Sixth Framework Programme (2002-2006)

FIELDWORK MANUAL FOR THE STUDY OF LEAVES AND WOOD EDITED TO ESTABLISH DROUGHT EFFECTS

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Based on the original protocol established by
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Sampling of Leaves and Wood

The objectives of the sampling of leaves and wood within the 1 ha parcels are:

1. To estimate the area and morphology of the leaves,
2. To determine the distribution of the of the foliage density,
3. To calculate the specific area of the leaves (SLA; g m^{-2}),
4. To estimate the carbon contents and nutrients in the foliage biomass and wood in relation to the branch's position and leaves present in the canopy (i.e. tall or sun recipients, half from top and low section from top),
5. To determine the wood density (specifically from the xylem or conductive tissue) of the sampled trees,
6. To calculate the radio area of leaves: wood area to determine the trees' hydraulic properties,
7. To indirectly estimate the leave area index, LAI or the leaf area container in one square ground meter (m^2 m^{-2}),
8. To multi-correlate the nutrients' results, LAI; SLA, top position, density, physiological properties to ground properties (nutrients, density, etc.) and climate.

a. Fieldwork protocol

Branches of more or less one meter length should be cut from 20 trees. The branches should be cut by the same person who should climb in-between 3 to 5 trees per parcel.

Selection of trees (for climbing) - criteria:

1) it can be climbed i.e. it does not have ant, wasp or termite nests it does not have thorns;

2) it should have more than 10 cm of DAP and it is high enough for the top to be exposed to the sun;

3) it should have at least 4 trees with 10 cm or more of DAP close or surrounding it, with the top exposed to the sun and those should be accessible enough for the person who climbs be able to collect a branch of the exposed top from the climbed tree;

4) it does not matter if the neighbouring trees belong to the same species;

5) those should be inside a permanent parcel and should be distributed in such a way that those include the parcel's soil and topographic variations.

Collections:

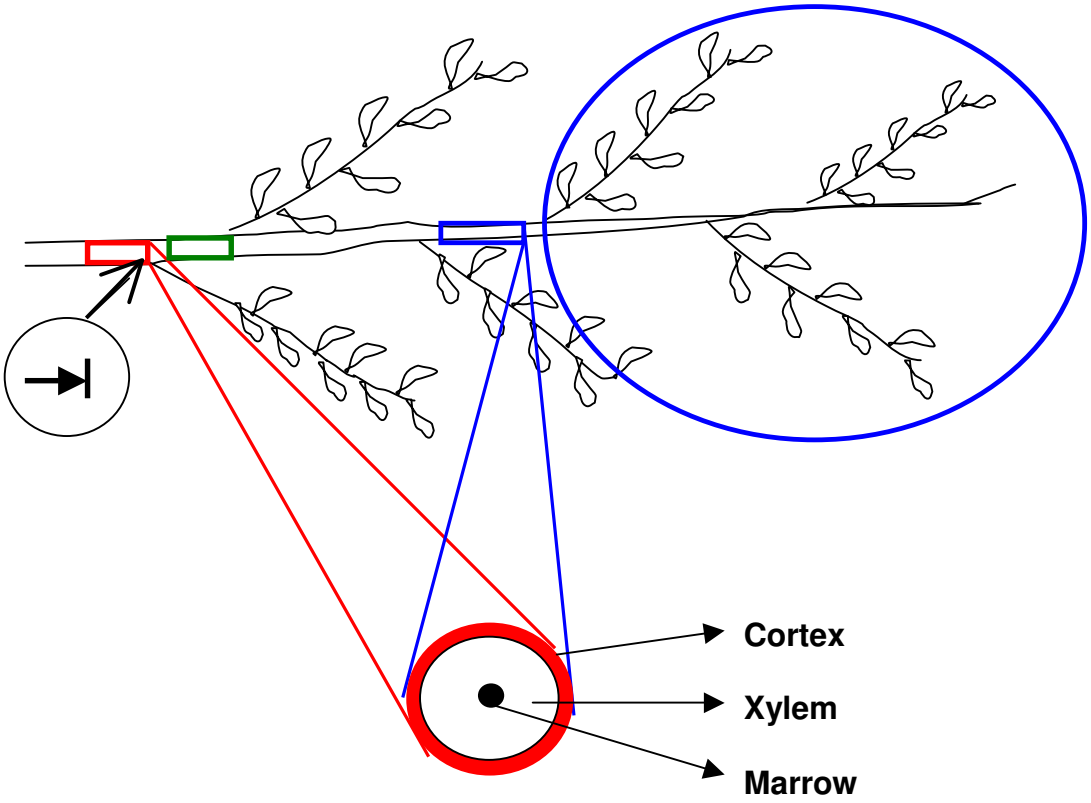
From every climbed tree, three branches are collected: one from the highest part of the top (branch exposed to the sun), one from the center of the top (sun/shadow) and one from the lowest part of the top (shadow). And from the same tree one branch from the exposed top of four neighbouring trees are collected.

