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CONTENTS

WINGFIELD MJ. Why IUFRO really matters to tropical forestry research	393
BUAJAN S, PUMIJUMNONG N, LI Q & LIU Y. Oxygen isotope ($\delta^{18}\text{O}$) of teak tree-rings in north-west Thailand	396
ZAPFACK L, NOIHA NV & TABUE MRB. Economic estimation of carbon storage and sequestration as ecosystem services of protected areas: a case study of Lobeke National Park	406
COURARD-HAURI D, CHANCELLOR R, RUNDUS A & BOLAND A. A method for estimating the current and future carbon content of standing biomass applied to Gishwati Forest Reserve, Rwanda	416
DO TV, CAM NV, SATO T, BINH NT, KOZAN O, THANG NT & MITLÖHNER R. Post-logging regeneration and growth of commercially valuable tree species in evergreen broadleaf forest, Vietnam	426
YANG L, CHEN Y, HUANG Y, WANG J & WEN M. Mixed allelopathic effect of <i>Eucalyptus</i> leaf litter and understorey fern in South China	436
HERNÁNDEZ-MÁXIMO E, LÓPEZ-UPTON J, SÁNCHEZ-MONSALVO V, VARGAS-HERNÁNDEZ JJ & SALAZAR-GARCÍA JG. Early performance and genetic gain of <i>Cedrela odorata</i> families from wide-ranging sites in Mexico	446
RIBERA J, TANG AMC, SCHUBERT M, LAM RYC, CHU LM, LEUNG MWK, KWAN HS, BAS MC & SCHWARZE FWMR. In-vitro evaluation of antagonistic <i>Trichoderma</i> strains for eradicating <i>Phellinus noxius</i> in colonised wood	457
SAIFUDDIN M & NORMANIZA O. Rooting characteristics of some tropical plants for slope protection	469
SANN B, KANZAKI M, AUNG M & HTAY KM. Assessment of the recovery of a secondary tropical dry forest after human disturbance in central Myanmar	479
MOHD-SHAHWAHID HO, MOHD-IQBAL MN, AMIRAMAS-AYU AM, RAHINAH I & MOHD-IHSAN MS. Social network analysis of Kampung Kuantan Fireflies Park, Selangor and the implications upon its governance	490
LEE CJ, CHUNG MJ, CHENG SS, CHANG TC, YANG TH & CHANG ST. Effects of Dricon treatment on surface properties and fire resistance of Taiwan and China moso bamboo (<i>Phyllostachys pubescens</i>)	498

ARELLANO G, CALA V, FUENTES A, CAYOLA L, JØRGENSEN PM & MACÍA MJ. A standard protocol for woody plant inventories and soil characterisation using temporary 0.1-ha plots in tropical forests	508
JARA AA, DARACAN VC, DEVERA EE & ACDA MN. Techno-financial analysis of wood pellet production in the Philippines	517
SUKADARYATI, DULSALAM & YUNIAWATI. Effects of tapping pattern and wood vinegar, lime and galangal stimulants on sap production of swamp jelutong (<i>Dyera lowii</i>) in Indonesia	527
ROSZAINI K, HALE MD & SALMIAH U. In-vitro decay resistance of 12 Malaysian broadleaf hardwood trees as a function of wood density and extractives compounds	533
THANKS TO REVIEWERS (VOLUME 28)	541
AUTHOR INDEX (VOLUME 28)	542
CONTENTS OF VOLUME 28	545

A STANDARD PROTOCOL FOR WOODY PLANT INVENTORIES AND SOIL CHARACTERISATION USING TEMPORARY 0.1-HA PLOTS IN TROPICAL FORESTS

