SECTION

4

MEASURING IMPACTS OF LAND-USE CHANGE ON THE SOIL

A preliminary approach to quantify processes in the rhizosphere, such as below-ground biomass in terms of root biomass and greenhouse gas emissions were explored. Simple calculation tools based on observed branching properties were exercised using fractal analysis. Flux calculation of a large number of gas samples was also demonstrated using statistical procedures based on the goodness of data fitting following mass-flow calculation.

4.1. GHG FLUXES

4.1.1. FIELD PROTOCOLS

By:
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The sampling protocol for gathering field data as developed for ASB project was presented in the workshop. The protocol is to assure comparison of data from the different sites. Field sampling and data structure are designed in accordance with the algorithm of the data processing developed at IC-SEA (see Section 4.1.2). The intention of this protocol is to provide a simple means of gas collection that does not require sophisticated equipment to be transported to all of the sites but at the same time will provide reliable and comparable data. In addition to the inventory samplings, some of the benchmark sites will be taking more detailed measurements of diurnal and seasonal gases fluxes to provide information on the factors and processes that control emissions.

During the fieldwork in the workshop, participants were introduced to the sampling protocol in measuring GHG emissions, including sampling time, sampling frequency and interval, replicate, sample handling, intensive measurements, sampling chamber and chamber's base, field incubation and gas sampling, bulk samples, and ancillary measurements.

The principle of the measurement is to replace the free exchange between soil and atmosphere by that between (undisturbed) soil profile and a sample chamber, recording the gas concentration in the chamber. Decrease of gas concentration in the chamber indicates sequestration, increase indicates a net emission.

4.1.1.1. SAMPLING STRATEGY

Sampling time

A specific stage in a land-use type (LUT) at a particular soil series is usually considered as treatment. If there are a number of LUTs to be sampled in a single field campaign, a reasonably easy movement from one land-use to another is required so that the sampling time can be optimised and data compatibility ensured. If measurements will be carried out twice a day, the morning measurements may be carried out between 9 and 10 a.m., and afternoon measurement between 3 and 4 p.m., avoiding the hottest part of the day.

Sampling frequency and interval

The interval of taking the samples in the gas chamber is 0, 10, 20, and 40 minutes, after enclosure. This time period is considered sufficiently to develop the slope of the changing concentration of the gases in the chamber, yet short enough not to influence the rate of gas exchange (by depletion of the source or product inhibition).

In order to show seasonal variation of GHG fluxes, monthly measurements may be planned, or at least wet and dry seasons measurements should be scheduled. However, additional samplings in between are also encouraged. It is also possible to do experiments on the direct effects of wetting and drying the soil.

Replicate

The replicate is meant to capture the spatial variability within each land-use type. A minimum number of replicates at each LUT or treatment of three is suggested.

Sample handling

Samples will be collected using 20 ml evacuated vials, which will be filled with 30-ml air samples for laboratory analysis. It is suggested that the samples should be analysed within two weeks.

The time of day affects gas flux, with the maximum flux occurring at the hottest part of the day. Ideally sampling should be repeated at different times of the day for each land-use system but unfortunately that would generate too many samples, so the morning and the late afternoon periods are selected, which may approach the daily average.

4.1.1.2. INTENSIVE MEASUREMENTS

If intensive measurements are possible, the following experiments may be carried out:

- soil wetting and drying;
- diurnal fluctuation;
- monthly fluctuation; and
- agronomic treatments, including N-fertiliser use.

4.1.1.3. SAMPLING CHAMBER AND CHAMBER'S BASE

The chambers are made from white PVC tubes with 12-inch (30.5-cm) external diameter and a height of 10-cm (Figure 4.1). The chambers are not vented and equipped with a sampling port fitted with Teflon septa on top of the chamber.

In order to ensure rapid sampling with minimal disturbance of the soil and litter layer, a metal ring is inserted about 2-3 cm in the soil as chamber base placed in the soil at least two days before the measurement. These rings can be left on the site for repeated measurements. During incubation, the chamber is placed on the base, which has a groove on the top rim, which can be filled with water to ensure a good sealing when a chamber is placed on it. In one particular land-use there are three bases installed permanently. Three chambers are, therefore, needed in each land-use, so that one cycle of sampling in that land-use type can be completed in about an hour.

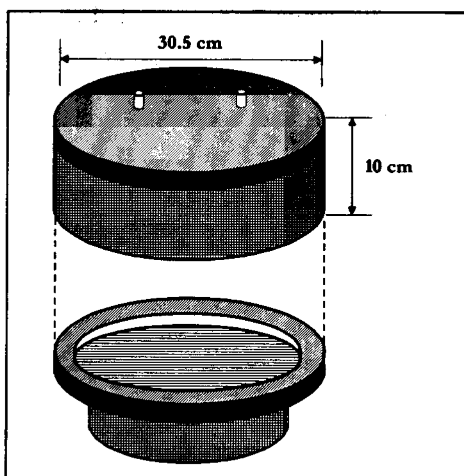

Figure 4.1. Chamber design for gas sampling and the relative size of the base permanently installed in the field.

4.1.1.4. FIELD INCUBATION AND GAS SAMPLING

The site should be geographically referenced. Be sure that the chambers are separated by 20 to 50 m, to get a representation of the variability. In order to facilitate sampling the three chambers it is best to have at least two people sampling and to stagger the installation of the chambers by 5 minutes. Immediately after the chamber has been located collect a gas sample from the chamber, this is the time zero (T0) sample. A sample is taken by inserting the 30-ml syringe into the septum, be certain that the syringe is pushed completely closed so that no air enters the chamber. Once the syringe is inserted mix the air in the headspace by pulling and pushing the plunger of the syringe several times. Finally, extract 30 ml of air into the syringe and remove the syringe. The use of stopper valve is recommended in order to avoid leakage. Inject the sample into the pre-evacuated 20-ml vial. The extra air will cause the plunger pushed back. Again, to avoid this the valve should be able to resolve. Write on the label necessary coding, which indicates site reference and intervals. Use the field sheet (see Section 4.6, Sheet 4.1). The 30-ml sample will exert an over-pressure in the vial but this is necessary to be able to extract the sample later. Sheet 4.2 (Section 4.6) may be used to record the corresponding results from Gas Chromatography analysis.

Follow the above sampling procedure at 10, 20, and 40 minutes after locating the chamber and put them in vials marked T10, T20, and T40 respectively. Do not forget to fill in the sheet with the date, site, land-use type, geographic location, and air and soil temperature readings.

