INFLUENCE OF SAMPLING STRATEGY
ON THE DISTINCTION BETWEEN TROPICAL PIONEER
AND NON-PIONEER FOREST SPECIES

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Abstract. It is widely accepted that tree species from tropical old-growth late-successional forests (natural forests) differ markedly in their leaf morphological and physiological attributes from tropical pioneer (early-successional) trees, which often form secondary forests in clear-cut areas. For example, higher foliar nitrogen contents, higher photosynthetic activities, and a larger leaf size have frequently been reported for pioneer tree species. Since most tropical forests are rich in tree species, we hypothesize that the results of a morphological and functional comparison between natural forest (NF) and secondary forest (SF) species may largely depend on the sampling design, i.e. the number of tree species sampled and how these are selected in the stands. To test this hypothesis we compared three different leaf-sampling strategies (SA: species average based on a large random sample; NSA: species average based on a small random sample; WSA: species average SA weighted by tree species frequency) with the results of a nearly complete stand inventory. The study was conducted in NF and SF stands in the lower montane rainforest of Central Sulawesi, Indonesia. As expected, the SA, NSA and WSA sampling strategies gave different results with respect to the assumed functional dichotomy between NF and SF trees. Our data indicate that, for many leaf attributes, more than 20 or 25 species per forest type are required for detecting significant differences between NF and SF trees. Leaf size was the only parameter that was significantly affected by a sampling effect, resulting from the absence of very large-leaved species in small samples. If the focus of interest is on the leaf traits of the 'average tree' of a forest type, as in biogeochemical studies, the weighted species average (WSA) approach, which also considers species abundances, should be given preference over simple species averages (SA and NSA). *Accepted 29 June 2006.*

Key words: *Foliar nutrient content, leaf size, random sampling, sampling effect, species average*, δ^{13}C.

INTRODUCTION

Recent years have seen increasing interest in the functioning of tropical trees and forests, which are encroached and converted at an unprecedented rate (Achard *et al.* 2002). With an estimated 50 000 or so tree species, the tropical rainforests of Asia, Africa and the Americas harbor a highly diverse flora of woody plants and, most likely, a great wealth of different tree functional types which may differ in growth rate, water and nutrient economy, and stress tolerance (Turner 2001). It is convenient to distinguish two principal groups of trees in tropical moist forests based on morphological and functional attributes, i.e. light-demanding, fast growing and relatively short-lived pioneer trees which often form early-successional forest communities, and more or less shade-tolerant,

slower growing, medium to long-lived non-pioneer trees which dominate old-growth, late-successional forests (Swaine & Whitmore 1988, Bazzaz 1991, Strauss-Debenedetti & Bazzaz 1996). Although this assumed functional dichotomy is not undisputed (e.g., Turner 2001) it can be helpful in formulating testable hypotheses on trade-offs between tree physiological and morphological traits.

When intending to study leaf functional traits in species-rich tropical forests, there are some decision steps to take. First, the study parameters have to be selected. In many cases, they should be reliable predictors of leaf and tree physiology because they should allow inferences from leaf attributes on processes at the tree or stand level (Garnier *et al.* 2001, Cornelissen *et al.* 2003). Leaf and seed properties have been used much more frequently than attributes of stems or roots or whole-tree growth patterns because they are easier to measure. Second, one has to select an ap-

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propriate sampling design that meets the purpose of the study and allows statistical analysis when comparing different samples.

In species-rich tropical moist forests, leaf sampling in the sun canopy of mature trees is often a difficult and labor-intensive task. Thus any reduction in labor effort during fieldwork is highly appreciated; however the sample must remain large enough to be representative of the stands. Most investigations of leaf traits in tropical trees that compared different forest types sampled a few, more or less arbitrarily selected tree species per stand, and calculated averages at the stand or forest type level. This procedure may be severely biased, not only by the subjective selection of tree species, but also by the sampling effect because typically not more than 10 or 20 % of the tree species of the stand are included in a sample. As far as we know, there is no systematic investigation which compares the results of different leaf sampling strategies in tropical forests with leaf trait data obtained in complete stand inventories, i.e. studies on the floristic composition of the forest.

In this paper we present the results of a joint study by tree ecophysiologists and taxonomists conducted in several species-rich, lower montane forests of Central Sulawesi, Indonesia. We sampled old-growth forests with very low anthropogenic disturbance (hereafter termed 'natural forest') and young secondary forests which had established after clear-cut of the natural forest. The study objective was to compare the results of three different random sampling strategies that differed considerably in the number of tree species and specimens collected, and thus contrasted in terms of labor effort. In all sampling strategies, a broad range of leaf traits (14) was investigated. The results from the three random sampling strategies were compared with the data of a near-complete leaf sampling of all trees in the stands, which was conducted in the context of a floristic analysis in collaboration with the herbaria in Bogor (Indonesia) and Leiden (the Netherlands).

