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Original article

Inclusion criteria influence species richness, diversity and forest structure with implications to deforestation permits in Rio de Janeiro State, Brazil

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ABSTRACT

Deforestation requests to government decision-makers aim for the legal permission to remove secondary forests for economic reasons. In the State of Rio de Janeiro, Resolução Conama nº 06/1994 drives the analysis of successional stages of such forests. It does not define which inclusion criterion (diameter at breast height) should be used in forest inventories. We used Floresta da Cicuta as a case study to evaluate the influence of different inclusion criteria on species richness, diversity and structure parameters having Resolução Conama nº 06/1994 as background. Using Hill numbers, we found that the DBH ≥ 2.5 cm highlighted species richness. Species richness is improperly addressed in Resolução Conama nº 06/1994. The use of DBH ≥ 2.5 cm detected 30 individuals of threatened species, while DBH ≥ 10 cm detected only nine. Basal area was the most accurate parameter to identify the successional stage. Mean DBH and mean height misidentified successional stage. We strongly advise environmental authorities to demand the use of DBH ≥ 2.5 cm in forest inventories for deforestation requests. We strongly encourage the review of Resolução Conama nº 06/1994 by environmental authorities in order to reduce the risk of mistaken classification of successional stage of secondary forests.

Keywords: Floresta da Cicuta, Diameter at breast height, Resolução Conama nº 06/1994, Forest inventory, Successional stage, Hill numbers; Threatened species.

Introduction

Atlantic Forest is one of biodiverse forests in the world, with over 15,000 angiosperm plant species (BFG 2015), being considered a world's biodiversity hotspot (Myers *et al*. 2000). Most of the Atlantic Forest is destroyed and the remaining areas encompass secondary forests in general (Dean 1996; Ribeiro *et al*. 2009). Deforestation requests to government decision-makers (environmental branches of Rio de Janeiro State and municipalities) aim to obtain the legal permission to remove such forests for economic reasons (Fonseca 1985; Ruggiero *et al*. 2021).

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Secondary forests in Rio de Janeiro State have been deforested to give place to roads, crops, cattle ranching, mining, water artificial reservoirs, distribution power lines, residential areas, and a great number of public and private infrastructures particularly in the last century (Fernandez *et al*. 2018). These forests differ in successional stages and levels of disturbance with implications on species diversity and structure parameters (Chazdon 2012).

Floristic/structure forest inventories are paramount studies (Freitas & Magalhães 2012) required by government decision-makers as part of deforestation permit requests. In the State of Rio de Janeiro, a federal legal instrument (Resolução Conama nº 06/1994) drives the analysis of successional stages of secondary ombrophilous and semideciduous forests providing definitions and measurable parameters (Brasil 1994) as commanded by the Federal Law nº 11.428/2006 (a special legal protection to the Brazilian Atlantic Forest).

The most critical aspects of Resolução Conama nº 06/1994 are related to quantitative parameters, especially due to the indefiniteness of inclusion criterion (diameter at breast height – DBH) to be used in forest inventories for deforestation requests (see Siminski & Fantini 2004). Inclusion criteria such as DBH ≥ 2.5 cm, DBH ≥ 5 cm or DBH ≥ 10 cm are among the usual measurements of woody individuals (Moro & Martins 2011; Arellano *et al*. 2016). Depending on the inclusion criterion to be used in a forest survey, different interpretations of the successional stage may rise according to the definitions in Resolução Conama nº 06/1994. This particularity may lead to dubious analyses on the successional stage, which ultimately may end up in bad decision-making of environmental authorities, with implications to diversity and conservation of species.

In fact, the misuse of legal instruments related to environmental permits has been discussed lately showing failures in deforestation control by governments with disastrous implications to Brazilian Amazon (Carvalho *et al*. 2019). The inadequateness of legislation enforcement has been pointed out to pose a threat to biodiversity and conservation of Campo Rupestre (Miola *et al*. 2019). Moreover, a fuzzy modeling was proposed as an alternative approach to identify the successional stage of São Paulo secondary forests (under Resolução Conama n° 01/1994) in order to mitigate the subjectivity and uncertainty of forest surveys in deforestation requests (Mota *et al*. 2019).

