

# Taxonomic Identification, Sampling Methods, and Minimum Size of the Tree Sampled: Implications and Perspectives for Studies in the Brazilian Atlantic Rainforest

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## ABSTRACT

Our aim is to assess the main problems in the studies of tree communities in the Brazilian Atlantic Coastal Rainforest by analyzing methods and sampling criteria used by different authors. We surveyed the literature and built a databank, which included 225 phytosociological tables obtained from 113 publications. Most publications are theses and papers published in non-indexed journals of restricted circulation. The studies concentrate in the southeastern states, and there are large non-surveyed stretches, mainly in the northeastern states. Botanical families with identification problems were always among the ten most abundant ones in every survey. The size of the smallest individual sampled exerted more influence on the results than the sampling method (plots or point-centered quarters), thus making a standardization necessary in future studies. Considering a similar sampling effort, the quarter method sampled a larger number of species than the plot method. The number of individuals sampled was a better indicator of sampling effort than the area sampled. Considering our present knowledge, there is still a great need to survey the Brazilian Atlantic Rainforest and publish the results in adequate journals. However, each survey should obey a minimum set of criteria that yields reliable data. Analyzing the variation of community structure in space and its relation to abiotic variables allows for hypothesis testing and provides key information for planning conservation of natural areas and remediation of degraded vegetation.

**Keywords:** phytosociology, bibliography, *sensu stricto* Atlantic Forest, tree flora, survey protocol

## INTRODUCTION

Originally, the Atlantic Rainforest *sensu stricto* (*s.s.*) or Atlantic Dense Ombrophilous Forest (Veloso 1992) continuously spread over the Brazilian coast since the Cape of São Roque (07° S), in the state of Rio Grande do Norte, until the municipality of Osório (29° S), in the state of Rio Grande do Sul (Andrade-Lima 1966). Its original area has been estimated at 237,530 km<sup>2</sup> (Câmara 2005), of which only 7.5% presently remains (Myers *et al.* 2000). The remaining area is scattered in many fragments, the largest ones occurring in hardly accessible terrains on the Southern and Southeastern coastal mountains (Hirota 2005). The Atlantic Rainforest *s.s.* has a very heterogeneous tree flora, which is characterized by the prevalence of species with low constancy and restricted geographic range (Scudeller *et al.* 2001). Many sites present values of Shannon's H' diversity that are greater than those estimated for the Amazonian Terra Firme Forest (Silva and Leitão-Filho 1982; Martins 1989, 1991; Baitello *et al.* 1993; Leitão-Filho 1994; Jesus and Rolim 2005), most of them ranging from 3.71 nats.individual<sup>-1</sup> in Teresópolis, Rio de Janeiro state, to 5.04 nats.individual<sup>-1</sup> in Linhares, Espírito Santo state. Salomão and Lisboa (1988) calculated values of Shannon's H' for many Amazonian forests and found a range between 3.58 and 4.76 nats.individual<sup>-1</sup> in sites of Terra Firme forests. Due to its high conservation value, Myers (1990) classified the Atlantic Rainforest *s.s.* as one of the 18 diversity hotspots of the world. The diversity hotspots are geographic areas sheltering almost 50,000 endemic plant species (about 20% of the world flora), but comprising only 0.5% of the entire world land surface. Myers *et al.* (2000) enlarged the number of world hotspots from 18 to 25, confirmed the prominent position of the Brazilian Atlantic Rainforest, and emphasized the priority of its

conservation.

Our knowledge of the Atlantic Rainforest *s.s.* is still incipient, especially considering: a) the nature (composition) and the number (richness) of the species that co-exist and constitute a local community, and how the composition and the richness vary among communities; b) the distribution of abundance among the species in a local community (equitability) and how equitability varies among different communities; c) the measurement of the local diversity (alpha-diversity) and how the alpha-diversity varies in space and time; and d) how the composition, richness, equitability, and alpha-diversity can be influenced by environmental variables. Although still poorly known, these issues about the Brazilian Atlantic Rainforest *s.s.* have been assessed with the application of Phytosociology.

Phytosociology was firstly defined in 1896 in Jozef Paczoski's paper *Zycie gromadne roslin* (The social life of plants) as the study of all the sociological relations among plants in their groupings (Maycock 1967). As a subdiscipline of Plant Ecology, Phytosociology investigates the co-occurrence of plant species in communities (Ewald 2003). Phytosociology may be defined as the study of the causes and effects of the co-habitation of plants in a given environment, the assembling, constitution, and structure of the plant groupings, and the processes that imply in the continuity or alteration of a plant grouping in time and space (Martins 2004).

Phytosociology relies on a set of methods, theories, and concepts that range: a) from the description of a local community to the detection of recurrent patterns in many communities over a large scale; b) from the analysis of the relation between an environmental variable and a species abundance in the space of a local community to the establishment of limiting conditions for species and communities in

their entire geographic range; c) from a description of the instantaneous structure of a local community to the integration of its variation within a time interval; d) from the classification of local-scale vegetation stands to the detection of relationships among entire phytogeographic formations (Martins 2004). Due to its wide range, Phytosociology has been divided into different fields of study (Braun-Blanquet 1979), each one equipped with a terminology and a set of methods that are internationally recognized. The property that is common to all these different fields is the quantification: the abundance of species and their relationships are expressed in numerical terms, so as to allow mathematical treatments and statistical comparisons (Martins 2004). An appraisal of publications by Brazilian authors who cited Phytosociology as a key-word leads to the conclusion that, in Brazil, Phytosociology is considered as the quantitative description of taxocenoses and their relationships in space and time. According to Hurlbert (1971), a taxocenose is a set of individuals more or less related phylogenetically that have similar forms and exploit similar resources in a similar way.

A present-day tendency in phytosociological studies is the analysis of metadata organized in electronic databanks. The organization of biological information as electronic databanks is important, since a databank centralizes information, reduces or eliminates the overlapping of efforts and decreases the costs of a research (Scudeller and Martins 2003). Data from individualized surveys can, and should, be systematized in order to gather sparse knowledge, allow the construction of working hypotheses, and lead to future researches, thus enlarging our scientific knowledge (Torres *et al.* 1997). In Europe, the recent development of large databanks has represented an objective way towards the ecological analysis of species or communities over large territories, thus improving the knowledge of vegetation (Gégout *et al.* 2005).