METHODS

Study area. The study was conducted inside and along the borders of Lore Lindu National Park in Central Sulawesi, Indonesia (districts of Donggala and Poso, 01°25'S, 120°20'E). With an area of 217 000 ha, the National Park is one of the last larger blocks of tropical montane rain forest in the area (Whitten *et al.* 2002). It was declared a Man and Biosphere Reserve due to its outstanding importance for biodiversity

conservation in Wallacea. However the park is subject to rapid encroachment from the surrounding communities (van Rheenen *et al.* 2004). The study plots were selected in the Napu and Palolo valleys close to the village of Wuasa at an elevation of about 1100 m a.s.l.

This study was conducted as part of the interdisciplinary, long-term research program "STORMA – Stability of Rain Forest Margins in Indonesia" which aims at identifying the socio-economic and ecological key factors and processes that lead to destabilization and forest degradation in the rainforest margin zone of Central Sulawesi.

Climate and soils. The study region is influenced by the Australian-Asian monsoon system throughout the year. Mean air temperature in Wuasa (Napu valley) was 21°C, mean air humidity was 82.6 %. Yearly rainfall from December 2001 to December 2002 was 1596 mm, without a pronounced dry season (data provided by H. Kreilein & G. Gravenhorst, University of Göttingen). A characteristic feature of the local climate is thunderstorms with heavy precipitation in the afternoon.

The soils of the study plots are predominantly fertile Cambisols, with the majority of investigated profiles having pH(KCl)-values above 5 and base saturations > 80 %. Carbon and nitrogen stocks in the secondary forests were significantly lower than in the natural forests. However, burning of logs after clearcut resulted in enrichment of calcium, potassium, and magnesium in the topsoil ash of the secondary forests.

Forest types. We studied two types of forest that differ greatly with respect to species composition, forest structure, and human impact. Lower montane, old-growth late-successional forests with only minor human influence were termed 'natural forest' (NF). The respective stands had a closed canopy of about 35 m in height with single emergent trees (mainly of *Ficus benjaminii* or *Polyalthia canangoides*) reaching 45 m or more. The canopy consisted of 2–3 strata which were not very conspicuous. In the sparse shrub and herb layers, seedlings and regrowth of the canopy species prevailed. According to Whitmore's (1984) classification, the forests are humid submontane tropical rainforests. In general, the disturbance intensity in the natural forest plots was low. However, rattan collection and, very locally, logging of single trees was observed. To exclude these influences as far as possible we chose all study sites at a minimum distance of 1 km from the forest border.

Secondary forests (SF) occupy a substantial area in the Napu and Palolo valleys. They develop on former slash-and-burn fields at distances of a few up to 2000 m from the recent forest border. The dense 3 to 5-yr-old stands had a closed canopy and were 4–7 m high. This is a typical age for secondary forests in the area because many stands are re-cut for wood production before they reach 10 years. After clear-cut of the natural forest, annual crops (mainly maize) are cultivated for a few years, with the fields left for secondary succession thereafter. Pioneer trees establish rapidly on these fallows, forming dense thickets already in the first year of colonization. In most stands, the understory was dominated by a more or less closed grass layer with scattered shrubs and forbs of Urticaceae, Zingiberaceae, and other families. The secondary forest stands typically had a size of 4–10 ha and were located in the middle of fields with annual crops and agroforestry plantations, mostly cocoa. The secondary forests studied were all similar in stem density and canopy structure. To avoid edge effects, all plots were situated at least 50 m from the forest margin.

Other land-use types in the study area include paddy rice fields and forest gardens, i.e. planted crops under a remaining sparse forest canopy. The size of the study region's local tree species pools for natural forest and secondary forest can be only estimated. With about 150 species ha^{-1} (Kessler *et al.* 2005), the natural forest is undoubtedly much richer in tree species than the secondary forest, where an estimated 50–60 species are found per hectare.

Plot selection. Defining plot size was complicated by the fact that secondary forests represented small stands < 5 ha in size, with a large part of the forest being close to the forest margin. To exclude edge effects and to avoid very high stem numbers in the samples, we decided to choose different plot sizes for natural forest (2500 m^2) and secondary forest (400 m^2), following the recommendation of Mueller-Dombois & Ellenberg (1974). As a consequence, stem numbers were similar in the NF and SF samples. However, plot-size-related diversity measures could not be compared between the two forest types with this procedure.