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ARELLANO G, CALA V, FUENTES A, CAYOLA L, JØRGENSEN PM & MACÍA MJ. 2016. A standard protocol for woody plant inventories and soil characterisation using temporary 0.1-ha plots in tropical forests. The use of both uniform woody plant inventories and laboratory soil analysis methods facilitates data sharing and improves the understanding of large-scale biological patterns in tropical forests. Temporary small 0.1-ha plots, fast and cheap to install, are increasingly employed in the tropics. This study presents a standard protocol for woody plant inventories and soil characterisation using 0.1-ha plots. The protocol gives specific recommendations on the size and shape of a 0.1-ha plot, taxa to be included in the inventories, minimum stem diameter cut-offs, evaluation of multiple stems and height estimation. In addition, we present a number of recommendations on soil sampling and analysis, whose standardisation is much required in tropical forest research. We suggest to measure Al and nutrients simultaneously after Mehlich-3 extraction, followed by inductively coupled plasma spectrometry, and to measure C and N through total combustion. The pH, texture and bulk density can be measured with standard manual methods. The study also includes guidelines to create and maintain a standardised database and metadata. All the proposed recommendations are compatible with those already employed in the standardised establishment of large plots. Each recommendation represents a reasonable trade-off between investment and data quality and is oriented to obtain low-cost standardised baseline data, useful to a broad range of studies.

Keywords: Census methods, edaphic assessment, floristic assessment, floristic standardisation, lianas, plant biodiversity, plant-soil relationship

INTRODUCTION

Floristic inventories are fundamental for tropical community ecology, including understanding diversity gradients, mapping vegetation units across scales and modeling plant species distribution. Basic data on species occurrence, abundance and habitat conditions are important to conservation and management and provide context for long-term, more complex research (Phillips et al. 2003a). Despite their importance, basic quantitative inventories are relatively scarce to date due to the large amount of species within tropical biotas, which remain under-collected (Feeley & Silman 2011).

When it comes to performing an inventory, researchers often choose a method based on

the objectives of a given project or research. However, data gathered for a particular purpose, in most cases, is compatible with other analyses for general purposes. Standardised methods, imperfect but fulfilling specific objectives, are preferred to developing new methods so as to avoid data incompatibilities. For example, it is better to measure the diameter of trees at 130 cm (good for a specific objective while being compatible with other data) than at 120, 135, 137, 140, 150 or 160 cm (Brokaw & Thompson, 2000).

This study presents recommendations for the standardised establishment of 0.1-ha plots in tropical forests. Alwyn H. Gentry was the first researcher to introduce temporary 0.1-ha plots

with composed transects in the tropics during the 1980's and employed a standard cut-off at 2.5 cm dbh, providing information on species unlikely to grow into larger size classes often used by previous standards (Phillips & Miller 2002). This method records much of the site diversity quickly, efficiently and inexpensively. Rectangular 20 × 50 m plots embrace Gentry's quick-and-inexpensive philosophy and are similar to his transects except in being contiguous. In fact, it is easier to establish rectangular plots than composed transects, especially on mountainous conditions or rough terrains, and rectangular 0.1-ha plots are widely employed by many research groups. In academic studies, > 400 plots have been established in tropical montane forests and > 500 plots in lowland rainforests (Duque et al. 2002, Sánchez et al. 2008, Duivenvoorden & Cuello 2012, Baraloto et al. 2013, Arellano & Macía 2014, Arellano et al. 2014). However, convergence of methods towards a full standardisation is still clearly needed.

Few research groups using small temporary plots have collected and analysed soil samples, which is common practice in the study of larger, permanent plots, e.g. The Amazon Forest Inventory Network (RAINFOR) and Center for Tropical Forest Science (CTFS). When soils are analysed, it is impossible to find two research groups using the same sampling and laboratory methods. Thus, it is difficult to generalise the relationship between soil variables and community composition, which is key to understand the relationship between environmental conditions and forest vegetation (Sollins 1998, Chave 2008, Phillips et al. 2003a).

The aim of this paper is to propose guidelines that would facilitate convergence of sampling methods based on 0.1-ha plots, with focus on its use in floristic inventories and community ecology research of tropical forests, including soil sampling and characterisation.

