5. *Push soil particle through the top sieve while washing, spray with water until the water passing the sieve has become clear.*
6. *Collect all of the organic material present on both sieves, and bring into a bucket of water, and swirl thoroughly to bring all organic material into suspension,*
7. *Separate organic material and mineral material by decantation, repeating step 6, until a MACRO-ORGANIC fraction (including closely associated soil particles) is separated from a MINERAL (sand) fraction with negligible organic content.*
8. *The macro-organic matter needs further treatments (2nd step), while mineral fraction is discarded.*

<u>Second Step</u>: Density fractionation in LUDOX

9. *Put all macro-organic matter on a tray with a mesh screen 150 μm, place in Ludox suspension with a density 1.3 g cm^{-3}, and mix it several times during a 10 minute period,*
10. *Collect the floating and suspended material using a 'mesh-spoon' and move to a second tray for separating it into Light and Intermediate fraction*
11. *The remaining material from the dense Ludox is called* heavy fraction *(PD > 1.3 g cm^{-3}),*
12. *Place the second tray in a Ludox suspension with a density 1.13 g cm^{-3}, mix it several times during a 10 minute period to again separate between a suspended or floating and a sinking fraction. The floating fraction (PD < 1.13 g cm^{-3}) is collected as* Light Fraction *and the sinking materials as* Intermediate Fraction *(1.13<PD<1.3 g cm^{-3}).*

Box 4.9. Soil organic matter fractionation by the LUDOX method (contd.)

12. *Wash the three fractions with tap water and dry in an oven for dry weight determination; for chemical analysis that materials should be rinsed with demineralized water.*
13. *Determine total N, ash and organic-C content.*
14. *The physical density separation has made the C content of the fractions reasonably predictable (30-40% for light, 15-30% for intermediate and 5-15% for Heavy); for some studies such default values can be used and checked on composite, in stead of individual samples*

Note:

- Standardization of immersion time and stirring method is necessary as the viscosity of especially the heavy suspension leads to incomplete separation
- All fractions are express in g kg^{-1}, converting the soil fresh weight on the basis of the subsample