Prior to plot selection, we inspected a number of suitable forest patches in the study region for localities that would meet the following criteria: (1) sufficient homogeneity in overall stand structure, (2) absence of larger gaps (larger than 2–3 fallen trees), (3) absence of obvious signs of major anthropogenic disturbance, and (4) structural comparability to the other plots of the forest type. A further selection criterion was elevation: all plots were chosen at 900 to 1100 m a.s.l.

Based on this procedure, three natural forest stands (Gunung Potong, Napu I, Napu II) and four secondary forest stands (Watumaeta, Alitupu I, Alitupu II, Kaduaa) were selected for study. In these seven stands, we demarcated six rectangular plots of 2500 m^2 (50 m x 50 m) in the natural forest stands, and four plots of 400 m^2 (20 m x 20 m) in the secondary forest stands, in most cases one plot per stand. The exact location of the plots was chosen by a two-step procedure: first, forest patches fulfilling the above-mentioned criteria (1) to (4) were identified, and second, a conspicuous thick stem was selected in the plot as the north-eastern plot corner in an attempt to demarcate the 10 study plots by a quasi-random procedure in the seven forest patches. In the Gunung Potong natural forest only, we placed four plots each of 2500 m^2 side by side because we also aimed at sampling a complete 1 ha (100 m x 100 m) plot. Only this plot was large enough to analyze the species/area relationship in the natural forest. Thus, four of the six 2500 m^2 plots in natural forests were spatially independent of each other; they can be treated as replications. In contrast, the Gunung Potong plots must be viewed as pseudo-replications. In total, we investigated 1600 m^2 of secondary forest and 15 000 m^2 of natural forest, and sampled more than 1000 trees with a dbh >10 cm.

Using compass, ropes, and wooden sticks, we established rectangular grids of 5m mesh-width on all plots with the x-axis in a north-south direction. This procedure resulted in grids with 121 nodes in the 2500 m^2 plots, and grids with 25 nodes in the 400 m^2 plots. These grids formed the basis for a random selection of trees by identifying the mature or pre-mature tree with fully sunlit leaves being closest to a node.

Leaf sampling. For obtaining data on various leaf morphological and chemical traits, fully sunlit, mature and vigorous leaves were collected from the trees. In the secondary forests, leaves could be accessed at heights of 3–6 m with a branch cutter on an extendable stick. For sampling the natural forest trees at 15–25 m height, local tree climbers and the staff of the Herbarium Celebense in Palu were employed, using climbing equipment with ropes and iron hooks or by freely climbing the trunks. Depending on leaf size, 4 to 30 leaves per tree were collected with their petiole, put in paper bags and transported to the laboratory at

Tadulako University in Palu where further analysis took place. Sampling was conducted from February to October 2001 on the natural forest plots, and from March to July 2001 on the secondary forest plots.

Analysis of leaf morphological traits. Leaf size, leaf shape, and specific leaf area were investigated in four to ten leaves per individual tree. The leaves were scanned directly after sampling with a flat-bed graphics scanner (CanoScan N340P, Canon, Japan), and thereafter dried at 70°C for 48 h. The dried leaves were weighed for calculating specific leaf area (SLA). Leaf area, and length and width of the leaves were determined from the digital images with the program Win-Folia (Régent Instruments Inc., Quebec, Canada). In the case of compound leaves, only one leaflet was analyzed. These data were treated as equivalent to those from simple leaves throughout the study.

Analysis of leaf chemical traits. For chemical analysis, the petioles were cut off and the leaves dried at 70°C for 48 h, and thereafter ground to fine powder. One sample per tree, consisting of 4–30 fully sunlit leaves, was analyzed according to the following protocol.

The total concentrations of Ca, K, Mg, and P in the leaf dry mass were detected through HNO_3-pressure digestion and subsequent atomic emission spectrometry (Optima 2000 DV, Perkin Elmer). The total carbon and nitrogen concentrations were determined using gas chromatography in an elemental analyzer (Vario EL, Elementar). The $^{12}C/^{13}C$ and $^{14}N/^{15}N$ isotope signatures were analyzed in ground leaf material with a gas isotope mass spectrometer (Finnigan, MAT 251) at the KOSI facility of the University of Göttingen.