One sample of the ambient air may be collected at this stage to represent the three replicates at that particular site. The amount of air taken using the syringe should be the same, 30 ml, injected in 20 ml vials marked A (for ambient) and other necessary coding referring to the respective site.

4.1.1.5. COMPOSITE SAMPLES

If we decide to take composite samples, immediately after locating the chamber collect a gas sample from the chamber, this if the time zero (T0) sample. A sample is taken by inserting the 25-ml syringe into the septa, be certain that the syringe is pushed completely closed so that no air enters the chamber, and mix the air in the headspace by pulling and pushing the plunger of the syringe several times. Finally, extract 10-ml of air into the syringe and remove the syringe. Inject the sample into the pre-evacuated vials marked T0. Collect a T0 sample from each of the other two replicate chambers and inject the 10ml sample into the same vial marked T0 (this will serve as a composite sample). The three 10-ml samples will exert an overpressure in the vial but this is necessary to be able to extract the sample later.

The same procedure may be applied for T10, T20, and T40. Coding on the sheet and on the vials should be made accordingly. Please note that this is an alternative of the previous sampling method. Comparison between these methods may be carried out occasionally to give us the idea on how variable the data is.

4.1.1.6. ANCILLARY MEASUREMENTS

The production, consumption, and flux of gases depend on several soil properties, including the porosity and aeration of the soil. These factors are determined by the texture, bulk density, and water content of the soil. In addition, the nitrate and soluble C content of the soil have been shown to influence nitrous oxide production. For each land-use replicate sampled for gas fluxes it will be necessary to take:

1) A bulk soil sample from 10 and 20 cm, below the litter layer, this soil will be sampled for extractable mineral N, mineralisation (incubation), and carbon fractionation (soluble C, microbial C, light fraction C). This requires about 350-400 g of soil. The sample must be taken immediately adjacent to each of the chambers. The sample for C fraction can be air-dried; for mineral N cool storage or direct extraction with KCl is desirable.

2) A bulk density sample at 3-8 cm, using standard soil physical ('Lutz') sampling rings (volume=100 cm^3). Soil can be transferred to a closed plastic bag from the rings. The sample can be taken immediately adjacent to the chamber. The bulk density sample will also be used to determine the soil moisture content by weighing it when wet, then drying at 100°C and reweighing.

Necessary samples coding is also needed and the sampling should also be recorded in the field sheet.

4.1.2. ESCAPE

By:
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ESCAPE — GHG Emission-Sequestration CAlculation ProcedurE — was developed under Pascal for Windows to handle the problems in analysing large number of GHG samples and data, with the main objectives to provide speedy calculation and quality control of the data.

ESCAPE was designed to handle the calculation of GHG flux based of curve fits of the data sets. The flux and the simple statistical analysis of the data are then reported by

ESCAPE. It is also capable of removing some "odd data" encountered in the laboratory analysis known as outlier(s).

The format of the data input considers the number of replicates in each land-use type (LUT), the number of samples taken within an interval, and the dimensions of the sampling chamber (height). However, other arrangement may be structured followed by the recompilation of the programme list. As far as the kinds of GHG are concerned, ESCAPE calculates the fluxes of CH_4, CO_2 and N_2O based on the constants being installed in the programme.

In calculating GHG fluxes, it needs variables like gas concentration analysed from samples, which are collected at a certain interval using incubation chambers. The change of concentration with time (dc/dt) is shown by the slope of the scatter diagram and the coefficient correlation indicates the quality of the data (Figure 4.2). As part of quality control, the user can drop or include individual data sets.

In GHG ESCAPE, all data are transformed into standardised normal variates (Z=(value-mean)/standard deviation); highly positive or strongly negative Z-values indicate 'outliers' and the user can set a critical value for excluding part of the data set.

Further application is being explored when spatial variation of GHG emission or sequestration will be evaluated in terms of gas exchange between sources and sinks.

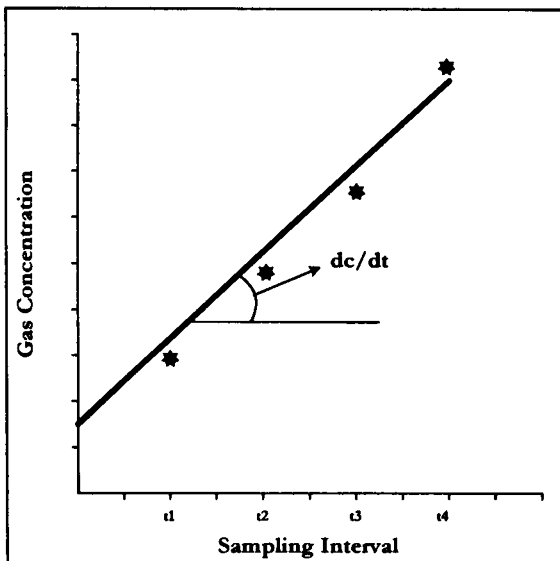

Figure 4.2. Scatter diagram of the data set showing the change of gas concentration with sampling time.

Other variable collected is the chamber's temperature, which determines the air density. The constants that will be used to calculate the flux include chamber's height, Avogadro number, molecular weight of the respective gas as shown in the following equation:

$$\Phi = \rho . \frac{M.V}{N_o.A} . dc/dt$$

where:

Φ	: ghg flux rate;
ρ	: air density;
M	: molecular weight of the respective gas;
V	: chamber's volume;
No	: Avogadro number;
A	: chamber's basal area; and
dc/dt	: rate of change of the incubated gas concentration.

The equation shows that the V/A ratio or height of the chamber is important for the flux calculation, rather than chamber surface area. In the exercise, sampling data of CH_4, CO_2, and N_2O from various land-cover types in Jambi were used. For methane fluxes study, only *Paraserianthes* plantation is considered as source (Figure 4.3).