MATERIALS AND METHODS

The guidelines presented here are based on 20 years of field experience performing quantitative inventories in tropical forests, an extensive literature review, and ample discussion with other researchers, technicians and local inhabitants. The following sections describe

a final consensus that have been applied in the field and laboratory, which is in general agreement with how other researches implement this method as well.

The objective of a 0.1-ha plot was to study a piece of forest, as homogeneous as possible in physiography and structure, to record abundance of local species. Plots of 20 × 50 m were preferred over transect-like shapes, e.g. 10 × 100 m, to obtain maximum internal homogeneity. In exceptional situations, such as sampling on ridges, plots were transect-like to fulfill the intra-plot homogeneity requirement. This limited the opportunities for comparison with other plots. If a plot was established on a slope, the longest side followed the contour line as far as possible. All distance measurements referred to the horizontal plane rather than the slope of the terrain, as in large plots (Condit 1998, Dallmeier 1992). Maps, aerial photographs and remote sensing data refer to horizontal areas. Many potential uses of plot data require field data expressed in 'per horizontal area', such as the estimation of individual density, soil nutrient stock or ground-truthing of remote sensing.

Measurement of woody individuals

At each plot, all woody plant stems rooting within the limits of the plot, dbh ≥ 2.5 cm at 130 cm from rooting point were measured (Brokaw & Thompson, 2000). Irregular trunks, e.g. buttresses and swellings, were measured at a representative normal part of the trunk, usually above the deformity but as close as possible to 130 cm, recording the exact point of measurement. When the trunk was inclined or on steep slopes, the dbh was measured from above. Many exceptions were encountered in the field (Condit 1998).

Woody taxa included trees, palms, tree ferns, lianas, woody hemiepiphytes and lignified bamboos (the life-form was recorded to allow separate analyses). In the case of hemiepiphytes, e.g. *Ficus*, distinguishing roots and stems was difficult or impossible. For these, we measured the dbh at 130 cm from rooting point without distinguishing roots and stems and estimated the dbh at 130 cm from the point where a regular stem began. Other woody structures were not inventoried, such as the large

woody petioles of acaulescent palms, which clearly had functions different than a stem. Herbaceous individuals were excluded even if they had dbh \geq 2.5 cm, e.g. *Zingiberaceae* and hemiepiphytic *Araceae*.

Stems with dbh \geq 2.5 cm were measured individually and assigned to individuals. Branches below 130 cm were considered as multiple stems, but distinguishing between low branches growing from the main trunk and stems rooting at the same point. Two stems connected underground with a reasonably obvious connection were assigned to the same individual. In the case of many clonal species, two stems within 1 m of each other are likely the same individual. However, different rules were established in the field, depending on the biology of the species (Condit 1998, Condit et al. 2014).

Height is rarely used in floristic assessments, but is necessary to describe forest structure, characterise species traits and estimate biomass of individuals. The height of each stem was estimated visually, with some prior training or experience. If cost and time permitted, we followed the protocols of RAINFOR and CTFS for an accurate measurement of height (Chave et al. 2005, Larjavaara & Muller-Landau 2013, Phillips et al. 2015). In the case of trees, the height can be interpreted in terms of success in the competition for light and reflected the individual size. In contrast, the length of a liana stem is impossible to be evaluated in the field and cannot be interpreted in terms of success in the competition for light (Gerwing et al. 2006, Schnitzer et al. 2008). For both reasons, the maximum height attained by any liana was estimated, i.e. how far the top of its crown was from the ground.

Information about the plot and plot metadata

Since meta-analysis is limited to the lowest common denominator, description data had to be collected systematically and carefully for each plot, including the precise geographical coordinates, compass directions, elevation, exposure and degree of slope. Other relevant qualitative information was recorded, such as topographic position (e.g. ridge, valley or slope, following the Food and Agriculture Organization of the United Nations guidelines for

soil description [FAO 2006]), soil structure and drainage, forest type and habitat particularities. Dates, names of field workers and indications to locate the plot were also recorded. Finally, plant vouchers were collected, labelled and stored in the herbaria.