Four different sampling approaches. We applied four different sampling approaches on the natural and secondary forest plots which differed in terms of sample size, labour effort and selection criteria. The approaches SA, NSA, and WSA (see below) considered all trees reaching the upper canopy and randomly selected a subsample of it. The three approaches differed with respect to sample size and data averaging procedure. In the fourth sampling approach (INV), we attempted to investigate all trees >10 cm dbh in the stands by conducting a more or less complete floristic and ecological forest inventory. These data were used as a reference in the analysis.

(1) – Species average (based on a large random sample; SA)
Since both forest types had a characteristic tree species density of 35 to 50 species per 1600 m^2, we aimed at investigating about 30 species per forest type. The

tree individuals were selected at random in the plots of a forest type until about 30 tree species were included in the sample. Due to the very different abundances of the species in the plots, one to ten individuals per species were sampled. In a first step, averages for each species were calculated from these conspecific samples. In a second step, a species average was obtained for each forest type by averaging over the *ca.* 30 species. Since the diversity-frequency relationships were quite different in the two forest types, our selection procedure resulted in160 tree individuals of 35 species in the secondary forest, but only 119 individuals of 28 species in the natural forest.

(2) – Species average (based on a small, normalized random sample; NSA)
For investigating the influence of the sampling effect on our leaf trait analysis, we created small subsamples from the larger random samples SA. This was done by randomly selecting n = 18 tree species per forest type from the SA sample. Thus, the NSA samples were obtained from the SA samples after normalizing them to 18 species in both forest types. As in the SA sampling approach, the data were first averaged over one to 10 different individuals per species for obtaining species means and thereafter used for calculating the 18-species-mean for each forest type.

(3) – Weighted species average (based on a large random sample; WSA)
Based on the SA random sample (1) with 160 or 119 trees of *ca.* 30 species, we also calculated leaf trait averages for the two forest types by simply averaging over all individuals in the sample irrespective of species. This mean value can be taken as the 'average tree' of the random sample, or the species average of the SA approach weighted by the species abundance in the sample. Clearly, this approach assumes that the sample is large enough to reflect differences in species abundance in the stands.

(4) – Stand average (forest inventory data; INV)
A complete inventory with respect to species identity, stem diameter, phenological status (presence of fruits and flowers), and leaf morphological and chemical traits was conducted in several of the study plots covering all stems > 10 cm in dbh. The fieldwork was conducted in a period of about 5 months by a team of 4–5 persons, including one taxonomist with profound South-East Asian expertise, and two additional botanists from Germany and Indonesia. Leaf, flower, and fruit samples (if available) were mostly collected in the sun crown by engaging local tree climbers (see above). All specimens were taken in triplicate num-

ber and transferred to the Tadulako University in Palu (Herbarium Celebense), to Bogor (Herbarium Bogorense), and to the National Herbarium of the Netherlands in Leiden for identification and deposition. Because of the large number of specimens, tree species identification was very time-consuming and took more than one year. Of the 1171 trees sampled in total, 1152 specimen could be identified to genus level, 7 only to family level, while the remaining 12 remained unidentified. For the large majority of the 1152 specimens, the species was also identified; the others were recognized as morphospecies. The scientific names follow the recent checklist of woody plants of Sulawesi compiled by Kessler *et al.* (2002).

Due to the very labor-intensive process of sampling and species identification, we were only able to conduct the inventory in a total plot area of 5000 m² in the natural forest, and in an area of 1600 m² in the secondary forest. These plots were thought to be representative of the 10 plots of the study. Further, we were not able to sample all mature trees in these plots completely because we failed to climb the canopy in 10 to 20% of the stems present. This happened in cases where either the stems were too smooth for climbing, or a dense liana cover made the trees inaccessible. Because several specimens remained unidentified, the number of species given for the natural forest plots is a slight underestimation of the real diversity. Thus the inventory data cover the bulk of trees and individuals, but still contain data gaps. The trees included in this inventory differ from those covered by the random sample because (i) the number of species and individuals are different, and (ii) all strata were included in the INV sample whereas the latter only referred to the canopy-layer trees.

Stem diameter was measured with a fabric diameter tape (+ 1 mm resolution) at 130 cm height. Several trees had large buttresses that reached 1.3 m in height. In these cases, D was measured 0.2 to 0.7 m higher on the stem.

Statistical analysis. The statistical analyses were conducted with the program SAS release 8.1 (SAS Institute Inc., Cary, NC, USA). All data were tested for normal distribution using Shapiro & Wilk's test; the data showed non-Gaussian distribution throughout. A non-parametric, univariate Wilcoxon test was used to detect significant differences between natural forest and secondary forest samples for all 13 measured parameters ($p < 0.05$). This was done separately for the four sampling strategies.