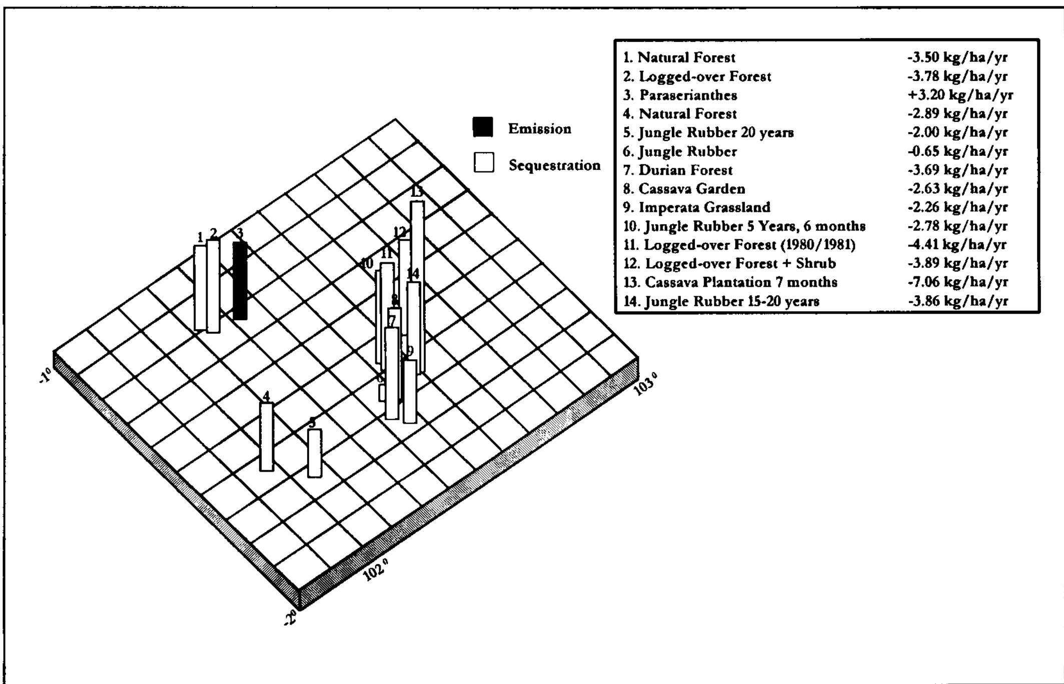

Figure 4.3. Methane fluxes (kg/ha/yr) from 14 sampling points in Jambi calculated using ESCAPE. The sampling was conducted on November 1996.

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

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 m^2 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 m^2 quadrats (5 m x 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 x 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

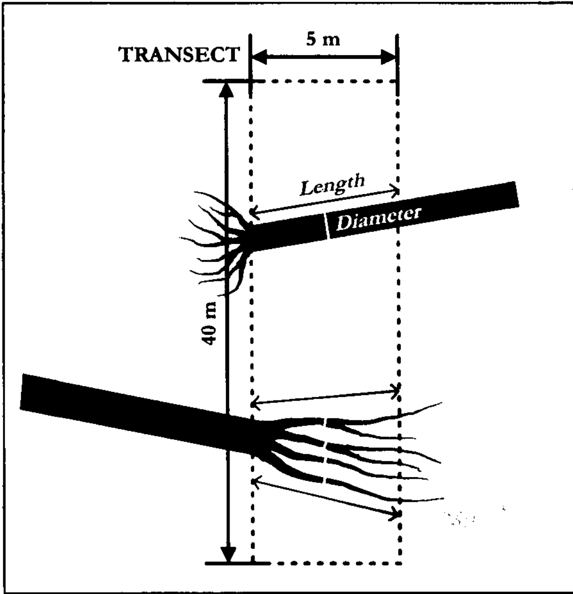

Figure 4.4. Measuring length and diameter to estimate biomass of fallen or felled trees in a transect after slashing and burning.

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.

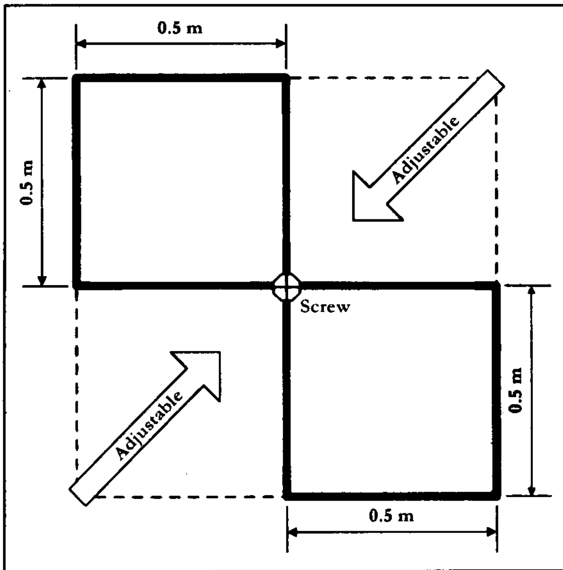

Figure 4.5. Design of a sampling frame, which can be used for 1 m x 1 m samples, or for two adjacent 0.5 m x 0.5 m samples.

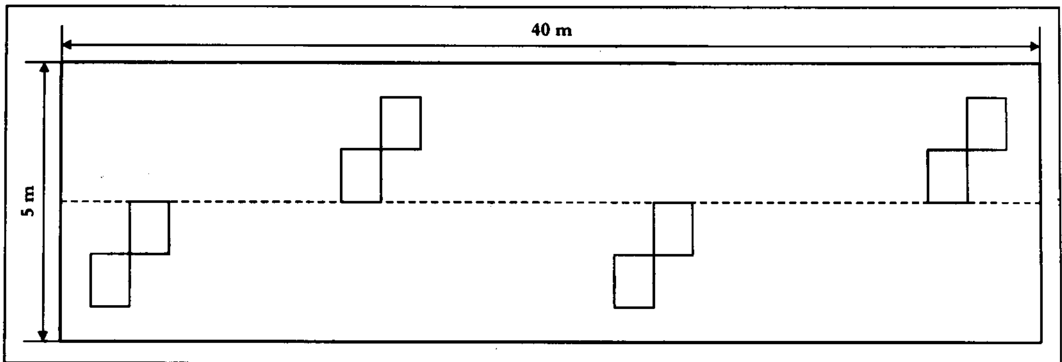

Figure 4.6. Position of understorey sampling within a 40 m x 5 m vegetation transect.