Special attention was paid to natural and human disturbances. We recorded the frequency and types of common disturbances and the extent to which human activities affected a site, e.g. wood harvesting or cattle grazing. We estimated the time since the last major disturbance using aerial photographs, satellite images or interviews with landowners and neighbors. In general, the implication of local inhabitants was very helpful. The successional phase of the forest was often easier to estimate than the time since a major disturbance. Based on Chazdon (2008) and Guariguata and Ostertag (2001), the following considerations are a general overview of tropical forest succession:

- (1) Stand initiation phase in young secondary forests is characterised by low basal area, low variation in stem diameters, even canopy, few gaps, lack of large trees or lianas (except for remnants) and re-sprouting of remnant trees and lianas.
- (2) Stem exclusion phase in old secondary forests is characterised by intermediate basal area, intermediate variation in stem diameters, development of canopy and understory tree strata, even canopy, common presence of small gaps and lack of large trees.
- (3) Understorey re-initiation stage in mature forests is characterised by large basal area, large variation in stem diameters, fewer but larger lianas, variable canopy height, high spatial heterogeneity in understory light levels, prevalence of large canopy gaps or other chronic disturbances and the presence of large trees.

When using plot data of disparate origin, these regeneration phases were assessed prior to any meta-analysis by considering the diameters of the largest trees and lianas, diameter distribution of a stand, quantity of lianas and abundance of pioneer taxa (e.g. *Cecropia* and *Ochroma* in Neotropical forests).

Proper safeguarding of standardised data is crucial to aid data-sharing, large scale comparisons and meta-analyses. Data was stored according to Condit et al. (2014) to minimise data redundancy and potential errors. We also stored adequate metadata along with the data, i.e. technical description of the data content, context, quality, structure and accessibility (Feagraus et al. 2005). Metadata took the form of a table with the names of the database fields, how the observation or measurement was performed, the units of each variable and references to more detailed methods, such as soil analyses protocols. Feagraus et al. (2005) discuss the Ecological Metadata Language but other standards for metadata creation and storage exist.

Soil sampling and analyses

We sampled superficial soil, 0–15 cm, below the organic litter layer with slightly decomposed organic material, such as leaves, flowers and seeds. Composite samples consisting of a mixture of several subsamples collected from different points of the plot were gathered, e.g. the center of five subplots arranged in a zig-zag. For chemical and physical analyses, a spade was used to collect the samples. The samples were air-dried and protected from the rain and direct sunlight. Samples were kept for chemical analyses in closed bags for a minimum time, during transportation from field to laboratory. After air-drying, the samples were sifted through a 2-mm sieve and stored in closed bags.

According to published results on soil-floristic relationships in tropical forests, the most influential soil properties are (1) available base content (Ca, Mg, K, Na), (2) texture, (3) pH, (4) total C and N, (5) available P, (6) available Al and (7) available micronutrients. Soil properties were measured in that order of priority according to available resources. Around 40 g of dried and sifted soil were required for the analyses.

A combination of the universal extractor Mehlich-3 and ICP spectrometry was used to measure Al, macronutrients (Ca, Mg, K and P) and micronutrients (Na, Fe, Mn, Cu, Zn). The Mehlich-3 method is widely used for the extraction of plant-available nutrients and Al over a wide pH range, and a trade-off exists in terms of laboratory time requirements, financial costs, effort and the extractability of critical

elements such as P and Al (Mehlich 1984). ICP spectrometry is a normalised and rapid technique allowing measurement of multiple elements, routinely used to measure nutrient concentrations. The combination of both methods was an efficient and reliable method for multi-elementary measurement, and is the standard of the CTFS protocol too (Chave 2008, Harms & Dalling 2004). If ICP spectrometry was not available, other determination methods were used on the Mehlich-3 extraction, such as molybdenum-blue for P or atomic absorption spectrophotometry for metallic elements (Murphy & Riley 1962).