Siminski & Fantini (2004) evaluated Resolução Conama n° 04/1994 (Santa Catarina State). They used plots in different successional stages of southern Brazil's Atlantic Forest fragments and measured all plant individuals. They realized that structure parameters like basal area, mean DBH and mean tree height were influenced diversely by different inclusion criteria with implications on identifying the actual successional stages of secondary forests. Species richness and diversity were not included in their study.

Here we used Floresta da Cicuta, a submontane semideciduous forest in Rio de Janeiro state that is legally protected as an "Área de Relevante Interesse Ecológico" (ARIE), as a case study to evaluate the influence of different inclusion criteria on structure parameters, species richness and diversity, with different sampling and statistical design than Siminski & Fantini (2004). Then we aimed to match the best possible use of inclusion criteria to reach the closest identification of the actual successional stages according to quantitative parameters in Resolução Conama nº 06/1994.

Materials and methods

Floresta da Cicuta is located between Barra Mansa and Volta Redonda, municipalities of Rio de Janeiro State (center at -22.549482° S, -44.091719° W, Sirgas 2000 *datum*). It is a federal protected area with 1.25 km^2 (Fig. 1). The previous land use in the surroundings of Floresta da Cicuta was coffee crops, but the core vegetation of the studied fragment remained under low disturbance since 1972 (Sardella & Nazareth 2016).

We used raw data from a structure survey in Floresta da Cicuta (Table S1) that used the plot method (30 randomized plots of 10x10 m; Mueller-Dombois & Ellenberg 1974), DBH ≥ 2.5 cm and by stem measurements in 0.3 ha sampling area. In despite of considering stems of multi-stemmed trees as different individuals, with subsequent overestimation of structure parameters (Moro & Martins 2011), it did not detract the analyzes below given that the overestimated structure results were still fit to evaluate quantitative parameters in Resolução Conama nº 06/1994.

In order to run the main analysis, we computed structure analysis using DBH ≥ 2.5 cm, DBH ≥ 5 cm and DBH ≥ 10 cm with Fitopac (Shepherd 2010) to generate species richness (including morphospecies) and the mean values of DBH and height per plot, and total basal area (m^2/hectare) per plot for each inclusion criterion. These are the most important quantitative parameters to identify the successional stage of a forest according to Resolução Conama nº 06/1994 (Tab. 1).

The presence of threatened species and the number of individuals measured were also recorded for each inclusion criterion. Such critical information was not considered in Resolução Conama nº 06/1994. We updated scientific names of species with Flora e Funga do Brasil (Flora e Funga do Brasil 2023; Tables S1 and S2). Supplementary data (Table S1 and Table S2) available at: https://doi.org/10.6084/m9.figshare.21960824.

Then we used the R package iNEXT (Hsieh *et al*. 2016) to test the hypothesis of significantly statistical differences in species richness and diversity obtained with the same plots, but using different inclusion criteria based on Hill numbers and abundance data. Hill numbers include the three most widely used species diversity measures (q= 0, 1, 2). Species richness (q = 0) counts species equally without