In Brazil, vegetation studies based on databanks are few and still have a regional bias. Silva and Shepherd (1986) performed the first study based on metadata in Brazil, which included Atlantic Rainforest *s.s.*, Seasonal Semideciduous Forest, and Amazonian Terra Firme forests. Silva and Shepherd (1986) made comparisons at the genus level with Jaccard Similarity Index (Sneath and Sokal 1973) applied to binary (presence/absence) data, and concluded that: a) climate, especially the occurrence of frost, was the main conditioner of the floristic similarity among the forests compared; b) the similarity of genera was very unevenly distributed among sites of the Atlantic Rainforest, some of which were similar to Amazonian Terra Firme forests, others were similar to Seasonal Semideciduous forests, and still others had a very low similarity with all the other sites. Comparing tree species similarity by means of binary data, Siqueira (1994) found that: a) most species had a very restricted geographic range; b) the central region of the entire latitudinal range of the Atlantic Rainforest *s.s.* had the maximum number of species; and c) most species were different at north and south from the center. She concluded that the tree flora of the Atlantic Rainforest *s.s.* is very heterogeneously distributed along the space. Also using binary data to compare species similarity, Ivanauskas (1997) found differences between the tree communities on the Atlantic Crystalline Plateau and sedimentary Coastal Province in São Paulo state. Applying multivariate analyses to a databank considering the Atlantic Rainforest, Seasonal Semideciduous Forest, Amazonian forests, and Cerrado, Oliveira-Filho and Fontes (2000) found a) a floristic differentiation between rainforest and seasonal semideciduous forest associated to rainfall regime; b) a significant influence of latitude, longitude and altitude on species distribution; c) greater species similarity between the Atlantic Rainforest and the Seasonal Semideciduous Forest than between any of them with either Amazonian or Cerrado; and d) great similarity, especially at genus and family level, between the Seasonal Semideciduous Forest and the Cerrado. Oliveira-Filho and Fontes (2000) concluded that, to a considerable

extent, the tree flora of the Seasonal Semideciduous Forest is a subset of the Atlantic Rainforest, constituted by species that are able to cope with a dry season. Scudeller *et al.* (2001) considered binary and quantitative variables of tree species occurring in the Atlantic Rainforest *s.s.* of São Paulo state to perform classification and ordination analyses. They found a similarity decay with geographical distance and a distribution of the tree species in complex gradients related to the distance from the sea, climate, and altitude, and concluded that the tree flora of the Atlantic Rainforest *s.s.* is very heterogeneous along the space, its species having narrow ecological niches and being finely tuned to environmental conditions. These few studies based on banks of metadata done so far in the Brazilian Atlantic forests are enough to highlight the importance and the need to perform field surveys and publish the results in good vehicles.

Martins (1989) divided the history of the Brazilian forest Phytosociology into the initial phase and the diversification phase. In the initial phase, phytosociological studies of trees in the Brazilian Atlantic Rainforest *s.s.* aimed to gather the information necessary for fighting yellow fever and malaria (Veloso 1945, 1946). After more than 60 years from this first phytosociological study in the Atlantic Rainforest *s.s.*, we think it is worthy having a look at the influence of the different methods and sampling criteria on the results, as well as to make an appraisal of the taxonomical difficulties, so as to provide elements to assess the results attained so far and suggest possible paths toward future studies. Our aim is to make an appraisal of the phytosociological methods of studying tree taxa in the Atlantic Rainforest *s.s.*, having the following questions as guidelines: 1) Where are the phytosociological studies done so far in the Brazilian Atlantic Rainforest *s.s.* published? 2) Are the surveys well distributed over the entire range occupied by this biome? 3) Are there taxonomic identification problems, and what are the most problematic taxa? 4) What was the influence of different sizes of the smallest individual sampled on the results? 5) Is there a sampling method that yielded a greater number of species in relation to the sample size? 6) Considering the number of species, would it be possible to indicate a minimal sampling effort? By answering these questions, we intend to identify the main problems of data surveying, facilitate the discussion of the methods used, suggest a minimal protocol for future studies, and provide the researcher with a fundamental literature.

## MATERIALS AND METHODS

We used the FITOGEO databank system, which was developed in Microsoft Access 97 to manage floristic and phytosociological information (Scudeller and Martins 2003). We considered as Atlantic Rainforest *s.s.* the Phyto-Ecological Region mapped as Atlantic Ombrophilous Dense Forest by IBGE (Veloso 1992). To update the databank we considered all quantitative tree survey done within this Phyto-Ecological Region. We performed the update in two steps. First, we filtered from the original databank all phytosociological tables referring to tree communities in the Atlantic Rainforest *s.s.* A phytosociological table is a single list of species with their respective phytosociological descriptors. A phytosociological table is analogous to a *relevé* (see Mueller-Dombois and Ellenberg 1974), but it is assembled with data collected by other methods that are different from those of Braun-Blanquet (1979) school. Each publication we gathered could have more than one phytosociological table referring to different sites, altitudes and soil types. In the second step we fed this databank with new phytosociological tables coming from theses, books and articles published in specialized periodicals. When a thesis was published as a scientific paper, we considered only the published article. We took into account only the periodicals that had an editorial board and ISSN, and we did not consider reports, undergraduate monographs and similar non-published studies. We considered only the references published until the beginning of 2005 that informed the number of individuals sampled in each species, the total sample size, and the size of the smallest individual sampled. We discarded studies of initial regeneration stages and those that did not sampled trees or

considered only a maximum height as the sampling criterion. We regarded as trees the taxa considered as such by the authors of the original studies, including arborescent ferns and palms. We then confirmed the arboreal habit by consulting specialized taxonomic literature.

For the data analysis we used the commands provided by Access for the use of simple or combined filters and for the generation of reports in order to quantify the number and percentage of phytosociological tables at distinct sites, different sampling methods, sample size, and the size of the smallest individual included in the sample, among other variables. To standardize the data, the area sampled was converted into square meters, and the PBH or CBH (trunk perimeter or circumference at breast height, respectively) was transformed in DBH (trunk diameter at breast height). We constructed the diagrams with Microsoft Excel 2003.

By pooling all phytosociological tables together in the databank we produced a preliminary species list. From this preliminary list we excluded the taxa with dubious identification (*cf.* qualification), but kept the ones with the qualification *aff.* and the taxa not identified to family, genus and species. Then we proceeded to taxon synonymization based on the specialized taxonomic literature (floras, reviews, monographs, theses, etc.), so as to combine the different synonymous of a same species in a single binome and dispose the taxa according to APG system (Stevens 2006). Based on this refined list we considered that the tree flora obtained from the samples taken in the Brazilian Atlantic Forest *s.s.* would have a number of species between a maximum and a minimum, as did Castro *et al.* (1999) for the Brazilian Cerrados. The minimum number considered that any non-identified taxon on any phytosociological table would be one of the already identified taxa on another table: the minimum number of taxa would just be the number of binomes, including those with the qualification *aff.* The maximum number considered that any non-identified taxon on any phytosociological table would be different from all identified taxa on all the other tables.