RESULTS

Species diversity in natural and secondary forest. Although the natural forest (NF) is undoubtedly much richer in tree species at plot sizes of 1 ha or larger than the secondary forest (SF), this difference in diversity was not found in an area of 1600 m² (40 x 40 m). Plots of this size contained 42–44 and 35 species in the NF and SF forests respectively (Table 1). However, the NF tended to be more diverse than the SF at the genus and family levels.

In the natural forest, the most abundant tree family with respect to stems was Lauraceae, to which 13.5 % of all recorded trees belonged (Table 2). This family was represented by 10 genera and 25 species in the study plots. The second commonest family was Meliaceae, followed by Urticaceae and Euphorbiaceae. The most abundant single tree species was an unidentified *Pouzolzia* species (Urticaceae) with 6.9 % of all trees, followed by *Aglaia argentea* and *Chisocheton* sp. (both Meliaceae).

In contrast to the natural forest, the Euphorbiaceae dominated the secondary forests with 45 % of all stems belonging to this family. The second most important family in terms of stems was Ulmaceae, followed by Annonaceae and Urticaceae. The three most abundant species were all Euphorbiaceae members.

TABLE 1. Number of tree individuals >10 cm dbh, and of tree species, genera, and families in two 1600 m² plots in the natural forest as compared with a 1600 m² plot in the secondary forest. Given are minimum values since 1–3% of the species remained unidentified.

Stand No.	Individuals	Species	Genera	Families
Natural forest No. 1	89	42	29	27
Natural forest No. 2	74	44	30	21
Secondary forest No. 1	89	35	20	15

TABLE 2. Most abundant families and tree species in the natural forest and secondary forest of the study region. Data refer to the forest inventory samples.

Natural Forest	Percent of stems	Secondary Forest	Percent of stems
Families			
Lauraceae	13.5	Euphorbiaceae	45.0
Meliaceae	12.7	Ulmaceae	9.3
Urticaceae	10.1	Annonaceae	5.4
Euphorbiaceae	8.8	Urticaceae	5.0
Species			
Pouzolzia sp. (Urticac.)	6.9	*Homolanthus populneus* (Euphorbiac.)	18.6
Aglaia argentea (Meliac.)	4.3	*Acalypha caturus* (Euphorbiac.)	12.4
Chisocheton sp. (Meliac.)	4.0	*Glochidium rubrum* (Euphorbiac.)	6.2

As is characteristic for species-rich communities, a diversity-abundance graph for the natural forest yielded an extremely left-skewed distribution, with the large majority of species being present only as one or two individuals per plot (data not shown). In the secondary forest, the most abundant species achieved a higher dominance whereas fewer rare species were present than in the NF.

Leaf traits of natural and secondary forest tree species. Interspecific variation among the species within a forest type was very different for the 13 morphological and chemical leaf traits investigated (trait no. 14, the abundance of compound leaves was not subject to statistical analysis). The variation among the species was very large for leaf size (coefficient of variation CV: 80–130%), intermediate for the leaf length/width ratio, the $\delta^{15}N$ signature, and for foliar Ca, K and Mg content (CV typically 40–80%), rather small for specific leaf area (SLA), foliar P content, and for C/N and N/P ratios (CV typically 5–40%), and very small (CV typically <5%) for the $\delta^{13}C$ signature and foliar N content (natural forest only). Comparing the two forest types with respect to interspecific variation of leaf traits showed no systematic difference. An exception was foliar N content, which was ten times more variable in the SF than in the NF sample (see Table 3).

In the large random sample of trees (SA: 119 or 160 trees per forest type in NF and SF respectively), we found highly significant differences (p < 0.01 or 0.02) between NF and SF trees in 10 of 13 leaf traits (Table 3). Only foliar Ca and Mg content and $\delta^{15}N$ signature showed no significant differences between

natural forest and secondary forest. Thus sun leaves of SF species were most different from the NF species in being, on average, narrower and thinner (see Fig. 1), and by having higher N and P contents, lower C/P and N/P ratios, and higher $\delta^{13}C$ values.