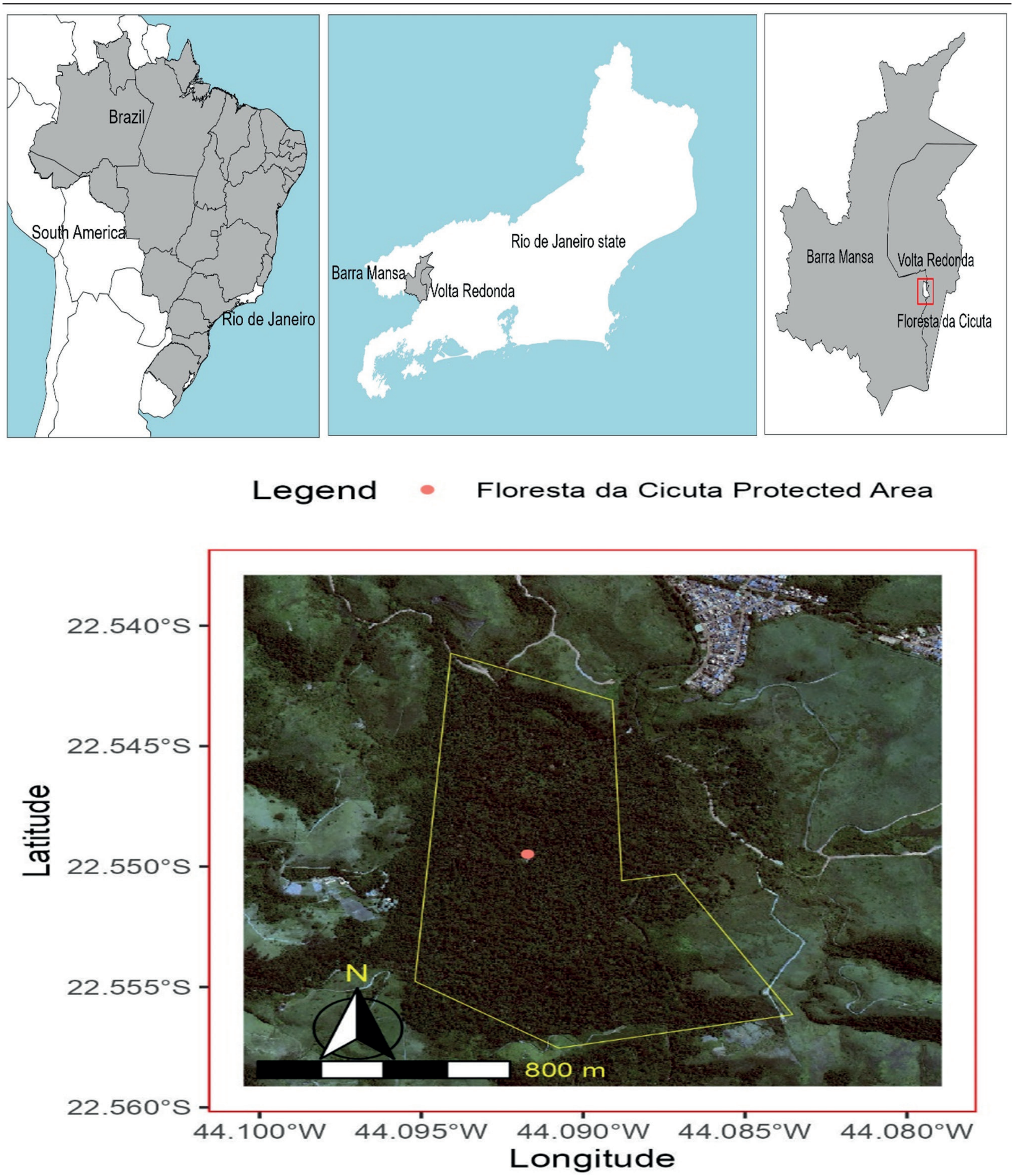

Figure 1. Map of Floresta da Cicuta Protected Area, located between Barra Mansa and Volta Redonda municipalities, Rio de Janeiro state, Brazil. CRS projection: Sirgas 2000/UTM zone 23S.

Table 1. Quantitative parameters of forest structure and species richness of secondary succession stages as in Resolução Conama nº 06/1994. SS stands for successional stages, BA for basal area, DBH for diameter at breast height, S for species richness and NI for non-informed.

SS	BA (m^2/ha)	Mean DBH (cm)	Mean height (m)	S
Early	0 to 10	5	5	≤ 20
Average	10 to 28	10 to 20	5 to 12	NI
Late	> 28	20	> 20	NI

regard to their relative abundances. Shannon diversity (q = 1) counts species in proportion to their abundances and can be interpreted as the effective number of common species in the assemblage. Simpson diversity (q = 2) discounts all but the dominant species and can be interpreted as the effective number of dominant species in the assemblage (Chao *et al*. 2014; Hsieh *et al*. 2016).

Rarefaction (interpolation) and extrapolation (prediction) curves were computed with iNEXT default arguments except endpoint at 1500 individuals (Hsieh *et al*. 2016). When the 95% confidence intervals were not overlapped, groups (inclusion criteria) differed significantly at $p < 0.05$. The unified interpolation and extrapolation procedures allow rigorous statistical comparison of species richness and diversity that can be performed with arbitrary sample size (Colwell *et al*. 2012; Chao *et al*. 2014). We predicted the decrease of species richness (q =0) and diversity (q = 1, 2) with the increase of inclusion criteria (DBH ≥ 2.5 cm to DBH ≥ 10 cm).