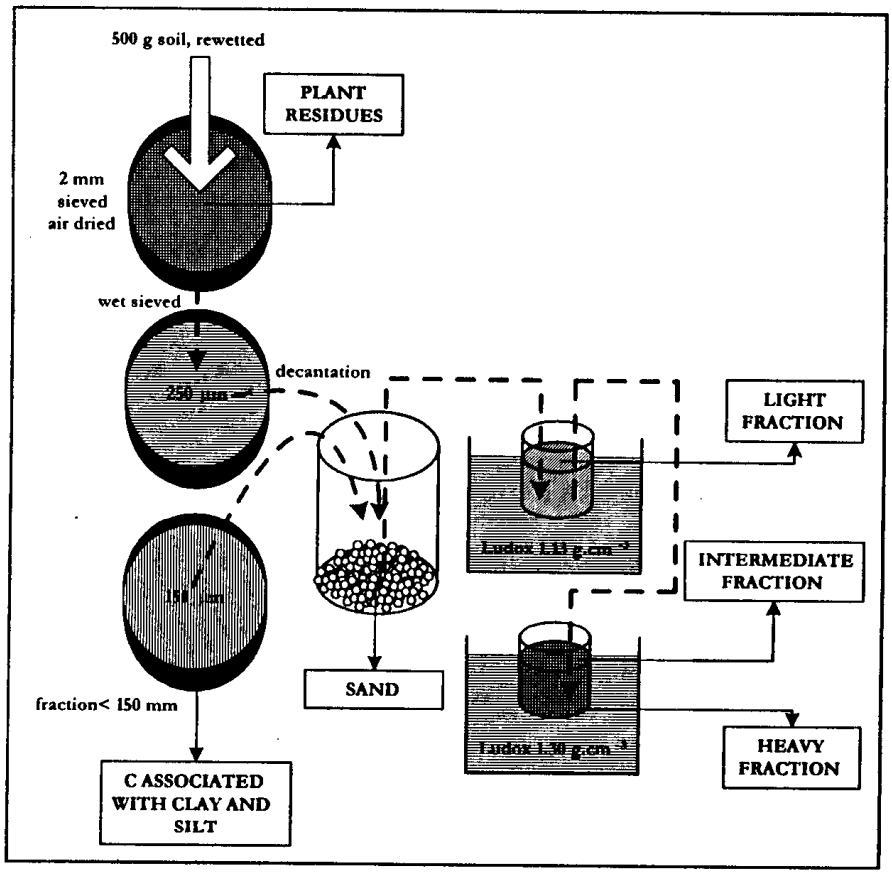

Figure 4.10. Schematic procedure of soil organic matter (SOM) fractionation based on size density in LUDOX suspensions.

Calibration for LUDOX particle density

After every 10 soil samples have been fractionated, the particle density of suspension needs to be calibrated as follows:

1) Hang a stone on the edge of the balance and weigh it (W_1, g), and put that stone in water and weigh it (W_2, g).
2) Put the stone into each LUDOX suspension ((W_3, g)

$$PD\ of\ Ludox = (W3-W1) / (W2-W1),\ g\ cm^{-3}$$

Volume of stone = $W_2 - W_1$ (cm^{-3})

PD = Particle density , g cm^{-3}

- If $PD_{ludox} > PD_{target} + 0.01$ → ADD SOME WATER, (where PD_{target} is 1.13 or 1.30 g cm^{-3})
- If $PD_{ludox} < PD_{target} - 0.01$ → EVAPORATE
- If $PD_{target} - 0.01 = PD_{ludox}$ or $< BJ_{target} + 0.01$ → Fractionation to be CONTINUED

Volume water to be added:

$$Vol.\ water = [(PD_{ludox} - PD_{target}) / (1 - PD_{target})],\ ml$$

Disadvantages of the LUDOX method

- Ludox suspensions solidify when evaporating water, so the jars should be kept closed when not in use, especially at high air temperatures,
- Charcoal separation remains as a serious problem, especially for areas which have been opened by burning; the charcoal particles can appear in all density fractions
- Ludox is relatively expensive

Advantages

- The results relatively accurate as this technique combined 2 techniques fractionation based on particle size and particle density.

4.2.7. DECOMPOSITION RATE OF ORGANIC SOURCES

Decomposition of dead plant material can have a direct effect on crop growth, by mineralisation of N, and an indirect one, by build-up of soil organic matter, which may increase future efficiency of nutrient use. Rapidly decomposing material of low C/N quotient contributes mainly by N-mineralisation and slowly decomposing litters contribute especially to the build up of the soil organic matter pool. Measurements of mass losses from unconfined litter under natural conditions had been demonstrated, by using a standard litterbag designed by TSBF.

Box 4.10. Litter bag decomposition studies to determine k-values

Equipment:

1. *Litter bag made of exuded polyvinyl with a 7 mm mesh, so its still allow free access to most groups of macro-fauna. The sides of litter bag is bent up to retain the shape of shallow box-like container, 30 cm 30 cm by 2.5 deep.*
2. *Balance*

What sort of organic materials should be used?

- *Forest studies: mixed samples of freshly fallen leaves, if necessary can be collected from the ground.*
- *Agricultural plots: crop residues (mixtures of stems and leaves).*

How many litterbags are needed?

- *At least five bags should be observed for every time of sampling, and at least four sets of samplings should be done before 50% of the original mass is lost.*

Procedure

1. *From the material used for the experiment, total N, C, lignin and polyphenolic concentrations for all sample materials should be analysed.*
2. *Fill the litterbag with a known amount plant material equal to normal inputs of that resource per unit area. For a fine plant materials, an extra finer plastic screen material needs to be placed in the bottoms of bags.*
3. *At sampling time (e.g. 2, 4, 8, 16 and 32 weeks after incubation), lift the bags carefully up, and put into plastic bag to avoid mass losses during transportation.*

Box 4.10. Litter bag decomposition studies to determine k-values (contd.)