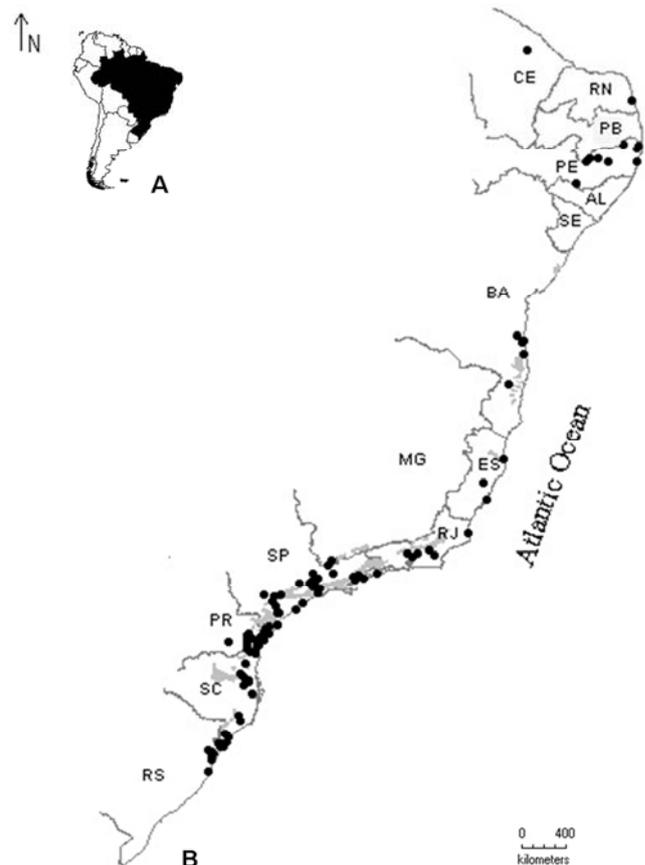
To test for differences of the total number of species sampled by the plot and the quarter methods in relation to the sample size expressed as number of individuals (N), we performed a linear regression analysis and then applied the *t*-test to look for differences between the slope coefficients (Zar 1999). To do this we considered only the phytosociological tables that sampled DBH  $\geq 4.8$  cm, so as to not have influence of any other variable but sample size.

Considering only the samples taken with the plot method including DBH  $\geq 4.8$  or 5.0 cm, we made scatter diagrams (Levine *et al.* 2000) and performed regression analyses of the number of species sampled (S) against the sample size in m<sup>2</sup> (A) or the number of individuals (N). We made goodness-of-fit tests for these regression curves considering different regression models and chose the best fit by considering the greatest coefficient of determination R<sup>2</sup> with the software BioEstat 3.0 (Ayres *et al.* 2003). Then we visually assessed the existence of an inflexion point on the curves by considering a change in the tendency from an initially great increment ratio to smaller and smaller increments, although the curve could keep asymptotic.

Considering only the phytosociological tables with sampling area from 7,000 to 10,200 m<sup>2</sup>, we used Spearman correlation tests (Zar 1999) to evaluate the relationship between the size of the smallest individual included in the sample and the total number of individuals or species sampled. With these data we also constructed scatter diagrams (Levine 2000) to allow for a visual assessment of tendencies.

## RESULTS

Our databank on the Brazilian Atlantic Rainforest *s.s.* included 225 phytosociological tables obtained from 113 publications, of which 52% were master or doctorate theses, 46% were papers in scientific periodicals and 2% were three books and a further qualification (livre-docência) thesis. The Atlantic Rainforest *s.s.* has not homogeneously been sampled over its remnant area (Fig. 1). In the Northeastern region, we gathered only 27 phytosociological tables: in the state of Rio Grande do Norte – the northern limit of the continuous biome range – we found only one publication in



**Fig. 1** Location of Brazil in South America. (A) Brazilian coast with remnant fragments of the Atlantic rainforest *s.s.* in gray (adapted from IBGE 2006). (B) The black points indicate the surveys used in our analysis. States: CE = Ceará, RN = Rio Grande do Norte, PB = Paraíba, PE = Pernambuco, AL = Alagoas, SE = Sergipe, BA = Bahia, MG = Minas Gerais, ES = Espírito Santo, RJ = Rio de Janeiro, SP = São Paulo, PR = Paraná, SC = Santa Catarina, RS = Rio Grande do Sul.

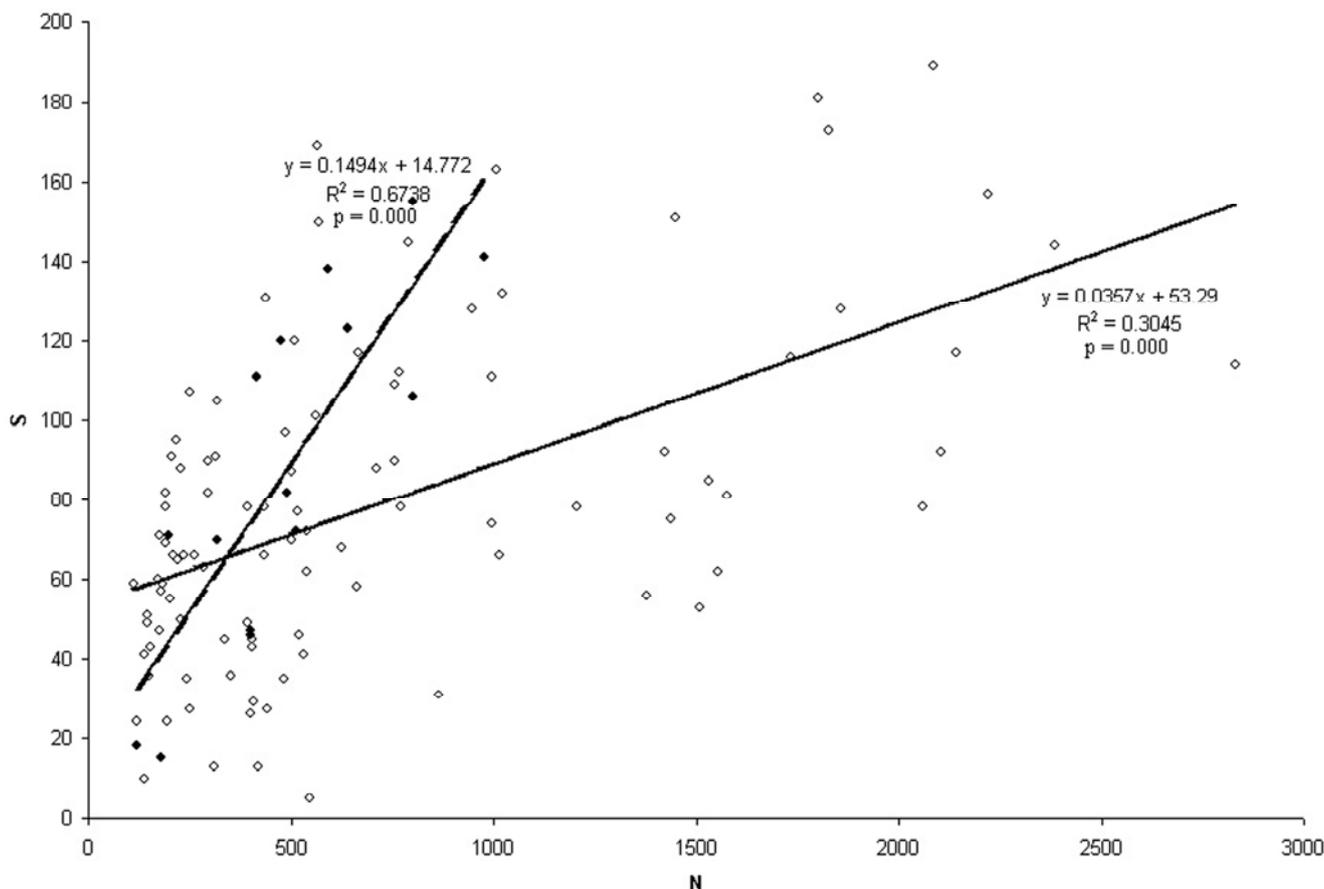
**Table 1** Families not identified to genus level with the respective number of references (NR) to unknown 198 genera in 225 samples taken from the Brazilian Atlantic rainforest.

