ANNEX 2: PRACTICAL FIELD GUIDE

PRACTICAL FIELD GUIDE FOR NON-TREE PLANT DIVERSITY ASSESSMENT

PROCEDURES

1. Structure based indicators (cover of shrubs, herbs and "giant herbs")

- Consider a 10 m radius subplot within each plot. Through visual estimation assess the cover of shrubs (separated for three layers: High: 2-3m, Middle: 1-2m, Low: 0-1m), herbs and "giant herbs" according the following 4 cover classes:

(1) <5%; (2) 5-25%; (3) 25-50%; (4) 50-75%; (5) 75-100%.

Definitions. Shrubs' category includes plant species shorter than three meters. Within this category we consider two life forms. The first includes actual shrubs (*chamaephytes*, woody plants with perennating buds on branches on or near the ground, <3m). The second considers only the juvenal or the seedling of tree species (*phanerophytes,* woody plants with perennating buds above ground, >3m) that constitute the forest structure.
Herbs are described as non-woody species with annual or perennial life cycle, generally lower than 1m. Most of them are *Hemicryptophytes* and *Therophytes*. *Hemicryptophytes* are plants with perennating buds at ground level, with or without stolons or rhizomes. Some examples are *Alpinia caerulea*, *Asplenium nidus*, *Imperata cylindrica*. *Therophytes* are annuals where the individual exists as a seed during the most unfavourable season. Some examples are *Ageratum conyzoides*, *Crassocephalum crepidioides*. In many cases one must rely on local knowledge to determine whether a species is a true annual and not bi-annual or tri-annual as is the case of some grass species.
Giant herbs. Even if most of the herbaceous species in tropical forests are represented by small plants not taller than one meter, there are numerous species/genera that are particularly tall. Examples of tall herbaceous plants are Musa (banana plants, Fig. 2A), *Araceae* (Fig. 2B), *Bamboos,* tree ferns, *Marattiaceae* (ferns, Fig. 2C), *Pandanus* and *Zingiberaceae*.

- The assessment, through visual estimation, of each species present in the 10 m radius subplot centered in the center plot will be conducted during the Taxon-based biodiversity evaluation, as described in the following paragraph.

Equipment: -

Time: 20 minutes (estimate, not tested in the field)

2. Taxon–based biodiversity indicators

Within the central plot of each cluster, consider a 10 m radius plot in 8 equal segments to simplify the collection procedure. In each segment collect all the species present, except the ones that have already been collected in the previous segments (if there is some doubt collect them again!). When possible, collect three samples for each unknown species.
Consider all the NTP (Non-Tree Plant species: herbs, ferns, shrubs, lianas, "giant herbs"), as well as all the trees below 10cm DBH.

Time: 1 h

Bring all the collected plants to the camp site. Insert data in the Collect mobile software:

1. Species name (if you are unable to ident)
2. Number of individuals (this is related to structural diversity but we inserted this information here to be consistent with field procedure and Collect Mobile database)
3. Life form and above-ground specialized roots
4. Specimen Code
5. Picture number (if a picture is taken)

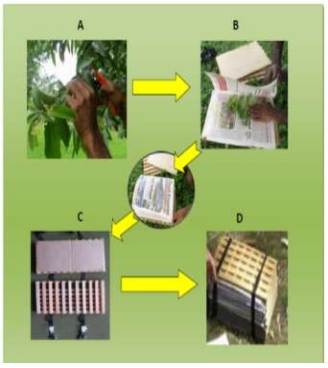
Store the specimens for shipment to Lae Herbarium (newspapers, cardboards, plastic bags, ethanol). Cut small samples (2cm x 2cm) for DNA analysis of "important" species listed by PNGFRI.

Special cases: if the Central plot is highly disturbed, next to a river or severely damaged by some unpredicted event, the operators have to collect NTP specimens in another plot of the cluster, proceeding in alphabetical order (East, North, West) until an appropriate plot is found.

Equipment: 20 plastic bags (1 m x 50 cm); 250 labels for specimens; ethanol (5 l); camera; compass; 2 roller tapes (at least 10 m long); telescopic pruner; slingshot with accessories (fishing gun rubbers, sinkers, fishing line 100 m x 20-30 lbs); secateurs; newspapers; scotch tape; plant press; plant press stripes; small plastic bags (Ziploc/Minigrip) for DNA samples (5 cm x 10 cm).