Influence of sampling strategy. Reducing the number of sampled tree species from 28 (or 35) to 18, and the number of sampled tree individuals from 119 (160) to 77/82, as done when shifting from the SA to the NSA sampling approach, resulted in a lower significance of leaf trait differences between natural and secondary forest in a number of leaf attributes (leaf size, SLA, foliar N and K content, C/N ratio; Table 3). The absolute difference between the means of NF and SF also decreased in five leaf traits when applying the NSA approach. For three parameters (Ca and K contents and $\delta^{15}N$) we observed a change in the ratio between the NF and SF means, i.e. opposite trends according to the two sampling strategies. However, neither the SA nor the NSA sampling approach yielded significant differences between the two forest types for these three traits. The size of the sample (28/35 species in SA *vs.* 18 species in NSA) had only a minor or no effect on the interspecific variation of the leaf traits within a forest type. However, several leaf traits tended to have larger absolute means in the secondary forest when sampled with the more comprehensive SA sampling strategy as compared with the NSA approach; this was valid in particular for leaf size, SLA, and foliar N and P.

In the WSA approach (weighted species average), the results of the SA approach were related to tree spe-

TABLE 3. Means and standard deviation (in parentheses) of 14 leaf traits in natural forest (NF) and secondary forest (SF) tree species according to four different sampling and calculation approaches with a variable number of tree species and individuals sampled. S = significance of differences between NF and SF ($p >$ 0.05). No statistical analysis was possible in the case of the compound leaves.

|	Species average (SA)			Normalized species average (NSA)			Weighted species average (WSA)			Stand average (Total inventory INV)		
	Natural forest	Second. forest	S	Natural forest	Second. forest	S	Natural forest	Second. forest	S	Natural forest	Second. forest	S
Species	28	35		18	18		28	35		53	23	
Tree individuals	119	160		77	82		119	160		198	78	
Leaf size (cm²)	159.7 (215.2)	222.7 (243.5)	0.02	142.7 (139.6)	161.6 (146.5)	–	156.1 (155.0)	188.9 (222.9)	–	167.6 (166.9)	113.5 (117.2)	< 0.001
Length-width ratio	2.4 (0.6)	1.5 (0.5)	< 0.001	3.0 (3.0)	1.7 (0.5)	0.003	2.3 (0.6)	1.9 (0.6)	0.03	2.4 (2.0)	2.0 (2.4)	< 0.001
SLA (cm² g⁻¹)	116.2 (36.5)	137.6 (33.0)	< 0.001	105.6 (30.4)	128.1 (35.5)	–	122.8 (36.7)	122.0 (33.4)	–	103.2 (22.6)	145.6 (38.2)	< 0.001
N (g kg⁻¹)	20.9 (0.7)	25.0 (7.8)	0.002	19.3 (0.6)	22.9 (8.6)	–	21.9 (0.7)	22.5 (8.6)	–	19.0 (0.5)	23.7 (7.4)	< 0.001
P (g kg⁻¹)	1.7 (0.7)	3.4 (1.0)	< 0.001	1.6 (0.6)	2.8 (0.8)	< 0.001	2.0 (0.9)	2.8 (1.3)	0.009	1.8 (0.8)	2.8 (1.0)	< 0.001
C/N	21.8 (6.4)	19.2 (6.3)	0.02	24.1 (7.4)	22.0 (7.1)	–	21.6 (5.9)	21.5 (6.7)	–	23.6 (5.6)	21.0 (6.1)	< 0.001
C/P	280 (140)	140 (54)	< 0.001	311 (96)	174 (57)	< 0.001	278 (148)	190 (75)	0.02	281 (110)	192 (107)	< 0.001
N/P	12.7 (4.6)	7.6 (2.2)	< 0.001	13.5 (4.7)	8.2 (1.6)	< 0.001	12.7 (5.1)	8.9 (2.4)	< 0.001	12.0 (3.1)	9.1 (2.7)	< 0.001
Ca (g kg⁻¹)	22.6 (13.9)	24.1 (11.8)	–	23.1 (14.4)	22.8 (13.9)	–	22.7 (11.1)	23.5 (15.8)	–	19.5 (10.7)	18.5 (7.8)	–
K (g kg⁻¹)	10.8 (4.7)	12.8 (4.9)	0.01	13.4 (7.0)	12.5 (4.5)	–	11.9 (4.9)	14.1 (5.3)	–	12.4 (4.7)	11.1 (4.4)	0.04
Mg (g kg⁻¹)	3.7 (1.7)	3.4 (1.3)	–	5.6 (3.1)	3.4 (1.8)	–	3.9 (1.3)	4.3 (2.2)	–	4.5 (2.5)	3.5 (2.0)	< 0.001
δ13C (‰)	−29.7 (1.4)	−27.8 (1.0)	< 0.001	−29.9 (1.3)	−28.1 (0.6)	< 0.001	−29.3 (1.5)	−28.4 (1.1)	0.03	−29.3 (1.2)	−28.4 (1.2)	< 0.001
δ15N (‰)	1.9 (1.9)	2.7 (2.2)	–	3.0 (1.2)	2.4 (2.3)	–	2.5 (1.6)	2.5 (2.0)	–	2.4 (1.2)	2.6 (2.1)	–
Compound leaves (%)	23	5		6	17		14	20		26	8	
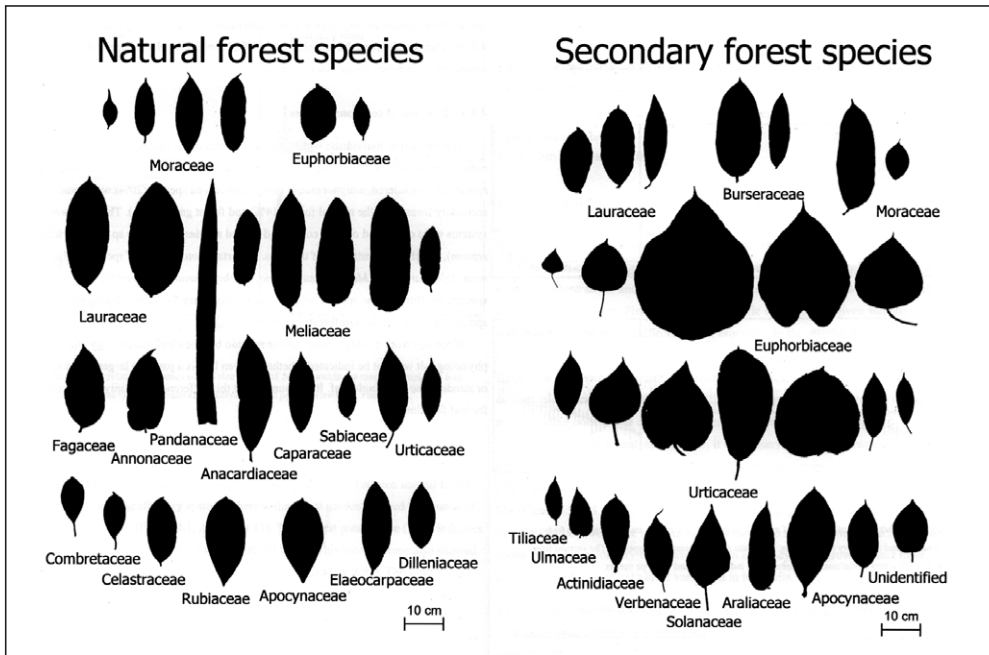
FIG. 1. Leaf silhouettes of 28 selected natural forest and secondary forest species from the study region.

