Protocol for screening functional traits of the tree community using understory material.

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Plant traits are normally collected at the species level, following protocols used world-wide (Perez-Harguindenguy et al. 2013, Cornelisen et al. 2003). This has many limitations, starting with the need of having all species previously identified to be able to start the sampling. Second, the species concept must be the same everywhere, which is improbable when several sites are being studied. Thirdly, species complexes in this approach will be lumped in a single taxon. Apart from the pure taxonomic problems, this approach of taking means over species ignores the intra-specific variability, which may be crucial to the adjustment of the species to different environmental conditions, and which may provide different responses to changes in these conditions. Considering that the landscape is the unit of management in most cases, it is important to understand the processes at this level, which implies understanding the behavior of individuals along the different environments across the landscape. Therefore, the sampling protocol to characterize PPBio plots is done at the individual level, including all species that may be present in the plots.

Collection of plant traits using the individual-based approach can be very time consuming, and limits the number of individuals that can be sampled. It is better to sample a smaller amount of the plants present in a single plot, and sample more plots, than the opposite. For trees, the best strategy to start is to sample all plants of the first size class (1 to 10 cm DBH) of a small strip (1 x 250 m) of each standard plot (250 x 40 m). In a place with a dense forest, such as Reserva Ducke, this will be around 70-150 individuals per plot. This amount of collections can be processed in 1 to 2 days per plot, with a team of 3 well trained people in the laboratory.

This first sampling can give a good representation of the trait variation per site and per environment within sites, and provide the data for evaluation of community assembly, community functional dynamics, and ecosystem processes such as growth, mortality, recruitment and productivity. If money and time are available, a second collection can be done of the bigger trees (>10 cm DBH) in a 10 or 20 m strip of the plot.

This sampling strategy is not adequate to represent in detail the variation within species, given that a small number of individuals per species will be collected in species-rich sites (this may not be the case in species poor sites, or those with monodominant species). If this is the interest of research, then a collection of individuals of the focal species in each plot must be done.

The measurements in the protocol presented bellow will allow the calculation of the following traits:

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Leaf Size (LS)
Specific Leaf Area (SLA)
Leaf Dry Matter Content (LDMC)
Petiole Dry Matter Content (PDMC)
Leaf Thickness (LT)
Leaf Density (LD)
Force to Punch (FP)
Specific Force to Punch (FPs)
Chlorophyll content per unit leaf area (Chl)
Branch Leaf Area (BLA)
Leaf number per branch length (LN_{bl})
Leaf area ratio (LAR)
Leaf Mass Fraction (LMF)
Wood Density (WD)
Wood Dry Matter Content (WDMC)
Branch Density (BrD)
Bark density (BD)
Bark Dry Matter Content (BDMC)
Specific Branch Length (SBL)
Branch Pith Proportion (PithProp)
Branch Xylem Proportion (XylProp)
Branch Bark Proportion (BarkProp)
Bark Thickness (BarkT)

SAMPLING STRATEGY

- Collect samples from all tree individuals of:
    - 1 to 10 cm DBH in each plot
    - In 1m of the strip used in the census plots for small trees (the strip is 1.5m, but we use only 1m). In the case of standard plots installed following recent protocols, this will be to the left side of the central line
    - Choose the branches in the more illuminated side of the crown, and with healthy leaves. Although it is best to always have mature leaves with no signs of herbivory or pathogens, it is hard to find all plants in this condition, so take the “best possible”.

IN THE FIELD

- Collect one branch for each tree (50 cm long) for leaf and branch traits. If the plant has only one branch, or if taking a branch 50 cm long will damage more than 10% of the canopy, skip the plant, and note in the field sheet that this was the case.
- Measure tree height, and the Clark and Clark (Dawkins modified) light index (see the protocol for this index in Keeling & Phillips (2007). Height can be measured with the collection pole, and for taller trees, with a hypsometer.
- Estimate also the height of the leaf sample, and Dawkins index of leaf sample if it is not the same as the plant

IN THE LAB

Here all the measurements follow the protocols of Perez-Harguindeguy et al 2013.

1. Measure branch length and count number of leaves. If possible, scan all the leaves to have a better estimate of branch leaf area. If not, follow protocol the below.