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 4.5. 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

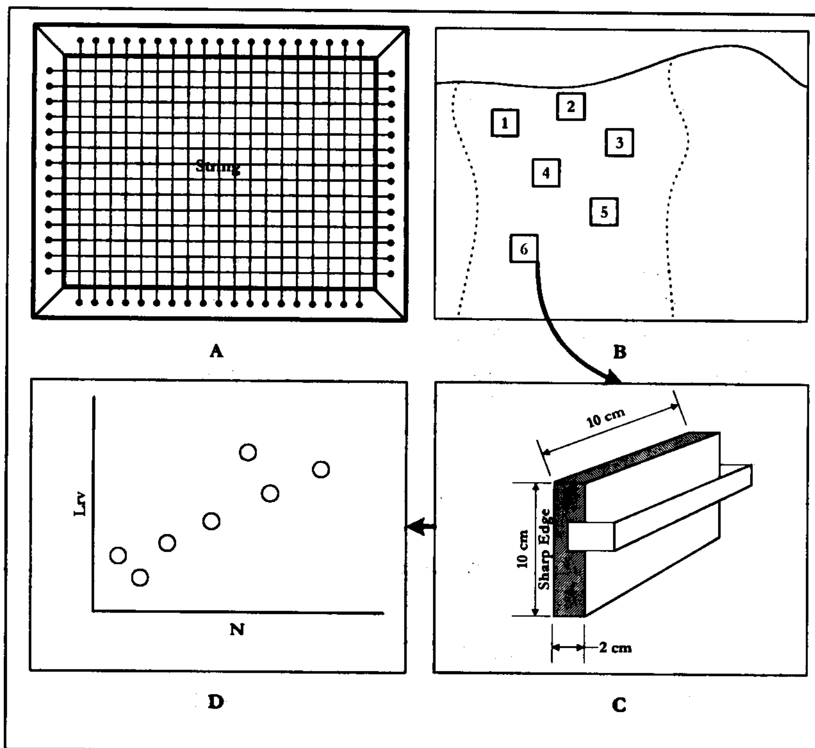

Figure 4.7. Root mapping. A. Frame with 10 x 10 cm grid of strings, B. Possible position of subsamples on the profile wall, C. Metal box for taking subsamples for washing fine roots and measuring their length, D. Calibration line of the number of intersections per unit area and root length density per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

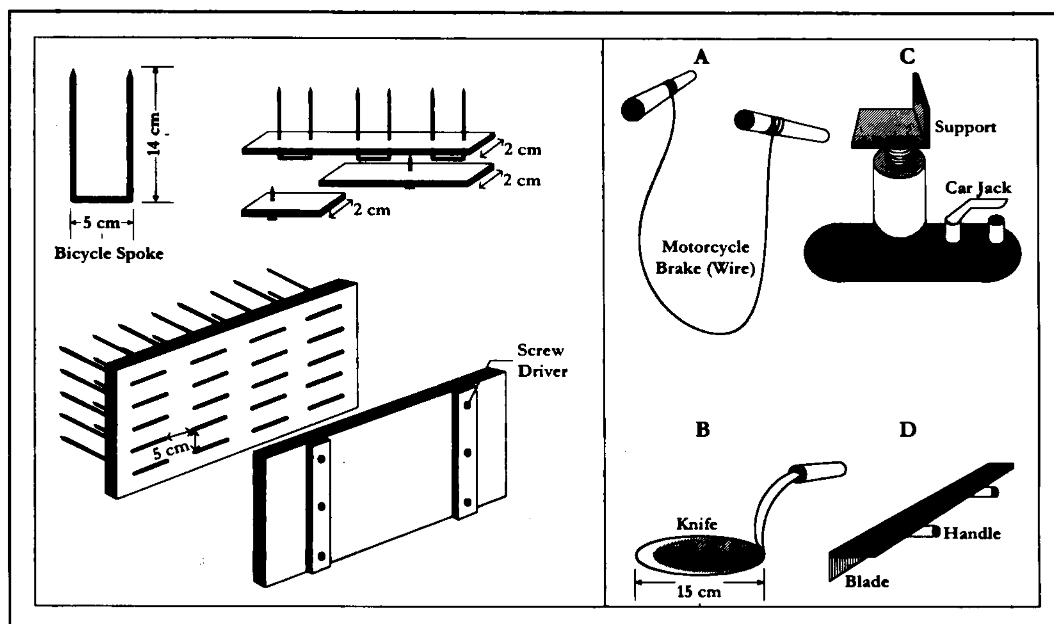

Figure 4.8. Left: Pinboard design. U-shaped pins are inserted into a board in order to hold the roots in place when soil is washed from a monolith. Right: Auxiliary equipment for taking root samples with pinboards: A. cutting wire, B. Knife, C. Jack to support the pinboard, D. Blade to smoothen a profile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

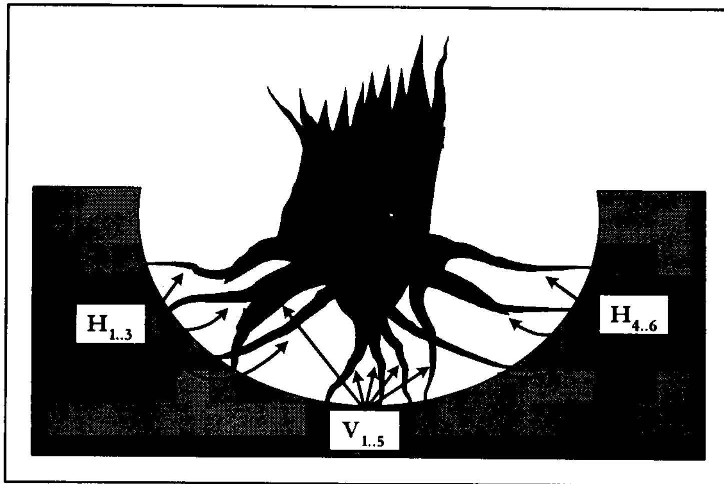

Figure 4.9. Exposing the proximal roots at the base of a tree stem and measuring root diameters of horizontal (H) and vertically (V) oriented roots, as well as that of the tree stem, can be used in FBA to estimate the overall shoot: root dry weight ratio, if the fractal branching parameters for stem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

Below-ground tree biomass = $\sum_i a\, D_i^b$ = *Above-ground Biomass/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.)