Time: 6 h

Total time: 7 h



2.1 Collection of leaf fresh material of Syzygium spp. For DNA analysis

The collection of leaf material is not related to any specific design (plots and cluster-plots), but it is opportunistic. Wherever the botanists find a Syzygium sp., they have to stop and collect leaf material, according the following procedure, and a specimen to confirm the field identification in the herbarium. Data about leaf material and specimen (ID, data and GPS coordinates) have to be inserted in the dedicated Collect Mobile software.

We recommend collecting 4–6 grams of fresh leaf material torn into pieces not exceeding 2 cm2. For storing and drying this material for DNA extraction we suggest to use empty teabags such as those that are widely available in supermarkets and specialist tea shops. The material is placed inside the teabag with a collector label placed against the wall of the bag so that it is easily legible (Fig. A) and sealed (usually by folding the top over). The teabag is then placed in an airtight container and completely submerged in silica gel until completely dry (Fig. B). The container is shaken frequently over the first day to make sure dry silica gel is always in close contact with the plant material. Once the material is completely dry, the teabag can then be removed from the silica gel and placed in an airtight container which has a fresh layer of silica gel at its base (Fig. C) for longer term storage. This silica gel layer can be easily replaced if it becomes hydrated.

Fig. A. Ripped leaf material placed in teabag with internal paper label. B. Teabag submerged in silica gel (regularly shaken). C. Longer term storage of teabags in sealable container with layer of silica gel at base. (Photos: P. Wilkie)

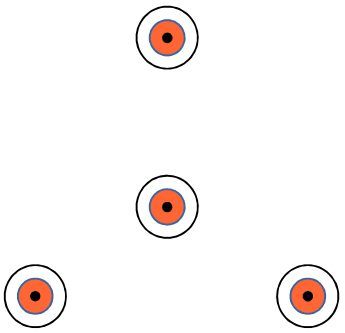
3. Functional diversity indicators

Leaf traits. Select the relatively young (presumably more photosynthetically active) but fully expanded and hardened leaves (minimum 5, preferred 10 leaves, per, if possible, 2-3 individuals of each species) from adult trees (dbh>10cm in 10m radius and all trees dbh>20cm in 15m radius sub-plot). Do not consider NTP (Non-Tree Plant species: herbs, ferns, shrubs, lianas, "giant herbs"). Wherever possible, avoid leaves with obvious symptoms of pathogen or herbivore attack, or with a substantial cover of epiphylls. LA is strongly affected by light intensity. Therefore, for many research questions it is best (giving the fairest comparison across individuals or species) to sample outer canopy leaves (also called 'sun leaves') using slingshot. For species that typically grow in the overstorey, take leaves from plant parts most exposed to direct sunlight. For true shade species (those that never grow in full sunlight), collect leaves from the least shaded parts found (but not from those that look light-stressed or bleached).
Store the specimens for shipment to Lae Herbarium as described before for Taxon based indicator.

WHERE DO THE PROCEDURES HAVE TO BE CARRIED OUT?

1. Structural diversity (cover of shrubs, herbs and "giant herbs")

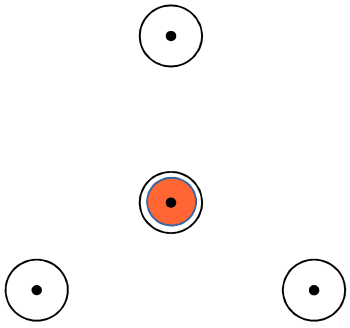
Where?
All clusters, all plots
subplot of 10m radius



What?
shrubs (separated for three layers: ***High: 2-3m, Middle: 1-2m, Low: 0-1m****), herbs and "giant herbs"*
Vegetation cover, according the following 4 cover classes:
(1)<5% ;(2)5-25%; (3)25-50%; (4)50-75%; (5) 75-100%.

2. Taxon–based biodiversity indicators

Where?
Center plot of each cluster-plot,
subplot of 10m radius fragmented in 8 equal segments



What?
Consider all the NTP (Non-Tree Plant species: herbs, ferns, shrubs, lianas, "giant herbs"), as well as all the trees below 10cm DBH.

2.1. Collection of leaf fresh material of Syzygium spp. For DNA analysis

Where?
Wherever the botanists find a Syzygium sp.

What?
Collect 4–6 grams of fresh leaf material torn into pieces not exceeding 2 cm2.

3. Functional diversity indicators

Center plot of each cluster-plot,
subplot of 10m radius