cies abundance in the stand to reflect the properties of the 'average tree' of the stand. Compared to the results of the SA approach, most traits showed smaller absolute differences in their NF and SF means when calculated according to the WSA approach. Moreover, in 9 of the 13 parameters, the significance of differences between the two forest types decreased or even disappeared when shifting from the SA to the WSA approach. The average leaf of the SF trees appeared to be smaller, thicker and less N- and P-rich when sampled with the WSA approach than according to the SA approach.

In contrast to the SA, NSA and WSA approaches, which consider randomly selected subsamples of canopy trees in a plot, the total inventory (INV) data covered nearly all trees of a stand (canopy trees and subdominate individuals) and thus approaches the true stand averages of the tree populations for the leaf traits investigated. For most leaf attributes except for foliar K content, the inventory yielded equally high or higher significances for natural forest/secondary forest differences compared with the SA approach. The absolute means of leaf traits in the NF and SF obtained by the INV and SA approaches were similar

for most attributes. However, leaf size was a marked exception: the average secondary forest leaf was much smaller, thinner and narrower in the inventory data than it appeared from the SA data (Table 3).

DISCUSSION

Leaf sampling in tropical primary and secondary forests may serve different purposes. Tree ecophysiologists and foresters are mainly interested in leaf traits that characterize a tree species' carbon gain or growth capacity, its nutrient demand and economy, or leaf water regime and drought tolerance. Consequently, the focus is on tree species that evolved in the contrasting environments of primary (late-successional) or secondary (early-successional) forests. On the other hand, scientists working on regional or global biogeochemical cycles may be more interested in the 'average' tree of a forest in order to upscale from the tree to the stand and landscape levels (e.g., Schulze *et al*. 1994, Körner 1995). Different sampling strategies may be necessary to reach these two goals.

By comparing three different leaf sampling strategies with the results of a nearly complete stand in-

ventory, we were able to show that the outcome of a functional comparison of two forest types is, to a considerable extent, dependent on the chosen sampling approach.