First step: ***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.*

Second Step: ***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

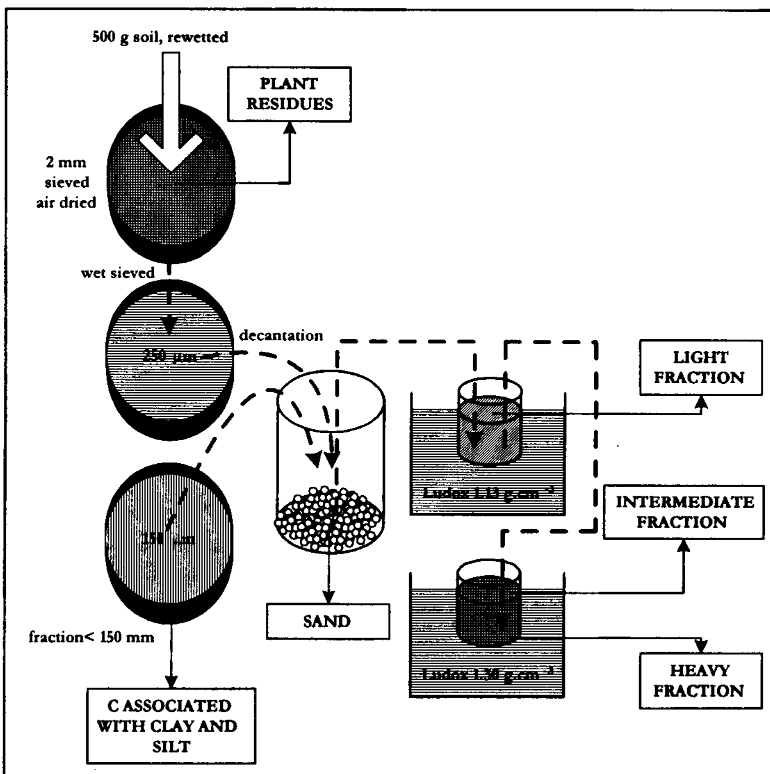

Figure 4.10. Schematic procedure of soil organic matter (SOM) fractionation based on size density in LUDOX suspensions.

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.

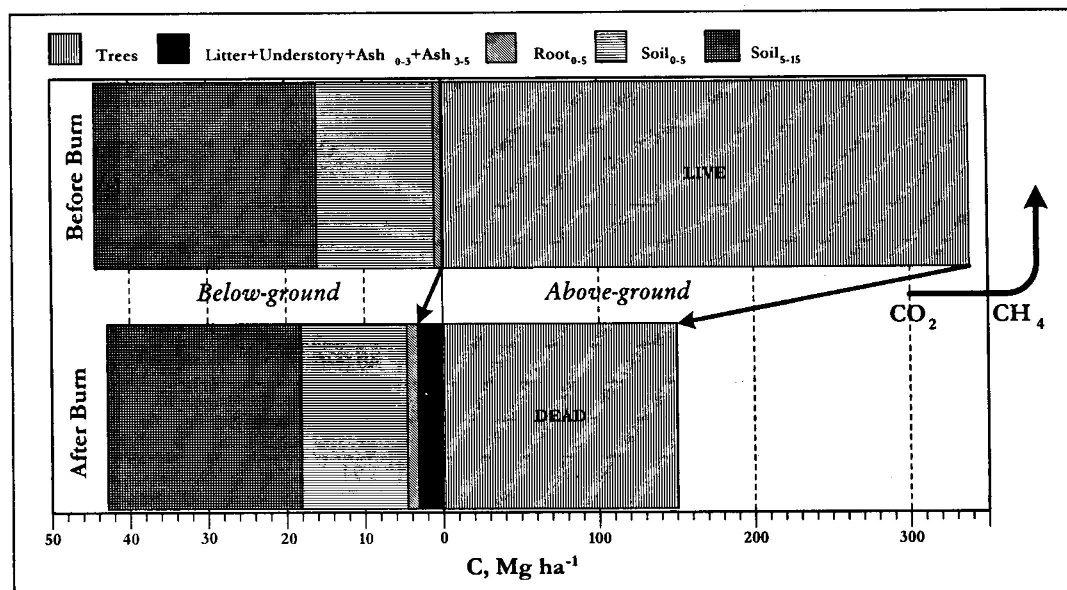

Figure 4.10. Carbon stock of secondary forest before and after burn in N. Lampung (Hairiah et al., 1996)

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).

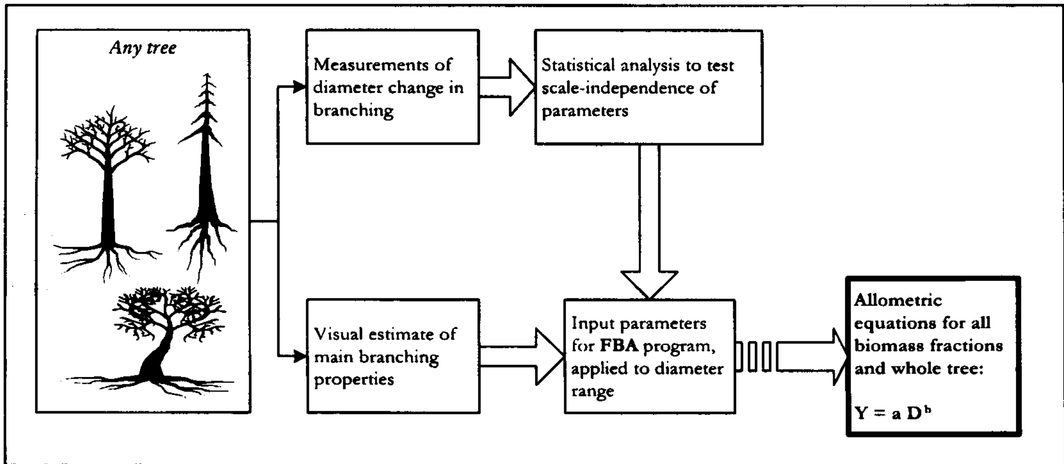

Figure 4.11. FBA estimates tree's biomass using a fractal branching model.

Fractal branching models make use of self-repeating properties in applying simple rules consistently across a range of scales. In trees above- as well as below-ground branching follows a simple logic in that the amount of transport tissue (functional xylem) where two roots come together or where two branches split has to be able to transport the same amount of water before and after the branching point. This consistency leads to a requirement of a near-constant cross-sectional area of xylem, and depending on the stem anatomy, to a proportional relation in the cross-sectional areas of the whole stem.

Any branching point can be described by a parameter for the change in total cross-sectional area (alpha = $D^2_{before} / \Sigma D^2_{after}$), one for the split of cross-sectional area over the branches (q = $\max(D^2_{after}) / \Sigma D^2_{after}$), one for the number of branches and the angle between the axes before and after branching. The angles are important for a 3D reconstruction of the tree, but not for total biomass. Direct measurement of diameter change at branching points and statistical analysis to test the independence of these parameters from diameter can establish the validity of the fractal model. All tests performed so far on above-ground trees as well as root systems confirm that the principle indeed applies, but with considerable variability in parameter values.

The more extreme cases can be directly recognised by looking at a tree (Figure 4.12), which leads to a considerable simplification of the procedure if an approximation is sufficient.