An auto-analyser was used to determine simultaneously total C and total N through total combustion and the measurement of the resultant gases. This method provided data only partially comparable with the manual, traditional methods. In the case of C, the traditional method of Walkley & Black (1974) measures only the organic C content, making the comparison with total C not straightforward. In the case of N, the traditional Kjeldahl method measures the organic N content plus the inorganic ammonium content (Van-Reeuwijk 2002). Inorganic N is commonly < 2% of the total N in surface soils and is represented by volatile compounds (Harmsen & Kolenbrander 1965), so the Kjeldahl method measures a pseudo-total N content comparable to total N using an auto-analyser. Since the samples were air-dried, the already-low concentrations of inorganic N were stable and minimum after several days (Turner & Romero 2009). In terms of ease of use, amount of soil sample needed, data precision and direct comparability, the automated methods are always preferred to manual methods.

To analyse soil texture, we used the hydrometer method, following H₂O₂ oxidation of organic matter and dispersion with hexametaphosphate (Day 1965). The pH was measured in a soil:deionized (1:2.5) water suspension (Van-Reeuwijk 2002).

To measure bulk density (BD) and stocks (the element content of superficial soil for a given area, e.g. g of C ha⁻¹) a second sample of soil with known volume (V) was gathered. After drying the sample, the sample was weighed before and after sifting (W_{total} and $W_{\text{fine-earth}}$). The BD was calculated as $W_{\text{total}} V^{-1}$. The fine earth content (FE) was calculated as $W_{\text{fine-earth}} W_{\text{total}}^{-1}$.

The stock of a given element per area was calculated as:

$$\text{stock} \left(\frac{\text{kg}}{\text{m}^2} \right) = \frac{\text{concentration} \left(\frac{\text{g}}{\text{kg}} \right) \times \text{BD} \left(\frac{\text{g}}{\text{cm}^3} \right) \times \text{layer thickness (cm)} \times \text{FE}}{100}$$

The quantity of fine roots, an important parameter for below-ground biomass estimation, was calculated by classifying the coarse matter (> 2 mm) that remained after sifting the sample. This estimate referred to the superficial soil (0–15 cm), the depth at which most fine roots appear in tropical forests.

RESULTS AND DISCUSSION

Using this inventory, all standing stems of trees, palms, tree ferns, lianas, woody hemiepiphytes and lignified bamboos with dbh \geq 2.5 cm were recorded in several tropical countries, at different elevations and in very contrasting habitats. An example of a specific project making use of this protocol is the Madidi Project (Jørgensen et al. 2001). Below, we discuss these results and those obtained with other methods, as well as potential opportunities for data-sharing and meta-analyses.

Comparison with other plot-based protocols

In the past few decades, floristic study of tropical forests increasingly used standardised methods that allow the comparison of data among different research groups and investigation sites (Condit 1995, Condit et al. 2002, Malhi et al. 2002). All methods of tropical forests inventory have pros and cons (Table 1). The most appropriate sampling protocol depends on the type of data needed to address the research objectives and the quantity of resources available.

Permanent plots are suitable for the study of species-level demographic rates and forest dynamics. They have undeniable advantages for the study of tropical forest ecology and comprehension of its functioning. However, they require the individuals to be tagged, rigorously measured and located spatially with enough precision within a permanently marked plot. For these reasons, they require a large amount of time and effort to be established and monitored. Temporary samples do not require permanent plot delimitation or individual tagging and

mapping, and measured each individual only once. Although unsuitable for the study of forest dynamics and local biomass estimation, the spatial variation in floristic composition was characterised at medium and large scales. Since the objective was to gather basic floristic information within a relatively large area, time and cost effective temporary samples were clearly preferable (Phillips et al. 2003a).