Family	NR	Family	NR
Myrtaceae	52	Malvaceae	2
Lauraceae	20	Meliaceae	2
Fabaceae	18	Myrsinaceae	2
Rubiaceae	15	Nyctaginaceae	2
Euphorbiaceae	8	Rhamnaceae	2
Bignoniaceae	7	Sapindaceae	2
Moraceae	7	Verbenaceae	2
Sapotaceae	7	Violaceae	2
Annonaceae	6	Asteraceae	1
Apocynaceae	5	Clusiaceae	1
Salicaceae	5	Combretaceae	1
Arecaceae	3	Humyriaceae	1
Melastomataceae	3	Icacinaceae	1
Monimiaceae	3	Myristicaceae	1
Rutaceae	3	Olacaceae	1
Celastraceae	2	Polygonaceae	1
Chrysobalanaceae	2	Simaroubaceae	1
Lecythidaceae	2	Solanaceae	1
Malpighiaceae	2	Symplocaceae	1

a journal; in the state of Bahia we found 14 tables, of which nine constituted Veloso's (1946) pioneer study; in the state of Pernambuco the publications are linked to the Plant Biology Graduate Course of the Federal University and to the Botanic Graduate Course of the Federal Rural University. In the Southern region, we found 89 phytosociological tables, most represented by Veloso and Klein's (1957,

**Table 2** Genera not identified to species level cited in more than 1% of the total number of references (NR) to 627 non-identified species in 225 samples taken in the Brazilian Atlantic rainforest.

Genus	Family	NR	Genus	Family	NR
<i>Ocotea</i>	Lauraceae	40	<i>Trichilia</i>	Meliaceae	9
<i>Eugenia</i>	Myrtaceae	32	<i>Casearia</i>	Salicaceae	8
<i>Calyptanthes</i>	Myrtaceae	25	<i>Chrysophyllum</i>	Sapotaceae	7
<i>Pouteria</i>	Sapotaceae	20	<i>Psidium</i>	Myrtaceae	7
<i>Myrcia</i>	Myrtaceae	18	<i>Alibertia</i>	Rubiaceae	6
<i>Cryptocarya</i>	Lauraceae	13	<i>Cinnamomum</i>	Lauraceae	6
<i>Miconia</i>	Melastomataceae	12	<i>Erythroxylum</i>	Erythroxylaceae	6
<i>Mollinedia</i>	Monimiaceae	10	<i>Faramea</i>	Rubiaceae	6
<i>Nectandra</i>	Lauraceae	10	<i>Ficus</i>	Moraceae	6
<i>Guatteria</i>	Annonaceae	9	<i>Guapira</i>	Nyctaginaceae	6
<i>Marlierea</i>	Myrtaceae	9	<i>Maytenus</i>	Celastraceae	6
<i>Psychotria</i>	Rubiaceae	9	<i>Myrceugenia</i>	Myrtaceae	6

**Fig. 2** Linear regression between the total number of species (S) and the total number of individuals sampled (N) with the quarter method (closed circles and hatched line) or the plot method (open circles and full line) in the Brazilian Atlantic Rainforest *s.s.*

1968a, 1968b) studies with small samples in many sites. In the Southeastern region, we gathered 102 tables, 80 of which refer to the state of São Paulo and 15 to the state of Rio de Janeiro. Considering the importance of the Atlantic Rainforest *s.s.* in the state of Espírito Santo (SE region), we expected to find many surveys, but we gathered only five phytosociological tables. In the state of Minas Gerais (also SE region), we found only two tables, but in this state only a small forest remnant occurs at the border with São Paulo state.

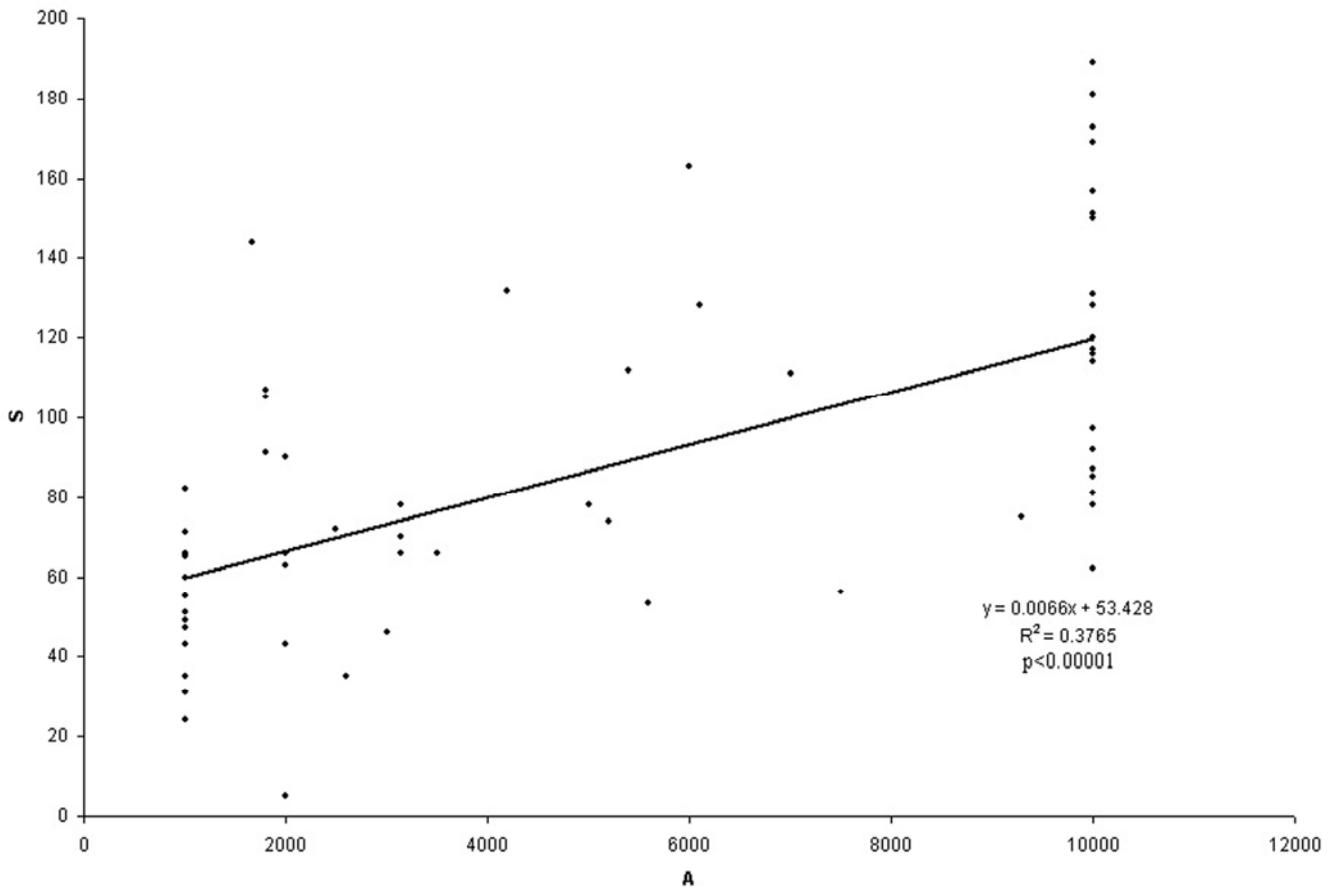
In Brazil, the main difficulty in phytosociological studies is the species identification. Generally, most individuals are in a vegetative phase during sampling and can remain in this state for years, thus causing great difficulty in their taxonomic identification. Out of the total 225 tables we analyzed only 41 presented 100% identification to species level. In the other 184 tables, the proportion of identification to binomes varied between 48.8 to 99.2%.