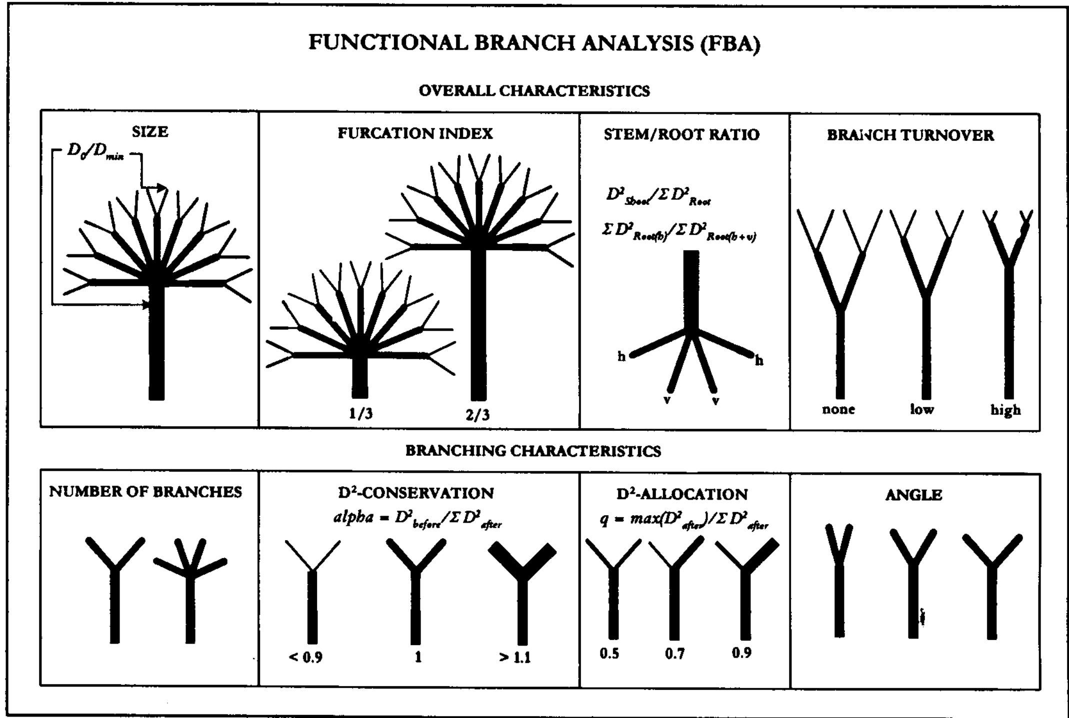

Figure 4.12. The main tree's branching properties used in FBA.

For actual measurement of the branching properties the basic data are collected for 20-50 'links' (sections between two branching points) on a tree, covering the whole diameter range present; Diam1 and Diam2 are measured halfway the link in two mutually perpendicular directions, reflecting the largest and smallest diameter for elliptical cross sections; the 'connected_to' column refers to the link number before the branching point and is used to sort the data in subsequent analysis. The scheme can be applied above- as well as below-ground, after parts of the root system have been carefully exposed. The last 4 columns are needed when total leaf areas or fine root lengths are to be calculated, or graphical tree reconstructions are to be made:

Link_no	Length *cm*	Diam1 *cm*	Diam2 *cm*	Connected_to:	No. of leaves or fine roots	End link? *Y or N*	Compass angle	Angle with normal
1	..	..	..	0	..	..	..	..
2	..	..	..	1	..	..	..	..
3	..	..	..	1	..	..	..	..
4	..	..	..	2	..	..	..	..

Experience with the FBA program so far shows that the 'branch turnover' parameter, which links to a relation between diameter and link length has the strongest influence on the overall allometric equation and may explain why equations for relatively young trees (secondary forests) differ for those of more mature trees. Further tests are needed. Programs for statistical analysis and the fractal branching model, programmed as macro's in an Excel workbook are available on request.

4.4. SOIL MICRO-FLORA

By:
Robert Simanungkalit

4.4.1. INTRODUCTION

The soil is inhabited by a vast array of micro-biota: the soil bacteria, *actinomycetes*, fungi, algae, and protozoa are all part of microbial diversity of the most dynamic sites of biological interaction. The forces that play an important role in the dynamics of soil micro-biota and their effects on the habitat are governed to a large extent by the physical, chemical, and biological components of a soil.

The intensification of agriculture in many developing countries is necessary to meet demands for foods due to population pressures. Agricultural lands have been extended by slah-and-burn land clearing, often followed by soil erosion, soil compaction, loss of biodiversity, environmental pollution and flooding.

Microbial diversity is an integral part of the entire biodiversity. It is generally accepted that biodiversity is of paramount importance to sustain agricultural productivity and maintain ecosystem functioning. However, our knowledge of biodiversity is still limited. It is estimated that only 13% of the earth's microbial populations are identified (Hawksworth, 1991). Table 4.8 shows the number of species in the major group of bacteria, fungi, algae, and virus.

Table 4.8. Number of species in the major microbial groups (Hawksworth, 1991).

MICROBIAL GROUP	NUMBER OF SPECIES COLLECTIONS			SPECIES IN CULTURE	
	Described	Estimated	Total Species (%)	Number	Total Estimated Species (%)
Bacteria	3,000	30,000	10	2,300	7
Fungi	69,000	1,500,000	5	11,500	0.8
Algae	40,000	60,000	67	1,600	2.5
Viruses	5,000	130,000	4	2,200	2

4.4.2. FIELD AND LABORATORY METHODS FOR MICROBIAL DIVERSITY

The methods presented in the workshop were those used in the two benchmark sites of the Alternatives-to-Slash-and-Burn (ASB) Project. The ASB Project funded by the UNDP-GEF has developed a manual describing the methods of sampling and laboratory assessment for the biodiversity of the key functional groups of soil biota. The following functional groups are included: earthworms, termites and ants, nematode, *mycorrhizae*, *rhizobia*, and microbial biomass.

FIELD METHODS

Soil Sampling. Sampling for soil biota takes place within 40x5 m^2 plots established for carbon budget estimations. The plot is replicated five times in each land-use system. Soil, plant biodiversity and C sequestration are characterised within these plots in advance of the soil biodiversity sample collection. The plot is then divided into three subplots. Before sampling, the litters on soil surfaces are removed. Four samples are taken in each subplot at 0-5 cm and 5-15 cm depth respectively making two composite samples. The four samples are then mixed and sieved to remove the roots. All samples are kept in cool conditions (4°C) until processed. The three composite samples from each location at each soil depth are then mixed in laboratory.