4. *Take the plant materials out of the bags by flotation and brushing the bags in water.*
5. *Rinse the materials with demineralised water, oven dry at 80°C and weigh.*
6. *Determine concentrations of total C, N, lignin and polyphenolic.*

Data analysis:

The remaining dry weight as fraction of the initial amount can be plotted as a function of time. An exponential decay model $Y(t) = Y_0 \exp(-k\,t)$ can be fitted directly (non-linear fit procedures) or after logarithmic transformation ($\log(Y(t)/Y_0) = -k\,t$). The k-values can be compared with those used in the CENTURY model for material of the same quality. TSBF has initiated a database of litter quality and decomposition values.

4.2.8. CASE STUDY OF TERRESTRIAL C STOCKS

As an example of the procedures for assessing terrestrial C stocks, results of C measurement of secondary forest before and after burn in N. Lampung are presented in Figure 4.10 and detail data is shown in Table 4.6.

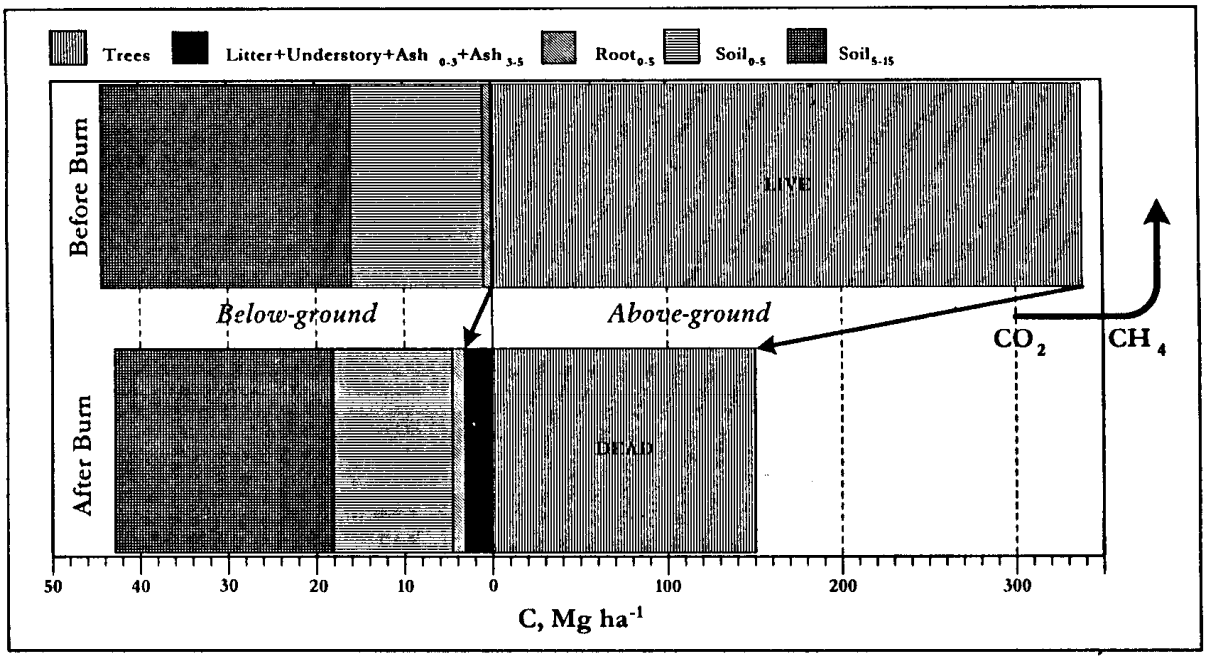

Figure 4.10. Carbon stock of secondary forest before and after burn in N. Lampung (Hairiah et al., 1996)

Table 4.6. Biomass measurement of secondary forest, before and after burn, in N. Lampung (Sept. 1994). (Note: Understorey + Brown litter are estimated, Water content of brown litter, roots: 5 %; Water content Green biomass: 20 % (for leaves) and 10 % (for stems))