We found 891 citations to non-identified species in the totality of the tables, of which 66 citations did not have

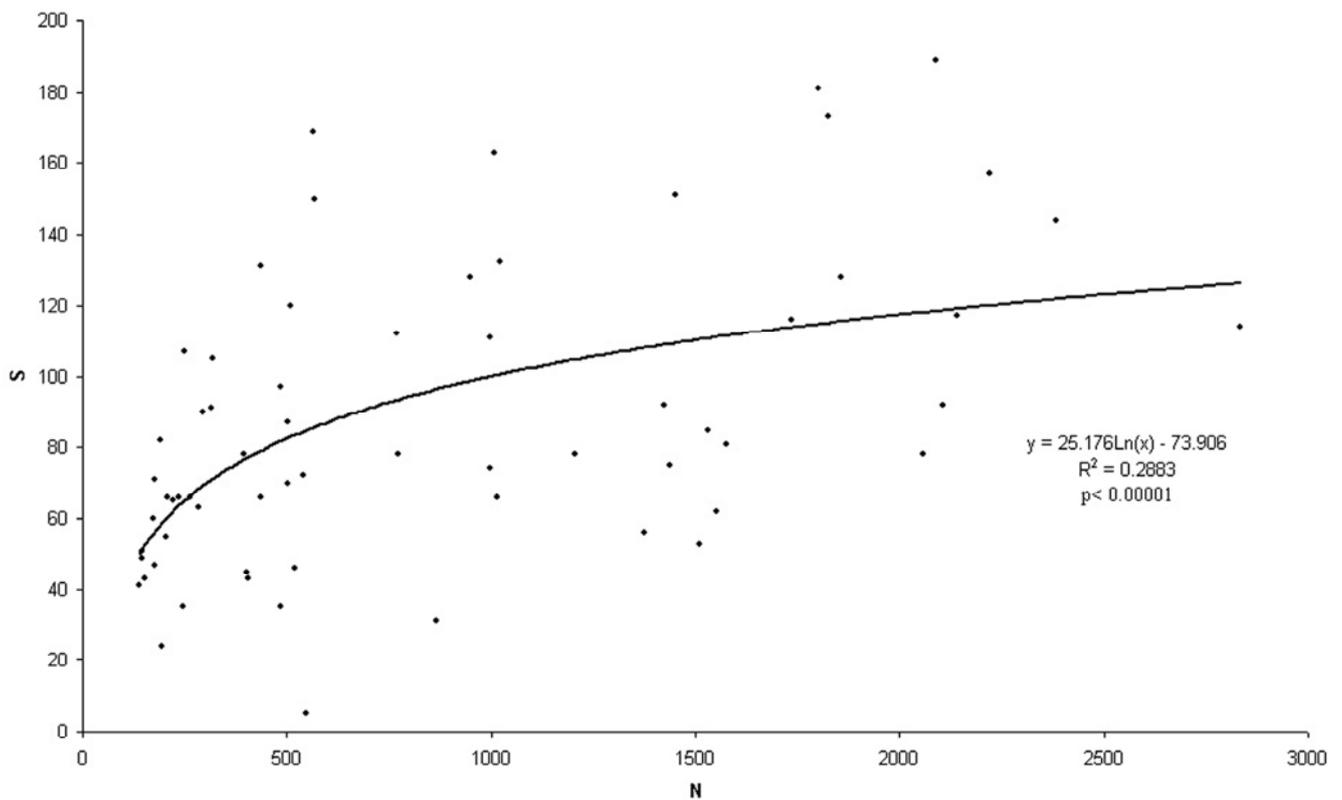
identification even to family level. Out of the remaining 825 citations to specific entities 198 were identified to family level and 627 to genus level. Out of the 198 citations identified to family level Myrtaceae appeared in 26.3%, Lauraceae in 10.1% and Fabaceae in 9% (**Table 1**). Out of the 627 citations identified to genus, *Ocotea* appeared with the greatest number (40) of non-identifications to species, followed by *Eugenia* (32), *Calyptanthes* (25), *Pouteria* (20) and *Myrcia* (18) (**Table 2**).

The minimum numbers of tree taxa we estimated for all the phytosociological tables in the Atlantic Rainforest *s.s.* were 98 families, 452 genera and 1,999 species; and the maximum numbers were 164 families, 713 genera and 2,891 species. The upper limits represent an addition of 44.6% of species, 57.3% of genera and 66% of families in relation to the lower limits.

The multiple plot sampling method (Daubenmire 1968) was used in 91% of all the phytosociological tables, and the quarter method (Cottam and Curtis 1956) was used in only 9%. The area sampled by the multiple plot method varied



**Fig. 3 Regression between the number of species (S) and the total area (A) in m<sup>2</sup> sampled with the plot method. The size of the smallest individual included in the samples was DBH ≥ 4.8 or 5 cm.**



**Fig. 4 Regression between the number of species (S) and the total number of individuals (N) sampled with the plot method. The size of the smallest individual included in the samples was DBH ≥ 4.8 or 5 cm.**

from 180 m<sup>2</sup> to 400,000 m<sup>2</sup>, whereas the number of points used in the quarter method varied from 24 to 266. Only Negreiros *et al.* (1995) used the variable angle method (Bitterlich 1984).

The linear regression line between the number of species sampled and the total sample size in number of individuals was significant for the data set surveyed by both the quarter method ( $R^2 = 0.674$ ;  $b = 0.149$ ;  $P = 0.000$ ;  $d.f. = 13$ ; **Fig. 2**) and the plot method ( $R^2 = 0.304$ ;  $b = 0.036$ ;  $P = 0.000$ ;  $d.f. = 96$ ; **Fig. 2**). The slope differed significantly between the two lines ( $t = 3.06^{**}$ ;  $d.f. = 109$ ), thus indicating that a greater number of species were obtained by the quarters than by the plots considering the same number of individuals sampled.