Nodule Sampling. Leguminous species able to nodulate or without information about this characteristic must be identified inside the transect. Whole root systems of the herbaceous ones can be carefully taken out from soil to avoid nodules to be detached off the roots. Care must be taken to avoid confusion with roots of other species, as sometimes

roots of different species grow closely together. When possible, a minimum of 50 viable nodules must be collected in each site. Nodules are stored individually in screw cap tubes with silica gel or anhydrous $CaCl_2$.

Plant Vouchers Sampling. Vouchers from nodulating species must be collected properly (if possible, including flowers and/or fruits) and sent to a herbarium for identification.

LABORATORY METHODS

Complete laboratory methods for *rhizobia* can be found in Somasegaran and Hoben (1995). Plant infection technique is used to estimate *rhizobia* population in soil. Further isolation and characterisation of *rhizobia* from plant nodules provide information about genera and species composition of these populations. The relationship among *rhizobia* strains with the different host are characterised to diverse degrees of specificity. Although some hosts are considered highly promiscuous, it no species is known that can establish symbiosis with any *rhizobia* strain and vice versa. Thus, to evaluate *rhizobia* biodiversity in soil, it is supposed that the higher the number of host species used, as 'traps' the higher the diversity among strains isolated from their nodules. *Siratro* (*Macroptilium atropurpureum*) is widely accepted as a promiscuous host (Vincent, 1970).

Rhizobia Counting. Soil samples are submitted to serial dilution to be inoculated in trap hosts. Base dilution ratios may vary from 2 to 14.5 depending on the expected concentration of cells in the soil sample to be analysed, *i.e.* soils with higher numbers of *rhizobia* cells must be diluted at maximum dilution ratios. The number of replicates can vary from 2 to 5. Non-inoculated controls must be replicated more than twice. Plants are grown in a growth chamber or a room with suitable controlled environmental conditions (Vincent, 1970). Records of the presence of nodules start to be made 15 days after inoculation.

Rhizobia Isolation and Characterization. The first step in *rhizobia* isolation is the surface sterilisation of nodules to remove contaminants. It starts with a brief immersion in alcohol (95%) followed by a longer immersion in $HgCl_2$ solution and successive rinses in sterile water. Then nodule is crushed in a few drops of sterile water with a pincers and a loopful of this suspension is streaked onto an agar medium. The identification of isolated strains at genus level is as follows:

- *Rhizobium and Sinorhizobium.* Colonies circular, 2-4 mm in diameter but usually coalesce due to copious extracellular polysaccharide production, convex, semi-translucent, raised and mucilaginous most with a yellowish centre, produce an acid reaction, time of appearance of isolated colonies (TAIC) 2 to 3 days (fast-growers).

- *Mesorhizobium* is treated the same as *Rhizobium*, but with TAIC 4 to 5 days (intermediate growers).

- *Bradyrhizobium*. Colonies circular, do not exceed 1 mm in diameter, extracellular polysaccharide production from abundant to few (generally in those strains taking more than 10 days to gross), opaque, rarely translucent, white and convex, granular in texture, produce alkaline reaction, TAIC 6 days or more (slow to very slow growers).

- *Azorhizobium*. Colonies circular 0.5 mm in diameter with a creamy colour, very few extracellular polysaccharide production (much less than in *Bradyrhizobium*

AM Fungi. Detailed description of procedures of spore isolation is given in Schenck (1982). Most probable number (MPN) is determined according to the method described in Sieverding (1991). Spore identification follows the taxonomic descriptions of Schenck and Perez (1990).

Microbial Biomass. Total microbial biomass is estimated by the fumigation-extraction method as described in Schinner *et al.* (1995).

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4.5. SOIL MACRO-FAUNA

By:
FX Susilo

4.5.1. INTRODUCTION

Land-use/-cover change is a major component of global change (Vitousek, 1994). In tropical regions, for instance Southeast Asia, land-use change is manifested in the forms of, among others, deforestation and agricultural intensification (GCTE, 1997). Extensive timber logging, short-cycled shifting cultivation, and slash-and-burn activities are responsible for deforestation as such that they have transformed the primary, intact forest zones in the region into logged-over and secondary forests, cropped land, tree-based agroforests, or *Imperata cyclindrica* grassland (van Noordwijk *et al.*, 1995).

Meanwhile, intensive agriculture offers a great potential of supplying food and other stuffs to match the needs of ever-growing human population. However, it often alters the biological regulations of the soil presumably via the depletion of the biodiversity of the soil biota as such that its contribution to sustainable agricultural productivity needs scrutinises (Swift, 1997). In the tropics the rate of intensification is greater than that in any other part of the world. Consequently, tropical ecosystems are getting more at risk of loss of their biological diversity. Conservation and enhancement of soil biodiversity are therefore relevant to lowering the risk and they are even more relevant to a significant number of farmers in the area who have limited access to agricultural inputs (Giller *et al.*, 1997).

This paper aims to share the information about the effects of land-use change or gradients of agricultural intensification on the biodiversity and other biological variables of soil biota, especially those of the soil macro-fauna. To achieve that goal, there is a need to present (1) what is meant by soil macro-fauna, (2) the existing data on the effect of land-use change on various attributes of soil macro-fauna, and (3) methods that have been standardised for assessing the impact of land-use types on soil macro-fauna in the tropics.

4.5.2. STANDARDISED METHODS TO ASSESS RESPONSE OF SOIL MACRO-FAUNA TO LAND-USE CHANGE

Macro-faunal sampling design should refer closely to those of Anderson and Ingram (1993 *cit.* Bignell, 1998). In a land-use type, sampling is centred on a single site of 40x5 m^2. It is recommended (Bignell *et al.*, 1998) that the mid-line of the site be taken as a transect for marking at least 5 soil monoliths of 25x25x30 cm^3. Parallel to the mid-line a second transect is set for placing at least 10 pitfalls of 14 cm diameter. If expertise are available, a still another transect of 100x2 m^2 can be set to specifically sample termites. The starting

point for the first transect ought to be selected randomly but its direction is taken based on the best judgement for representativeness of the land-use.

Having been tried out four times during rainy seasons of 1996 and 1997 in Jambi and Lampung, respectively, it was realised that rapid assessment for a site could be completed by 10 people in two working days (excluding night sorting and provisional identifications). It was recommended that the 10 people be divided into two groups, one of six is to do general macro-fauna (to handle the first and second transect) sampling. Both group can proceed to determine the second transect and directly fit all pitfalls on place. Pitfall containers are to be put at about 4 m intervals along its transect line and to be collected the next 24 hours. Each container contains about one-fifth volume of detergent solution to trap the falling invertebrates.