To record as much of the local flora as possible, Gentry's 0.1-ha samples were very efficient. These were small temporary samples composed by ten 2 m \times 50 m transects placed randomly within an area of 2–10 ha. However, Gentry's original sampling design was not suitable to answer some fundamental questions in tropical ecology. First, the area covered by each sample was neither constant in shape nor size, because of the non-standardised way of placing individual transects. As a result, diversity was not comparable between samples. Some samples included more diversity because they covered a greater area, or the different transects that composed the sample were distributed along a line. Second, these were spread samples, which means the diversity measures were affected by both alpha-diversity (local diversity recorded by a single transect) and beta-diversity at the hundreds-of-meters scale (differenced between each individual transect). While this was useful to rapidly gain a good understanding of the gamma-diversity of a site, it also blurred the inter-sample comparison needed to measure beta-diversity at larger scales (Anderson et al. 2011, Tuomisto 2011).

The area variability issue was resolved by a modification of Gentry's transects (Phillips et al. 2003b) by placing 2 m \times 50 m transects within a 100 m \times 180 m regular sampling grid, covering approximately 2 ha of terrain. Including original Gentry's transects and the later modification, there exist > 800 plots of 0.1-ha spread samples in the tropics (Baraloto et al. 2013, Phillips et al. 2003a). In contrast, the rectangular 20 \times 50 m plots that have been employed and described in this study were contiguous; the area covered by a sample was just the studied plot. Therefore, they were more useful for a neat and classical approach to alpha-diversity (within a sample) and beta-diversity (among samples). Moreover, the covered area, 0.1 ha vs. \sim 2 ha in the case of modified Gentry transects, was closer

Table 1 Summarised comparison of classical approaches to inventory woody plants in tropical forests

Attribute	0.1-ha plots (present work)	Modified Gentry-type transects	Gentry's transects	1-ha plots	20–50-ha plots
Shape	20 m × 50 m	Composed by ten 2 m × 50 m transects within a 100 × 180 m regular grid	Composed by ten 2 m × 50 m transects placed at random	100 m × 100 m	Variable, often 500 m × 500 m or 500 m × 1000 m
Area covered	0.1 ha	≥ 2 ha	Variable	1 ha	20–50 ha
Area sampled	0.1 ha	0.1 ha	0.1 ha	1 ha	20–50 ha
Permanent?	No	No	No	Yes	Yes
Focus on	Canopy and understorey trees, and lianas	Canopy and understorey trees, and lianas	Canopy and understorey trees, and lianas	Canopy trees	All woody plants
Cut-off	dbh ≥ 2.5 cm	dbh ≥ 2.5 cm	dbh ≥ 2.5 cm	dbh ≥ 10 cm	dbh ≥ 1 cm
Number of estimated individuals	200–500	300–600	300–800	300–800	Hundreds of thousands
Field effort in person-days (~ money effort)	5–20 for the installation and census	5–20 for the installation and census	5–20 for the installation and census	20–40 for installation and first census, 5–20 for other censuses	Hundreds to thousands for installation and each census
Internal heterogeneity	Low	Intermediate	High	Intermediate	High
Suitability for the study of alpha-diversity	High	Intermediate	Low	Intermediate	Intermediate
Samples per site and per region	Several per site, many per region	Several per site, many per region	Several per site, many per region	One or few per site, potentially many per region	One per region
Suitability for the study of beta-diversity	Between samples from local to regional scales	Within the sample and between samples	Within the sample and between samples	Within the sample or at regional scales	Within the sample
Suitability for the study of gamma-diversity	High	High	High	Low to intermediate (depends on the number of plots)	Low

Permanent plots require tagging and mapping of individuals and are suitable for the study of dynamics, unlike temporary samples

to the actual 'alpha' spatial scale of terrestrial plants (Rosenzweig 1995, Schmidta & Wilson 1985, Whittaker et al. 2001). The contiguous 20 × 50 m shape also guaranteed that the forest sampled was approximately homogeneous, unlike transects that tend to include environmental and floristic heterogeneity.