	Total DW *Mg ha^{-1}*	C- org %	Total C *Mg ha^{-1}*
Before Burn			
Understorey: Green Biomass	0.94	40 (est)	0.45
Roots (0-5cm depth)	0.04	40 (est)	0.02
Brown litter	6.34	40 (est)	2.01
Trees Biomass			
Living trees	249	40 (est)	99.6
Dead standing trees	11.0	40 (est)	4.40
Dead on forest floor	19.5	40 (est)	7.80
Stumps remain in forest (due to logging)	9.18	40 (est)	3.67
Forest Soil 0 - 5 cm 5 – 15 cm	 0.6 1.2	 2.44 2.12	 15.9 27.5
		Total	*161.35*

Table 4.6. Biomass measurement of secondary forest, before and after burn, in N. Lampung (Sept. 1994). (Note: Understorey + Brown litter are estimated, Water content of brown litter, roots: 5 %; Water content Green biomass: 20 % (for leaves) and 10 % (for stems)) (contd.)

	Total DW *Mg ha^{-1}*	C- org %	Total C *Mg ha^{-1}*
After Burn			
Ash (0-3 cm) ash (3-5 cm)	10.6 21.6	7.55 4.23	0.80 0.91
Brown litter	0.45	40 (est)	0.18
Roots (0-5 cm depth)	1.40	40 (est)	0.56
Small branches	8.08	40 (est)	3.23
Stump remains (after clearing)	0.16	40 (est)	0.06
Soil 0-5 cm depth 5-15 cm	650 1300	1.94 2.12	12.6 27.5
		Total	*45.84*

Loss of C due to slash and burn practices

Based on calculation of above data (Table 4.6, C-loss of above-ground was about 112.21 Mg ha^{-1}, and below-ground was about 3.3 Mg ha^{-1}. Total loss was 115.51 Mg ha^{-1} or about 72 % of total C-stock.

4.2.9. FIELD WORK EXCERCISE FOR CARBON STOCK

1) During the field work we are going to have exercise to measure C-stock of above-ground from three land-use types around BIOTROP:
   - Imperata
   - Cassava
   - Homegarden
2) Carbon content of biomass will not be measured during field exercise, as time is not permitted. In order to be able to calculate C-stock of above-ground, data of dry matter (%) and total C in Table 4.7 can be used.

Table 4.7. Estimation of Dry matter (%) and Total C (%) of plant material (Hairiah, 1997).

	*Dry Matter %	Total C %
Forest Understorey	20	40
Litter	30	40
Cassava		
• *Leaf*	15	40
• *Stem*	25	39
• *Tuber*	30	42
Imperata		
• *Green leaf*	20	42
• *Brown leaf*	25	41
	Average	*40*

**Dry matter (%) = FW (g)/DW(g) x 100*

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4.3. FUNCTIONAL BRANCH ANALYSIS TO DERIVE ALLOMETRIC EQUATIONS OF TREES

By:
Meine van Noordwijk

A major part of the carbon and nutrients in terrestrial ecosystems is in the tree component and it is therefore imperative to have appropriate methods for estimating tree biomass. To reduce the need for destructive sampling, biomass can be estimated from an easily measured property such as stem diameter, at specified height, by using an allometric equation. A substantial number of allometric equations have been developed for various climatic zones, forest types and tree species, using a variety of algebraic forms and parameter values. Anybody who wishes to use such equation for a new situation is faced with a difficult choice among the various equations, the result of which may vary over a factor 2 at least when applied to a specific data-set. Collecting more empirical equations will hardly reduce this uncertainty for any new situation, unless we can better understand the background of the allometric equations in its link with the shape of trees. The FBA (functional branch analysis) scheme was designed to generate allometric equations on the basis of easily observed properties of branched systems, in order to allow a more informed choice among empirical equations for forest types or even for individual trees in a sampling area (see Figure 4.11).