Four major conclusions can be drawn from our analysis:

(1) As expected, a significant sampling effect may exist when analyzing tree functional traits because, in certain variables, specific properties of individual species will have a large effect on the stand mean. This effect was most evident when comparing average leaf size of NF and SF trees as derived either from 18 (NSA) or from 28–35 species per stand (SA): the absolute means of the two forest types and also the difference between NF and SF were larger in the SA than in the NSA sample because additional large-leaved species were included in the SA sample which were missing in the more restricted NSA sample. Since leaf size typically showed a left-skewed frequency distribution in the secondary forest samples, this explains why a reduced sample size should be associated with smaller leaf size averages.

(2) In many leaf traits, rather large samples of more than 20 or 25 species are required for detecting significant differences between SF and NF tree species means, because interspecific variation within a forest type was substantial in our forests. This is crucial because the majority of studies investigating the putative functional dichotomy between NF and SF species did not analyze more than 3 or 5 species per forest type and thus may have missed important differences (e.g., Oberbauer & Strain 1984, Fetcher *et al.* 1987, Strauss-Debenedetti & Bazzaz 1991).

(3) It matters whether only those trees are sampled that form the upper canopy layer, or whether samples are taken from trees of all canopy strata. In the total inventory (INV) a few subdominate trees with dbh >10 cm were covered which thrived in the second or third stratum, among them *Homolanthus populneus* (Euphorbiaceae), *Grewia glabra* (Tiliaceae) and *Trema orientalis* (Ulmaceae), all with comparatively small leaves. In fact, only the fast growing trees in the upper canopy of the secondary forest had very large leaves, as one would expect for this forest type, whereas a number of mesophyllous and notophyllous trees (leaf size 20–182 cm^2) were also present in the lower strata. This explains why the SF had a smaller average leaf size (only 114 cm^2) than the NF (168 cm^2) in the total inventory data, which contrasts with the SA, WSA, and NSA data.

(4) A weighted species average (WSA), which considers the contrasting frequencies of the species in the stand, led to smaller differences between NF and SF trees than did a simple species average (SA), although both approaches used the same data basis. Thus the 'average trees' of natural and secondary forests are functionally less distinct than it would appear if the species averages (SA and NSA) of the two forest types had been compared.

Our analysis indicates that natural forest and secondary forest are functionally different primarily with respect to leaf conductance and P acquisition patterns: the average SF tree had a higher mean leaf conductance (as inferred from a smaller ^{13}C discrimination) and profited from a better phosphorus availability (or P acquisition) than the average NF tree (as indicated by a higher leaf P content and lower N/P and C/P ratios). Other functional attributes were not significantly different between NF and SF when comparing the average tree of the stand.

More recent work has raised doubts as to whether tropical trees can be clustered into two or more discrete functional groups. There is evidence that the physiological constitution of tropical trees may change more or less continuously along the pioneer-climax axis if growth rate, size at maturity, regeneration requirements and longevity are analyzed (Turner 2001). On the other hand, other functional attributes such as photosynthetic capacity and maximum leaf conductance seem to be clearly different between pioneer and non-pioneer tree species with minor or no overlap between the two groups, although considerable interspecific variation exists (Bohman 2004). This is also true for the foliar δ^{13}C signature as analyzed in this study (see Table 3). Apparently, these three variables are physiological character traits for pioneer and non-pioneer trees which are rather uniform in each group because the specific environmental constraints in secondary and late-successional tropical forests only allow rather limited variation among the species with respect to water loss and carbon gain regulation, calling for functional convergence (Meinzer 2003).

Other variables were much more flexible among the species of a forest type and consequently showed a clear overlap between the trees of secondary and natural forest; examples are leaf size and SLA. Indeed, many pioneer species possess relatively small leaves; on the other hand, there are also late-successional species with very large leaves in the upper canopy (Turner 2001). The same is true for shade tolerance, and for the often reported association of pioneers with r-selection, and of non-pioneer species with K-selection. This is because the light environment and the regeneration niches in secondary forest and natural

forest are sufficiently heterogeneous in space and time to allow for the coexistence of tree species with alternative traits in the same stand. We conclude that the notation of a character continuum, or alternatively a dichotomy between pioneer and non-pioneer trees, depends on the trait investigated. At least for leaf size, we found evidence against the existence of a continuum along the pioneer-climax axis despite considerable overlap of the values between the two forest types. In our data, the significance of the NF – SF difference in leaf size increased with the size of the sample, indicating that the two leaf morphological groups became indeed clearer, and not less clear, by including additional species. Thus a sufficiently large sample is needed to detect the difference.

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