Site design and distribution of plots

Using contiguous samples, the species turnover was recorded by placing several plots, so the placement and spatial distribution of plots was an issue. A clear definition of the objective of the study was required to find an optimal solution, in a case-by-case basis. If the objective was to inventory a small sized locality, e.g. < 25 km², a group of ten 0.1-ha plots with > 2000 individuals covering the whole environmental heterogeneity usually provided sufficient floristic information to reach an approximate saturation on a species accumulation curve (SAC), or at least to record all common species even in species-rich forests (Condit et al. 1998, Macía 2008). If the objective was to inventory specific environmental conditions, e.g. steep slopes, habitats or swamp forests, plots were placed differently, but with similar considerations and applications regarding SAC saturation and recording of common species. When elevational changes existed in a locality, plots did not differ > 300 m in elevation. Consideration of the targeted forest was also important to avoid potential biases in sample location selection, such as the 'majestic effect', a tendency to locate plots in attractive patches with exceptionally tall and continuous canopy (Sheil 1995).

The number of plots a project could install depended on the available resources. For a given number of plots, the inter-plot distances depended on the species turnover of the targeted forest. We had to consider the diversity of the ecoregion, changes in physiographic, edaphic or environmental conditions and research objectives and resources, to define the adequate size of a sampling site and the distance used to separate (a) two plots within a site and (b) two or more sites (groups of plots). A common thumb rule was to separate plots by at least 500 m in tropical lowland forests, and 250–300 m in heterogeneous montane forests.

Establishment of permanent plots and individual mapping

Since 'changes over time' was not an objective, temporary 0.1-ha plots were used. The method is rooted on field efficiency considerations that apply to temporary samples, but it is also possible to establish 0.1-ha plots as permanent plots at a moderate cost (Baraloto et al. 2013) and repeat censuses at different times. In that case, the large permanent plot standards contain many additional details needed, such as individual tagging and measurements (Condit 1998). For the proposed method, no precise mapping of the plot or individuals was required, although we often divided the plot into ten 10 × 10 m subplots to facilitate the inventory and soil sampling. Some researchers may need more spatial resolution, or even a precise mapping of individuals for particular purposes, e.g. neighborhood competition. For such aims, CTFS protocols are the main reference for permanent plots, whereas relatively inexpensive methods have been developed for small, temporary plots (Ledo 2015).

Smaller cut-offs

The proposed method employed a 2.5 cm cut-off. Some researchers recorded smaller stems. In the case of lianas, a cut-off of 2.5 cm dbh does not provide a detailed assessment of its diversity. There are standard protocols for lianas, using a dbh ≥ 1 cm at 130 cm from the rooting point, recording the greatest observed stem diameter (excluding deformities) (Gerwing et al. 2006, Schnitzer et al. 2008). In the case of young secondary forests (< 15 years in wet tropics) too many individuals are left out by a cut-off of 2.5 cm dbh, and a cut-off of 1 cm dbh would be more appropriate to document a similar proportion of woody plant diversity as in elder forests with a cut-off of 2.5 cm. Measuring individuals < 2.5 cm dbh with specific purposes implies more field effort, but does not prevent comparison to other datasets, as far as all individuals < 2.5 cm are excluded from the meta-analyses. Researchers should establish a trade-off between data comparability and suitability for specific purposes. However, it is not recommended to use cut-offs different from 1 cm or 2.5 cm dbh.

CONCLUSIONS

Inventories based on 0.1-ha plots are highly suitable to obtain accurate measures of floristic composition and alpha-, beta- and gamma-diversity, thus representing a useful trade-off between field-effort efficiency over a broad range of conditions and data quality in terms of a straightforward quantitative inventory of diversity at different levels. This method allows further refinements that do not hinder comparison with data obtained by other researchers. Overall, there exist hundreds of 0.1-ha plots in the tropics and their use has become increasingly widespread. Convergence of methods by following the guidelines proposed in the present study would aid to large scale comparisons and meta-analyses, increasing the value of dataset and improving collaboration opportunities between research groups.

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