Recommendations:

When a person is on a tree he should maintain certain distance because many dry branches can fall and at any time the branch cutter or any of the tubes can fall. Maintain communication with that person: identify together from which tree a fallen branch belong to. Mark the branch at the base with tape or sticking plaster, write the parcel code on the tape, the tree number and the position of the branch in the canopy (high, medium, low). Collect the branches in one place, because if those are left where they fall it is very possible not to find them when coming back to the site.

Branch processing:

From each branch we count all the leaves. Once the leaves are counted, we select one random sample big enough to fill up a plastic bag A4 size. When counting leaves one should take into account if the specie has simple or compound leaves. When a species has compound leaves, each compound leave counts as "one" and the approximate number of leaflets per leave. For example, one branch with 20 compound leaves, naturally will have a variable number of leaflets, let's say between 3 and 9, so this information should be registered in a field notebook, in the collected wood section (see it further in this document) and in the bag where the leaves are to be kept. The leave information for this example will be: 20 x 3-9). When the leaves are ready, two wood segments are collected from the same 10 cm (aprox.) branch. One should be the end segment: which "feeds" all the counted leaves (figure 1, red rectangle), this should be marked with an arrow and a line (see symbol enclosed in circle, Figure 1) that indicates that this xylem or wood supported the leaves, the other segment should be close (Figure 1, green rectangle).



Note: If the branch is very big (baseline diameter > 2 cm or it has too many leaves, a minor branch from it can be selected for leave counting and for collecting a wood segment of 5 cm that corresponds to the "xylem that feeds the counted leaves (blue rectangle and circle, Figure 1). This wood segment should carry the symbol with the line and arrow in order to allow the counted leaves' recognition.

One of the wood segments of 10 cm that are being collected is used to measure xylem density. Generally, the end segment is used, but if it is too deformed, short or broken, another segment from another side of the branch can be chosen (blue or green rectangle, Figure 1). When the wood segment that is used to measure density corresponds to the segment that feeds the counted leaves it should be labelled with the arrow and line symbol. Besides this, it should be marked with the name of the parcel, tree number, position of the branch in the leaflet (high, medium or low) and number of counted leaves, taking into account the compound leaves. The second 10 cm segment (a replica) is only labelled with the parcel code and tree number. Keep the wood in the same bag as the leaves and label the bag with the same information that the wood piece kept for density has. To label the bag

generally a piece of white plaster or masking tape will suffice. The bags containing the leaves and wood are to be taken to the laboratory at the end of the day trip.

The height to which the branches from the climbed tree are collected is measured with a 50m tape taken by the climber. The end of the measuring tape could be attached to the branch cutter and another person located below at the bottom of the tree can read the height. The height of the neighbouring trees could be estimated by the climber using the cutter and the measuring tape. Another option is to measure the height of the top and from the first branch of each tree using a clinometer.

Additional procedures for the drought project:

Before counting the leaves from the collected branches those should be separated based on age (Figure 2, three ages) and count the number of leaves under each age. If it is possible observe differences in the leaves' ages: old leaves are generally the ones located closer to the base of the trunk, are darker, could have lichens, the front is darker as if it were dirty or dusty, present more physical damage, like tears, not being necessarily more herbivoria than the young ones, these leaves theoretically represent the group of leaves that grew up before the drought period of August – September 2005. The ones of middle age are generally brilliant, cleaner, with minimum presence of lichens and could have herbivoria. If theoretically these grew during drought season they could present some symptoms such as

Figure 2

Photo from the different leave "ages" Manaus, Brazil December 2005. From left to right: old leaves, young leaves possibly developed during drought period and new leaves with possible develop after drought period. (Photo taken by Peter Vitzthum, please consult before publishing or distributing, e-mail: vitze-olvthueringen@web.de)

rolling, yellow colour, tightness, smaller size; observe and try to recognize these and other symptoms; after all the new leaves should have been born in between November (2004) and January (2005) or are starting to form at the moment of collection. These leaves can present herbivoria, shine, a softer green, cleanness in the front and closer to the tip. Thus, every group of leaves will go in a separate bag with the following information: parcel code, tree number, age, number of leaves of that age.