Considering the plot method, we did not observe any inflexion point on the regression curve between the number of species and the total area sampled, the best goodness of fit being the linear equation ( $R^2 = 0.376$ ; **Fig. 3**). On the other hand, we noticed a tendency towards an inflexion point on the regression curve between the total number of species and the total number of individuals sampled, the logarithmic equation being the best goodness of fit ( $R^2 = 0.288$ ; **Fig. 4**). The inflexion point on this curve was around 600 individuals, indicating that in sample sizes that were smaller than 600 individuals the number of species varied very much as the number of individuals sampled enlarged, but larger samples presented less variation of the total number of species sampled with the number of individuals. However, the low value of  $R^2$  indicated that, although an inflexion point could be present, the number of species sampled varied very much among samples taken with the same number of individuals.

The criteria to decide about the size of the smallest individual included in the sample varied very much among the phytosociological tables we compared. Some tables (2.2%) considered a minimum height, some (3.1%) considered a mixture of height and diameter, others (4.9%) considered trunk diameter at soil level, still others (23.6%) considered a range of the trunk diameter at breast height (DBH), but most (66.2%) considered only DBH. The smallest individual included in the sample had a DBH ranging from 1.5

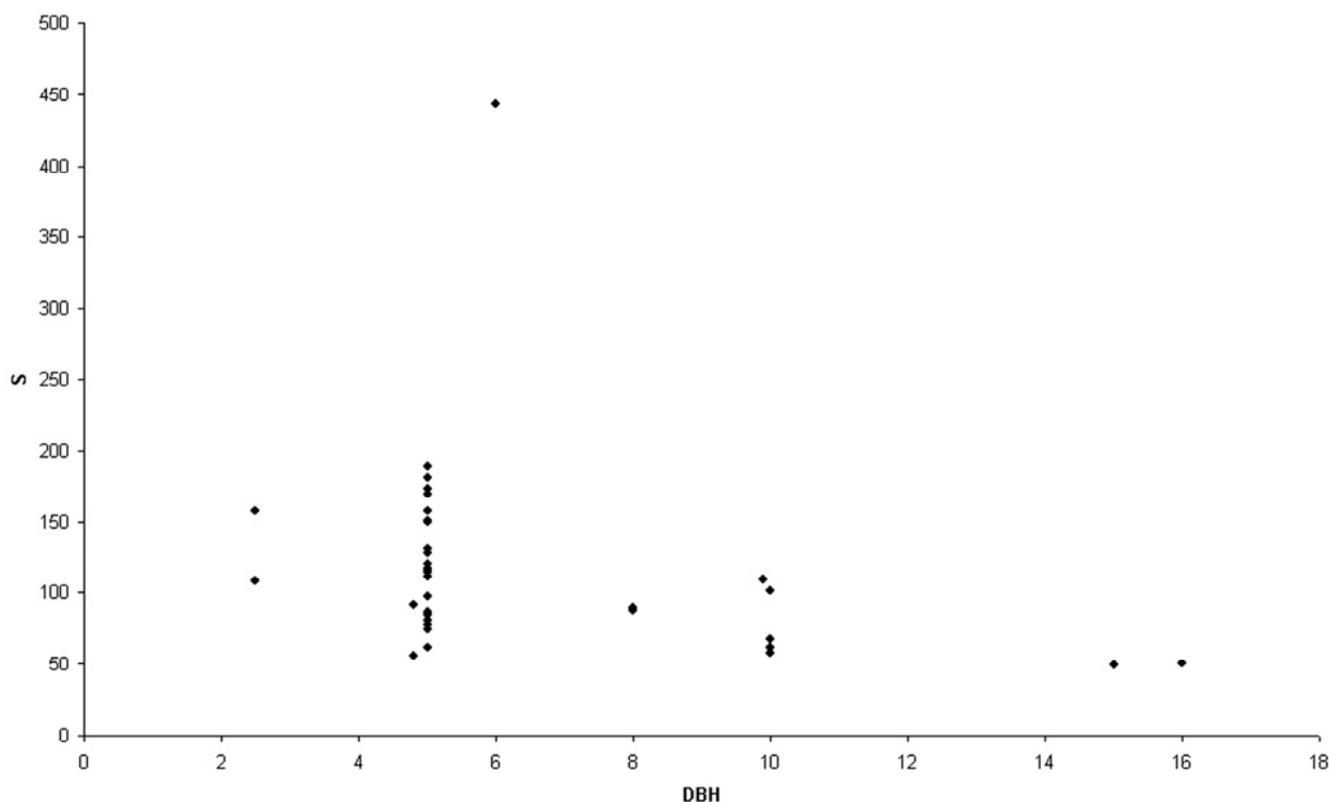
cm to 16 cm, but 79% of all phytosociological tables were based on a  $DBH \geq 4.8$  cm.

Both the number of species (**S**, **Fig. 5**) and the number of individuals (**N**, **Fig. 6**) sampled on a similar area tended to diminish with the increase of the size of the smallest individual sampled. We found the highest correlation between DBH and **N** ( $RS = 0.528$ ;  $P = 0.001$ ;  $N = 37$ ) and only a marginal significance between DBH and **S** ( $RS = 0.323$ ;  $P = 0.05$ ;  $N = 37$ ). Considering the same minimum DBH and a similar sample area, both the number of species and the number of individuals sampled varied very much, thus indicating that other variables, besides the size of the smallest individual included in the sample, can influence the results of the sampling.