LEAF PROCESSING

2. Take 2 good leaves (no or few signs of herbivory, epiphylls and disease) and measure:
    a. Thickness – one measurement per leaf, away from the main and secondary veins, preferentially in the middle of the leaf.
    b. Chlorophyll (with a SPAD) - two measurements per leaf, in the best places of the leaf
    c. Leaf area (scan the leaves). Area is calculated later with Image J.
    d. Leaf fresh weight (weigh petioles separate from leaf)
    e. Force to punch (only one leaf). An apparatus to measure this force can be built easily with simple materials (see Figure 2).
    f. Put samples in paper bag to dry (70 C for 48 hs)
3. Take one good leaf to store for anatomy
    a. Cut a strip including the main vein and store in FAA

BRANCH PROCESSING

4. Take 3 pieces of the branch, starting from base (see Figure 1)
    a. The first 2cm long, goes for anatomy
    b. The second, 0.5cm long, goes for NIR
    c. The third, 5cm long, goes for BWD
5. For the first piece of branch (anatomy):
    a. Under stereomicroscope, measure extension of pith, xylem and bark (Figure 3)
    b. Store sample in FAA
6. For the second piece of branch (NIR):
    a. Store piece in silica
7. For the third piece of branch (BWD):
    a. Record weight
    b. Measure length and diameter (in the center, avoiding parts with nodes)
    c. Measure volume (water displacement)
    d. Take bark out
    e. Measure diameter, weight and measure volume again
    f. Put sample in paper bag to dry (105 C)

DRY MATERIALS

8. After the drying period (48 hs for leaves, 72 hs for branches), weigh samples. Make sure the samples are kept in a dry environment (such as a box with silica) while they are waiting to be weighed, so they don´t regain humidity.

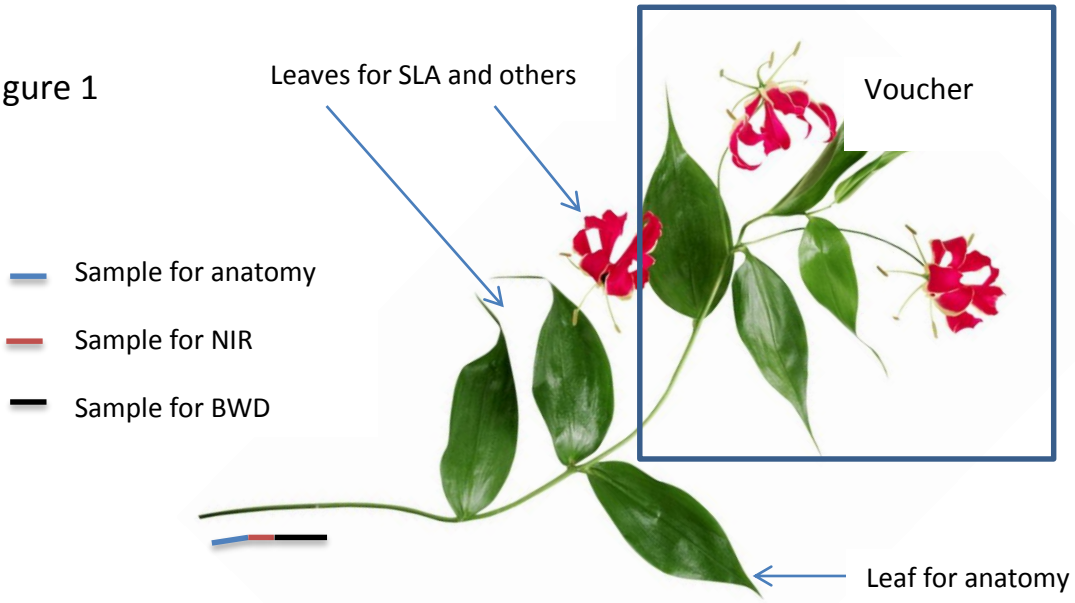
VOUCHER

9. With the remaining material of the branch, make a voucher (even if there are no flowers or fruits!). Make sure that it dries well, and leaves are flat, so leaves can be used for NIR spectrometry.

IMPORTANT TIPS

With so many samples, it is easy that some get lost or numbers are changed. Check processed samples at the end of the day, and don´t discard extra material until the end of processing (keep then closed in their original numbered bags into the lab), for any needed replacements.