b. Laboratory protocol

Scans. From the bag with leaves we select a random sub-sample of 5-60 leaves (depending on size, Fig. 3) to be scanned and to calculate area and morphology of each leave. For each branch a scan of at least A4 size should be performed. If the leaves are very big 5 leaves of

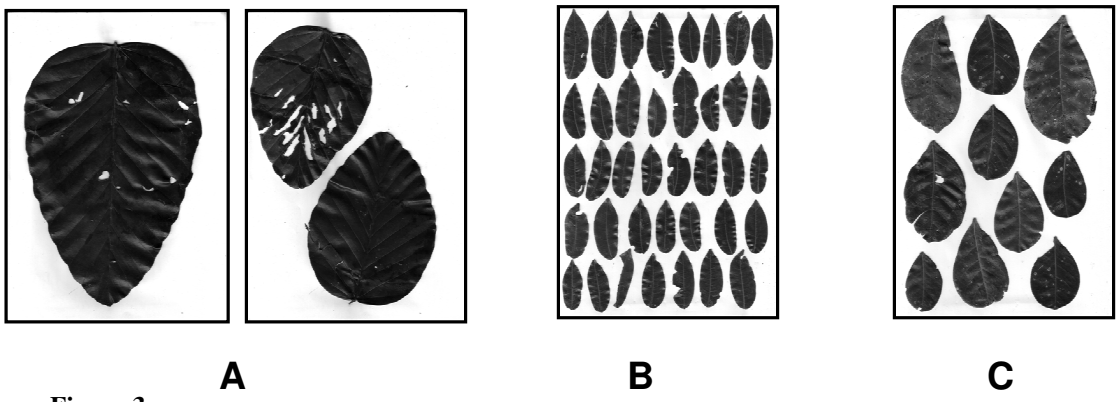


Figure 3

Scanned leaves' model showing different species and sizes. A- A specie with big leaves; B- Small leaves; C- Medium-size leaves

different sizes are to be scanned (Fig. 4 A) and if the leaves are very small all the ones that fit in an A4 sheet should be scanned (Fig. 4 B). The ideal situation is to choose 10 leaves that represent all the sizes that were present in a branch (Fig. 4C).

Note: For the scans do not chose leaves that are starting to develop form, close leaves completely formed.

If it is possible, the leaves are to be scanner the same day they are collected to avoid drying and canopy area reduction. If it is not possible, the leaves should be kept in sealed plastic bags in a fresh and dark place. When the scanning cannot be performed within five days a

mix of water and alcohol should be added to the bags in order to maintain them and avoid decomposition.

Before being scanner, the petiole should be cut off from the leaves. Alter being scanned, the leaves of each scan should be put in an envelope or paper bag labelled with the scan code and number of scanner leaves. After being scanned the leaves are dried up in a botanical oven for three days at 70 °C. When those are totally dried, their dried mass is measured in grams.

Additional protocol for the drought project:

Choose several leaves from different years (the number Hill depend on availability) and perform a scan for each different age.

Recommendations:

1) Keep the scanner clean. This can be accomplished by sticking a white paper sheet (office or A4 size) on the lid surface and changing it when spots develop. On the glass base from the scanner an acetate (transparent sheet) should be attached with transparent tape

2) Avoid the leaves to touch each other, (Figure 4.A);

4) When scanning keep the scanner lid in a soft and homogeneous position to avoid light entering in the moment the scanning runs. If light enters in the background of the scan will be grey or black, which will cause problems when analyzing leave's area (Figure 4B);

5) look at the correct scan (Fig. 4 C) 3) Put all the leaves vertically, not horizontally (Fig. 4 A), in case the scan is analyzed with WinFolia, this software analyzes the relationship between length and width;