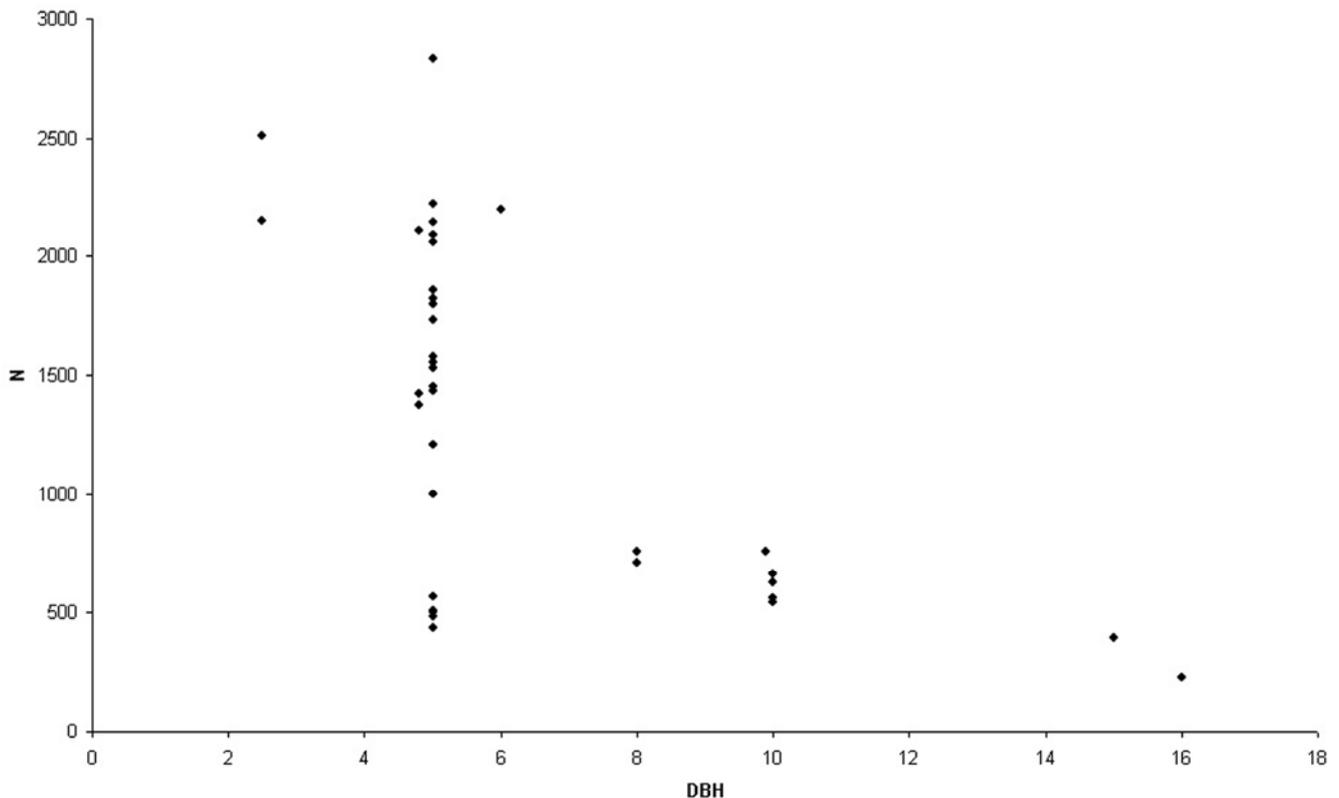
## DISCUSSION

### Where are the phytosociological studies done so far in the Brazilian Atlantic Rainforest *s.s.* published?

We found a relatively large number (113) of publications concerning quantitative studies of tree communities in Atlantic Rainforest *s.s.* fragments spread over the Brazilian coast, although they are not enough to provide a good basis to understand the diversity and structure of this biome. Over a half of these publications constitutes the so-called gray literature, represented by theses and studies published in non-indexed vehicles with restricted distribution (Scudeller and Martins 2003). This fact prevents, or at least renders very difficult, recovering information – the information becomes inaccessible to the scientific community, thus hindering other studies, actions or plans that need this kind of information. An effort by researchers and thesis supervisors is necessary for stimulating the publication of these studies in indexed vehicles of wide distribution. However, since these studies generally describe the structure of a natural vegetation stand and are classified as descriptive case studies, they do not reach from editors the priority level that is necessary to compete with studies in the deductive format, which disperse the scanty publication space available in



**Fig. 5** Scatter plot of the number of species (**S**) against the size (**DBH**) of the smallest individual included in samples taken in the Brazilian Atlantic rainforest *s.s.* The total sample area varied between 7,000 and 10,000 m<sup>2</sup>.



**Fig. 6** Scatter plot of the number of individuals (N) against the size (DBH) of the smallest individual included in samples taken in the Brazilian Atlantic rainforest *s.s.*. The total sample area varied between 7,000 and 10,000 m<sup>2</sup>.

highly impacting vehicles. Just accepting as an established fact that Brazil is the country of the megabiodiversity is not enough: it is necessary to qualify the diversity (what are the species? what is the richness?), to quantify it (what is the abundance of each species? how does the abundance vary? what is the numerical value of the diversity?), and to model it (where is the diversity? how does it vary in the space? is the diversity influenced by environmental variables?). The only way to get this knowledge is by surveying the vegetation in the field and publishing the phytosociological tables, so as to enable the construction of metadata banks.

#### **Are the surveys well distributed over the entire range occupied by the Atlantic Rainforest *s.s.*?**

Our results showed that the distribution of studies over this biome is not homogeneous, with gaps mainly in the Northeastern region (states of Bahia, Alagoas, Sergipe, Paraíba, and Rio Grande do Norte) and also in the state of Espírito Santo (Southeastern region). Considering the location of the remaining areas (IBGE 2006) and samples we analyzed (Fig. 1), it could be thought that the studies done so far encompassed almost all the largest fragments, but the mapping scale does not indicate the smaller fragments. Besides, it is possible that some fragments had been extinct, such as, for example, the forest described by Gandolfi (1991), which gave place to the Guarulhos International Airport in the São Paulo Metropolitan Region (São Paulo state). It is necessary to survey other fragments, document their diversity and put in practice the Brazilian laws concerning the conservation of natural vegetation. Studies in the Northeastern region are necessary especially to help establish the northern limits of the Atlantic rainforest *s.s.*

#### **Are there problems of taxonomic identification, and what are the most problematic taxa in the Atlantic rainforest *s.s.*?**

We observed that the families with the greatest identification problems were always the most abundant ones in every

sample taken in the Southeastern region (Guedes-Bruni *et al.* 1997; Pessoa *et al.* 1997; Guedes-Bruni 1998; Tabarelli and Mantovani 1999; Scudeller *et al.* 2001; França and Stehmann 2004; Jesus and Rolim 2005), Southern region (Jarenkow 1994; Jaster 1995; Negrelle 2002) and Northeastern region (Lobão 1993; Tavares *et al.* 2000). These families have also the widest geographic ranges in the Atlantic rainforest *s.s.* Siqueira (1994) found Lauraceae, Euphorbiaceae, Myrtaceae, Fabaceae and Rubiaceae as the most constant taxa in the samples, but the genera did not present the same pattern: their geographic range is restricted, and those having identification problems are different from one sample to another. Siqueira (1994) found that only 6% of a total of 460 genera occurred in more than 50% of the total number of samples, and concluded that the floristic heterogeneity in the space of the Atlantic rainforest *s.s.* is very high. We found phytosociological tables in which over 50% of the taxa were not identified to the species level. The huge proportion of taxa not identified to species points to the need of intensifying floristic collecting in the field, improving herbarium collections and stimulating systematical and taxonomical studies in the Atlantic rainforest *s.s.* (Scudeller *et al.* 2001). Assessing the real number of tree taxa that occur in the Atlantic rainforest *s.s.* is very difficult, especially considering that we included only quantitative surveys in our analysis and disregarded studies concerning floristic collecting. In a study aiming to evaluate the richness of the woody flora of Brazilian cerrados, Castro *et al.* (1999) enumerated many factors that can enlarge or diminish the estimates. Our conjectures about both our estimates are implausible, mainly the upper limit, in which all non-identified taxonomic entities would represent species, genera and families not previously identified. The lower limit seems to provide a more reliable minimum estimate, but it is not completely safe to assume that no unidentified taxon will be added to a new sample taken from the Atlantic rainforest *s.s.* In spite of these deficiencies, our estimate furnishes a way to establish the probable upper and lower limits for the tree species richness in this biome, especially in a scenario in which the field surveys are unevenly distributed and have a

large proportion of unidentified taxa. Moreover, an evaluation of the diversity is hindered by the great unfamiliarity with the taxa occurring in the Atlantic rainforest *s.s.* and by the difficulty to establish its precise geographic limits.