Figure 1



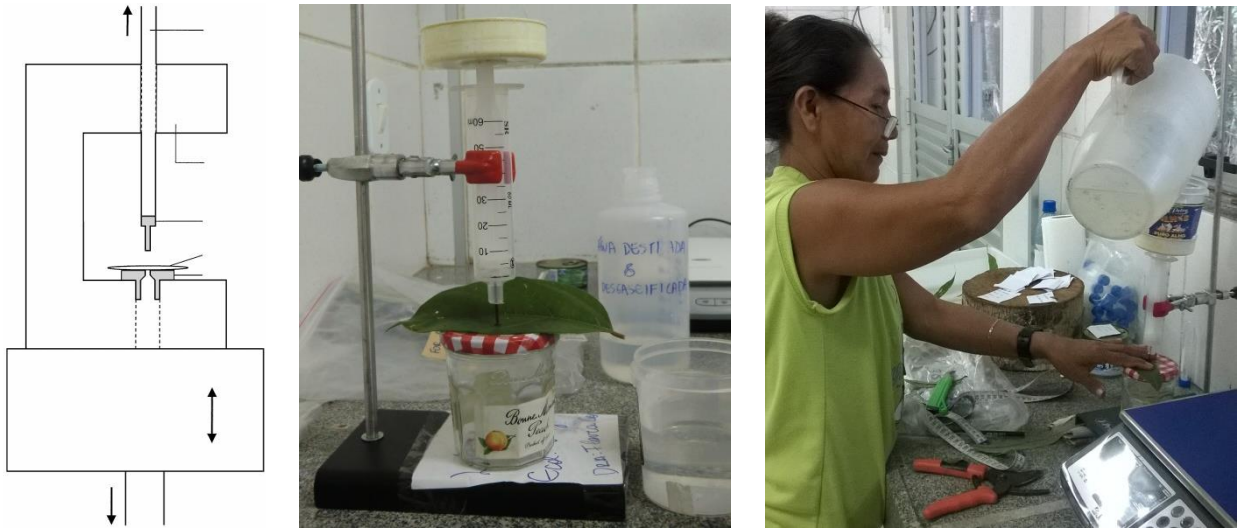


Figure 2. On left, the design of the Chatillon Universal tester to determine force to punch a leaf (figure copied from Arawella et al. 1999) and on the right a simple home-made version of it. A pot is fit over the syringe and gradually filled with water until the weight is enough to perforate the leaf. The leaf must be held in place by hand while pouring gently the water. The marmalade pot bellow the leaf has a hole in the lid, through which the nail attached to the syringe will pass.

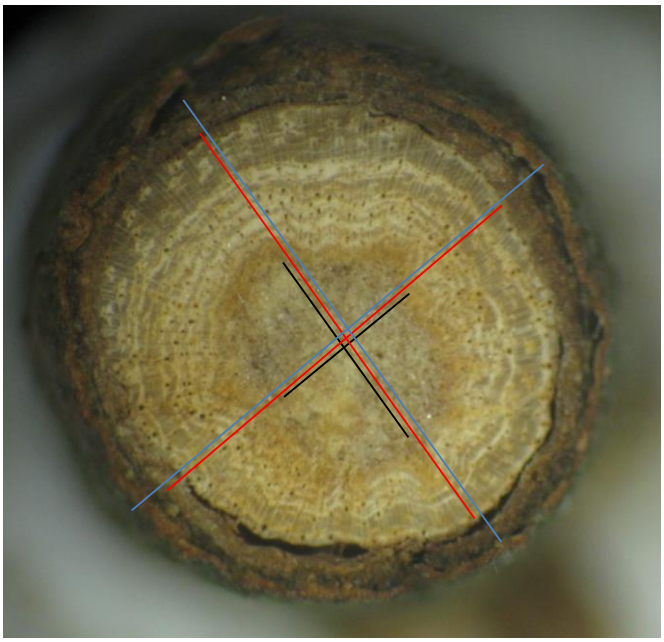


Figure 3. Measurements of macro-anatomy of the branch. Black lines are the measurements of the pith, red lines xylem + pith and blue lines the whole diameter of the piece. Measurements are converted to areas, and discounted from each other to obtain the area of xylem and bark.

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