After fitting the pitfalls, the first group should directly move to the first transect to do monolith sampling. As mentioned before, at least 5 monolith points should be sampled in a site. At each point, firmly place a 25x25-cm^2-ring sampler, remove all litters inside the sampler and hand-sort in situ (or put them in a plastic bag for sorting later in the day). Then isolate the monolith by making three-edged trench of about 20 cm wide and 30 deep around it. If the soil is sandy then there is no need to make the trench. Instead, directly take the monolith – layer by layer – place them into a plastic bag, and sort.

Divide the monolith block into three layers (0-10 cm, 10-20 cm, and 20-30 cm). Using a small spade, shovel, or *parang*, place the soil layers into plastic bags, and hand-sort them for any organism 2 mm in size or larger. Finish all monolith diggings and start sorting in the day (time is normally limited to sort all samples in the day). Complete the sorting and labelling in the next day. Finally, record the taxa, number, and post-blot weight of the recovered macro-fauna (by taxa).

For the second group (termite sampling), select another transect of 100x2 m^2 consisting of 20 serial segments or sections of 5x2 m^2. Equip with respectively a plastic tray, a strong and sharp *parang*, a small shovel, a pointed forceps, and a number of vials, two people collect termites in each section for 30 minutes totalling one hour sampling per section. Concentrate the search in soil surface to about 5 cm deep, litter and humus at base of trees, inside dead logs, under rotten logs, tree stumps, branches and twigs, nest mound, and galleries on vegetation up to the height of 2 m. In case of *Imperata* grassland or others with very little above-ground biomass, spend only 15 minute per section to ensure equal attempts to search for termites in the soil (Jones *et al.*, 1998). Make sure that the priority is to collect soldiers, workers, and reproductive (in that sequence). Also make sure to label the section and origin of termite finding, for example wood (w), soil (s), interface of wood/soil (I), and epiphyte (e).

Results can be presented as list of taxa, number (by taxa), biomass (by taxa), and diversity indices (Shannon's or Simpson's) versus land-use types and with appropriate statistical analysis. In case real replications are impossible to make, it is possible to generate

a rougher or semi-quantitative data of relative number (Jones *et al.*, 1998) from the single site land-use with sections. But it is important to sum up the final synthesis of the data using some kind of matrix to reach that, for example as in Table 4.9.

Table 4.9. Example of overall quantitative synthesis of response of soil macro-fauna to land-use changes (after Bignell et al., 1998).

	Land-use Types				
Variables	A Natural (Control Site)	B	C	D	E
Density	x=80	x=67	x=50	x=95	x=57
Probability of No Difference		p=0.10	p=0.04	p=0.11	p=0.05
Relative Change (%)		-16	-38	+19	-29

x = average (per monolith), p = level of statistical significance for comparison with the control site A – any appropriate test will suffice, % = percentage of difference of land-use type from the control site A (- indicates decrease and + indicates increase in response).

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4.6. FIELD WORKSHEETS

SHEET 4.1.
GHG FIELDWORK SHEET

Site : ....
Date : ....
Land-use type : ....
Air temperature : ....°C

Soil temperature : ....°C
Latitude : ....
Longitude : ....
Altitude : ....
Observer : ....

Air samples

INTERVAL (MIN)	REPLICATE	SITE	SAMPLE CODE	REMARKS
A			A	
0	1		T0/1	
10	1		T10/1	
20	1		T20/1	
40	1		T40/1	
0	2		T0/2	
10	2		T10/2	
20	2		T20/2	
40	2		T40/2	
0	3		T0/3	
10	3		T10/3	
20	3		T20/3	
40	3		T40/3	

Soil samples

SAMPLING DEPTH (cm)	REPLICATE	SITE	SAMPLE CODE	REMARKS
	1			
	1			
	2			
	2			
	3			
	3			

SHEET 4.2.
GHG LABORATORY ANALYSIS SHEET

Gas name : ....
Unit : ....
Land-use type : ....
Sampling date : ....

SAMPLE CODE	REPLICATE	INTERVAL (minutes)	CONCENTRATION	REMARKS
A				
T0/1	1	0		
T10/1	1	10		
T20/1	1	20		
T40/1	1	40		
T0/2	2	0		
T10/2	2	10		
T20/2	2	20		
T40/2	2	40		
T0/3	3	0		
T10/3	3	10		
T20/3	3	20		
T40/3	3	40		

SHEET 4.3.
CARBON STOCK – NONDESTRUCTIVE MEASUREMENTS

Site number : .... Location GPS : .... E, ...S
Land-use type : .... Farmer name : ....
Sample taken by : .... Sample area : ☐ 5 m x 40 m
Date : .... ☐ 20 m x 100 m

No.	Type	Branch-ed? *(Y or N)*	Tree diameter, *cm*	Tree height (h) or length, *m*	Wood Density (s) *H/M/L*	Estimated Biomass DW, kg/tree		
						Cylinder *$D^2 h s/40$*	For branched trees:	
							$0.092\ D^{2.60}$ (Brown, 1997)	$0.030\ D^{2.87}$ (Ketterings in prep.)
1	LT	Y						
2	DST	N						
Total per category: kg / sample area LT DST DFT BG								

LT = live tree, DST = dead standing tree, DFT = dead, felled tree, BT = big tree (tree diameter > 30 cm, in large sampling area), estimated wood density: H = high, M = medium), L = low (0.6, 0.4, 0.2 g cm^{-3})

SHEET 4.4.
CARBON STOCK – DESTRUCTIVE SAMPLES

Site number : ....
Land-use type : ....
Sample taken by : ....
Date : ....

Location GPS : .... E, ...S
Farmer name : ....
Sample area : ☐ 0.5 m x 0.5 m
☐ 1 m x 1 m

No.	Type	FW *(kg)*	SFW *(g)*	SDW *(g)*	Tot DW= FW x SDW/(SFW x area) *(kg m^{-2})*	Biomass DW= 10xTotDW *(Mg ha^{-1})*
1.	Biom (L)					
1.	Biom (S)					
1.	CLit					
1.	FLit					
2.	Biom					
2.						

NOTES: W = fresh weight; DW = dry weight; S = sub sample; Biom = green biomass leaf (L), stem (S), tuber (T); CLit = coarse litter; FLit = Fine litter