### What was the influence of different sizes of the smallest individual sampled on the results?

Different studies have shown that results vary with the size of the smallest individual included in the sample rather than with different sampling methods (Scudeller *et al.* 2001). In samples taken from the Seasonal Semideciduous Forest in São Paulo state, Dislich *et al.* (2001) found a negative correlation between the minimum size (PBH) and the total density of trees per unit area and emphasized that different minimum sizes adopted in different field surveys render it difficult to compare samples. Freitas (2004) also found a negative correlation between the minimum DBH and the total tree density per unit area in Atlantic fragments in Rio de Janeiro state. Our results showed a negative correlation of the minimum DBH with both the number of species and the number of individuals sampled in an area around 10,000 m<sup>2</sup>. Therefore, the size of the smallest individual to be sampled in a field survey is a variable of greater importance than the sampling method itself, and we suggest that it be standardized in all future phytosociological studies so as to allow more reliable comparisons.

### Is there a sampling method that yielded a greater number of species in relation to the sample size?

The comparison of phytosociological descriptors obtained with different methods in Brazilian forests has already been made by other authors. Gibbs *et al.* (1980) applied both the plot and quarter methods in a riparian forest in the municipality of Moji Guaçu (São Paulo state) and sampled a larger number of species with the quarter method. Cavassan (1990) mapped one hectare of cerrado in the municipality of Bauru (São Paulo state), digitalized the map, and compared both methods by simulating different sampling efforts in the digitalized community. He concluded that the quarter method does not yield less information when applied in tropical woody vegetation and has as an advantage the considerable economy of efforts during field work. He also concluded that the plot method is efficient in the study of small areas (about one hectare) because it provides more details on the vegetation, and the density estimate is less biased; whereas the quarter method can be used over larger areas, provides a more general idea of the community structure and is a good indicator of the number of species and probable gradients in the vegetation, but furnishes biased estimates of density. Analyzing metadata on the Atlantic rainforest *s.s.* in São Paulo state, Scudeller *et al.* (2001) did not find any influence of the sampling method (plots or quarters) on the results. Our results showed that considering a similar sampling effort, the quarter method yields a larger number of species than the plot method. Hence, we think that the sampling method (plots or quarters) does not represent an important factor in the variation of the results in samples taken from the Atlantic rainforest *s.s.*, but we recommend the quarter method for expeditious samplings in large areas where a general, fast survey is aimed and precise estimates are not required.

### Considering the number of species, would it be possible to indicate a minimal sampling effort?

There is little available information concerning the sampling effort that is required to warrant the “sufficiency” of a sample. Two aspects should be considered when we assess the sample “sufficiency”. One aspect refers to the proportion of species sampled in relation to the total of species present in the community. The restrict geographic range and the very low constancy of tree taxa in the Atlantic rainforest *s.s.* produce a decay of the floristic similarity with increase-

ing geographic distance (Scudeller *et al.* 2001), that is the tree flora is hugely heterogeneous in space. This means that the species-area curve would keep its steep slope as the sampling area enlarged, and even if the sample had a very large area the number of species sampled would still enlarge with enlarging sample area. The other aspect of “sufficiency” refers to the precision of the phytosociological descriptor estimates. Before starting sampling, we do not know the number of species or their abundance in the community to be studied; we come to know them only after the sample is taken. This means that the sample always is *a posteriori* stratified and, therefore, each stratum (species) will have been sampled with a different sampling effort. Vieira and Couto (2001) showed that the sizes and numbers of plots would be very different to attain statistical sufficiency for each species in the Atlantic rainforest *s.s.* and concluded that it is very difficult to standardize the size and the number of plots, so that we should always keep in mind the sample errors when we decide to use a unique plot size to sample all the species in the community. Cielo-Filho (2001) suggested that one hundred random plots summing up a total area of one hectare would be a fairly sampling effort in 6.5-ha stands in the Seasonal Semideciduous Forest in the interior of São Paulo state. However, considering the whole range of occurrence of the Atlantic rainforest *s.s.* in Brazil, it is not possible to recommend a fairly minimum sampling area. Our results showed that the variation of the species number was very high between samples, even if a sample area about 10,000 m<sup>2</sup> and a minimum tree size of DAP ≥ 5 cm were kept constant. This indicates that species richness would not be strictly tied to the total area sampled. Hence, we speculate that the environmental differentiations and variations – which are known to occur in the Atlantic rainforest (Oliveira-Filho and Fontes 2000; Scudeller *et al.* 2001) – would be more strongly associated with species richness, which is a component of species diversity (Wright 2002; Leigh *et al.* 2004). It is also likely that different disturbance histories could contribute to differences in species richness among the sites sampled, but unfortunately there is no local record that could support the inclusion of this variable in our analysis. We suggest that what we usually call sample “sufficiency” or “representativeness” may be considered a ratio of convenience between the sampling effort and the return of information, and that the mathematical modelling of the collector’s curve (Pielou 1977) may be an adequate tool to decide when stop enlarging sampling effort. Also, we suggest that the total number of individuals sampled is more important than the area sampled.

## CONCLUSIONS

Considering the present state of knowledge of the Brazilian flora and vegetation, we believe that field surveys of the community structure with the production of phytosociological tables are still very necessary. From our viewpoint, there is no other way to qualify, quantify and model the diversity of the different vegetation types of Brazil unless by means of the incentive not only to field works but also to the publication of the phytosociological tables in good quality vehicles. However, each field survey should obey to a minimum set of rules that can yield a threshold of reliability on the data sampled.

The greatest source of variation in the results among the different field surveys does not seem to be the sampling method (plots or quarters) but the different sizes of the smallest individual included in the sample, provided that the sample size is not smaller than a critical minimum. Therefore, it is necessary to standardize the size of the smallest individual to be included in the sample as well as to attempt to a minimum sample size, which should not be less than 1,000 individuals (Palmer *et al.* 2000). Information on the vegetation and the environment is also necessary, and we indicate as a guide the *Vade-Mecum pour le Relevé Méthodique de la Végétation et du Milieu*, which was extracted from the *Code pour le Relevé Méthodique de la Végétation*

*et du Milieu* (Godron *et al.* 1969). Every phytosociological survey should give information about the biome and classify the vegetation according to an internationally accepted system, as well as describe topographic characteristics (altitude, slope aspect, position on the local relief, declivity), soil characteristics (micro-relief, soil classification, texture and chemical variables), and climatic characteristics (monthly and yearly averages of the climatic elements, climate type). Martins (2004) highlighted that the description of the environment in field phytosociological works is a base to establish associations between species and environmental variables. Once established, these associations can be added to the ecological knowledge already published, and this can allow constructing hypotheses and expectancies on the presence and abundance of species and the magnitude of alpha-diversity. These are key-information concerning conservation of natural areas and remediation of degraded areas.

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