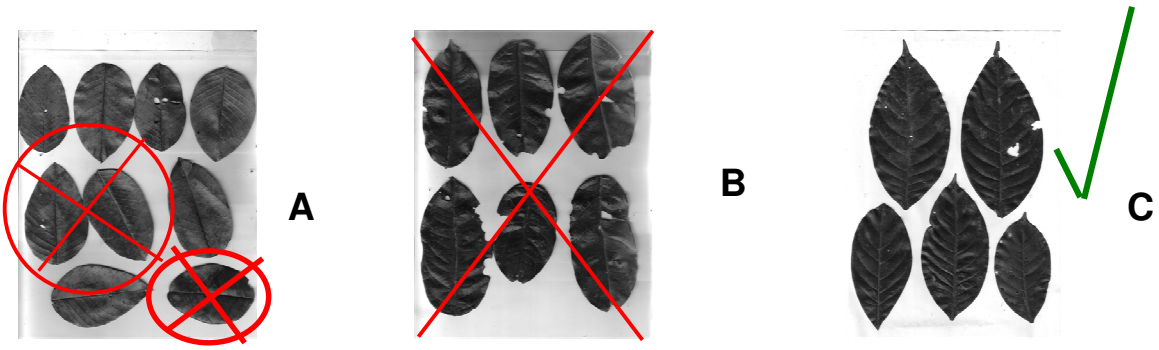


Figure 4.

Mistakes that should be avoided during the scanning process: A- Do not leave over-imposed leaves of out of the scanning area or touching each other; B- Avoid the dark shadows at the bottom of the scanner.

6) Seal the envelopes with staples or adhesive tape Carmelita type (for parking, it is the only one that does not comes off due to the oven heat) in order to avoid the loss of leaves' particles.

7) The scanner configuration is as follows:

Image type: grey scale

Image quality: 300 dpi

Paper size: A4

Format, image type: TIF

7) Type the following information in a notebook or in an Excel spreadsheet:

Parcel Code	**Tree Number**	**Leaves position**	**Scan code**	**Leaves number or scanner leaflets**	**Leave type** **If compound by leaflet number per leave**	*** Age of leave**	**Total number of leaves in the branch** ***Drought Project: total number of leave with the same age in the branch**	**Observations:** **Moss** **Lichens** **Herbivoria** **Dry yellow parts with parasites or gills** **Soft texture, leather like, etc.**
ZAR-01	34	low	Z-001	5	simple	young	60	herbivoria moss
ZAR-01	34	low	Z-002	5				
ZAR-01	10	high	Z-003	10	3-9	old	40	lichens, herbivoria

8) When finishing the scanning process, take off the white sheet and transparency, clean the surfaces with alcohol and block the scanner to facilitate transportation. Almost all the scanners have a block button located at the bottom.

Area and morphology. The leaves' area and morphology will be calculated analyzing the scans with the use of a software program (Win Folia Basic 2001a, Regent Instruments Inc., 4040 rue Blain Quebec Qc. G2B 5C3 Canada) or another one available.

Leaves preparation for nutrients' analysis. The surplus of leaves of each bag is packed in envelopes or paper bags to be dried in botanical dryers for three days at 60 ° C. After being dried, the central vein is cut, then cut in small pieces, and then they are mixed and they are kept in paper envelopes (letter size) to be sent to the respective laboratories for nutrient analyses. The envelopes are labelled with the parcel's name, tree number, position in the leaflet and country. It should be noted that the central vein removal is faster if it is made with the fresh leaves at the field site or on the same collection day at the laboratory.

Wood preparation for nutrients' analysis. One of the collected wood segments can be used for the analysis of nutrients. This one is packed with the leaves that are previously marked with the name of the country, parcel, tree number and leaflet position.

Wood density. The wood pieces used to measure density are carefully cut to avoid, if possible, shape imperfections.

From a fresh wood segment we take the following measurements:

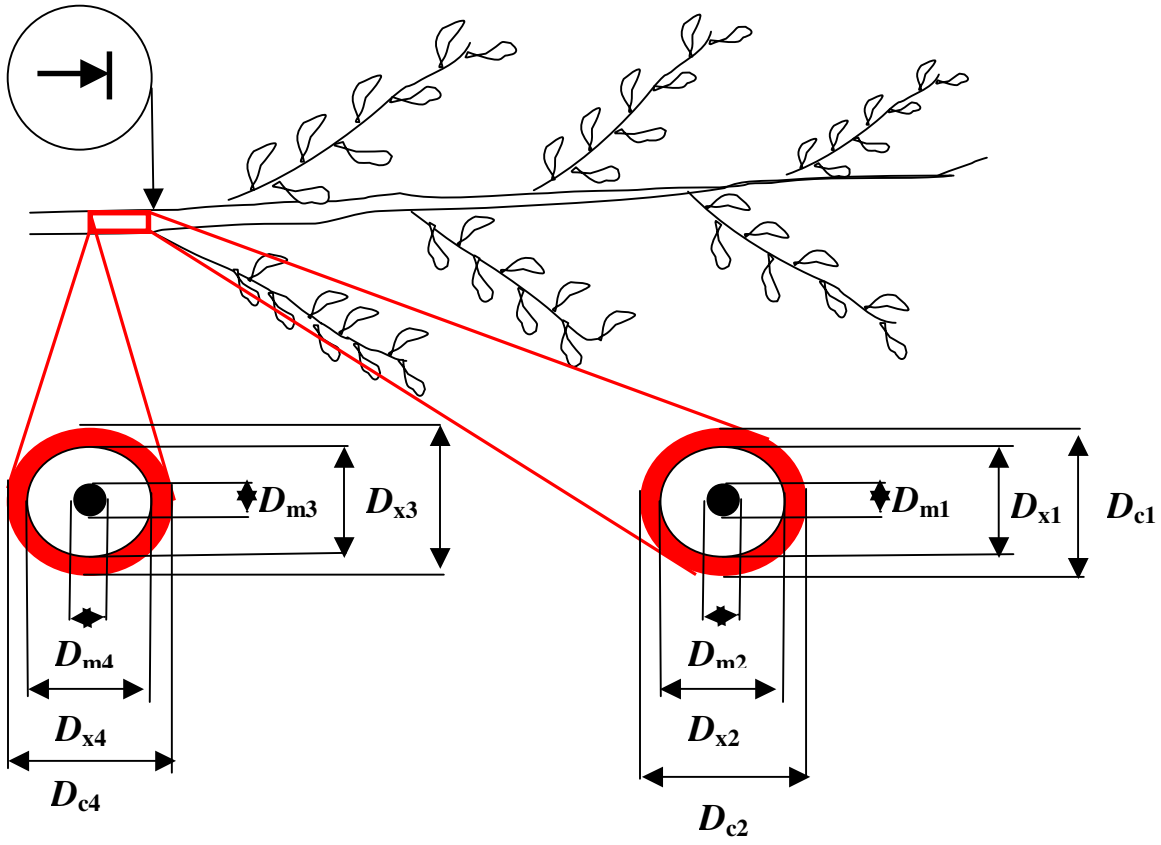


Figure 5

Diagram of the needed measurements to be taken from the wood segments

1. length
2. 4 diameters with cortex (D_{c1}, D_{c2}, D_{c3}, D_{c4}, Fig.5)
3. 4 diameters without cortex (D_{x1}, D_{x2}, D_{x3}, D_{x4}, Fig.5)
4. 4 diameters from the marrow (D_{m1}, D_{m2}, D_{m3}, D_{m4}, Fig.5)
5. fresh weight with cortex
6. fresh weight without cortex

The xylem's fresh volume is calculated multiplying the xylem's area times the longitude of the trunk without cortex, or using a scale taking the weight (g) of the volume of displaced

water in a glass when the trunk is introduced (Archimedes' Principle). From the trunk's fresh volume we subtract the marrow's volume, obtaining in this way the xylem's fresh volume. Once the fresh trunks are measured those are stored in a paper bag with its correspondent cortex and are left to dry in an oven for botanical simples at 80 °C for 3 or 4 days. When these are dry the cortex and wood are weighted by themselves.

Density is calculated with the following formula $\rho = \frac{m}{V}$, where ρ is density (g cm.$^{-3}$), m is dry mass (g) and V is the fresh volume. In the same way, we can calculate the trunk cortex density and the cortex density. These are data that are available and that can be useful in the future.

Relation leave area: wood area: The total canopy area of each branch is estimated using the measured area of the scanned leaves and extrapolating to the total number of leaves in each branch. The wood area that feeds that leave "area" is calculated with the xylem's diameter. With this in mind the wood diameters (xylem) are measured opposed to the Terminal end from the collected simple labelled with the symbol "arrow-line" (D_{X1} y D_{X2}, Fig. 5) and the diameters opposed to the marrow (D_{m1} y D_{m2}, Fig. 5). An average is taken from the xylem and marrow diameters, the marrow and xylem areas are calculated and the marrow's area is subtracted from the xylem's area. The areas are calculated with the formula: $a = \pi r^2$, where r is the xylem or marrow ratio.

Additional measures performed from the trunks to calculate density:

1) Volume with cortex

2) Volume without cortex

Recommendations:

Measure fresh volume in the field because it is easier to remove the cortex from the trunk the same day it is collected.

Foliage's nutrients analysis. The analyzes of foliage's nutrients Hill be done in Leeds following Standard protocols for analysis of carbon, nitrogen, phosphorus and other elements.