We also tested the hypothesis of significantly statistical differences between inclusion criteria using the results of structure parameters: the mean values of DBH and mean tree height per plot, and total basal area (m^2/hectare) per plot. As data were dependent (drawn from the same sampling plots in Floresta da Cicuta) and violated normality and heteroscedasticity assumptions, we runned the Friedman's Test with alpha set at 0.05 using rstatix R package (Kassambara 2021). The Friedman test procedure is an analysis of variance by ranks, i.e., observed rank scores or rank scores obtained by ordering ordinal or numerical outcomes (Friedman 1937). Thus, we used the numerical outcomes of each plot (n= 30) as rank scores for each inclusion criterion according to each of the structure parameters above in a repeated measures design.

We used Kendall's W (Tomczak & Tomczak 2014) to check the effect size of variables (structure parameters) on groups (inclusion criteria), the "Wilcox.test" (signed rank test) function for multiple pairwise comparison between groups with "p.adjust.method function" using Bonferroni for multiple test correction to control the familywise Type-I error rate (Kassambara 2021). We predicted the increase of basal area, the decrease of mean DBH and mean tree height with the decrease of inclusion criteria. We also predicted that decreasing inclusion criteria would increase the detection of threatened species.

All plots were created with ggplot2 (Wickham 2016) and all tests were computed in R environment (R Core Team 2021) with RStudio (RStudio 2021).

Results

The use of DBH ≥ 2.5 cm detected 176 species in total, 68 more species than the DBH ≥ 5 cm, which recorded 108 species, and more than twofold species than the DBH ≥ 10 cm, where 73 species were recorded (Tab. 2; Table S2). The species richness mean per plots (Fig. 2) also decreased from 12.8 ± 5.6 standard deviation (DBH ≥ 2.5 cm), 7.5 ± 2.5 (DBH ≥ 5 cm), to 4.9 ± 1.8 (DBH ≥ 10 cm). With concern to abundance, the use of DBH ≥ 2.5 cm (n= 968) recorded almost twofold the number of individuals of the DBH ≥ 5 cm (n= 488), and more than fourfold the number of individuals measured with DBH ≥ 10 cm (n= 236).

Species richness decreased with the increase of inclusion criterion as expected. The comparison between different inclusion criteria (Fig. 3; Tab. 2) resulted in statistically significant differences (confidence levels interception) for interpolation of species richness (q= 0). On the other hand, the extrapolation showed that DBH ≥ 2.5 cm was statistically different from the other inclusion criteria, which showed to be statistically equal. Species richness put Floresta da Cicuta in average to late successional stage according to Resolução Conama nº 06/1994 regardless of any DBH size (Tab. 1).

Shannon diversity (q= 1) was statistically equal between inclusion criteria both for interpolation and extrapolation. Simpson diversity (q= 2) showed that DBH ≥ 10 cm stood

Table 2. Abundance-based interpolation and extrapolation of species between inclusion criteria using Hill numbers (q^0: Species richness; q^1: Shannon diversity; q^2: Simpson diversity) showing statistically significant differences when existent ([a,b,c]). Spp. stands for species, LCL for low confidence level and UCL for upper confidence level.

Parameters		Interpolation			Extrapolation		
Order (q)	DBH ≥	Spp.	LCL	UCL	Spp.	LCL	UCL
0	2.5 cm	176 [a]	163.75	188.24	207.26 [a]	191.75	222.78
	5 cm	108 [b]	99.01	116.98	147.86 [b]	123.37	172.36
	10 cm	73 [c]	65.50	80.49	95.15 [b]	54.18	136.13
1	2.5 cm	39.88 [a]	35.33	44.43	42.24 [a]	37.37	47.11
	5 cm	30.36 [a]	24.87	35.86	35.28 [a]	28.62	41.94
	10 cm	36.52 [a]	30.74	42.31	45.93 [a]	38.23	53.63
2	2.5 cm	10.28 [a]	8.68	11.88	10.32 [a]	8.71	11.93
	5 cm	10.20 [a]	8.43	11.97	10.33 [a]	8.51	12.15
	10 cm	17.94 [b]	14.24	21.63	19.10 [b]	14.89	23.30

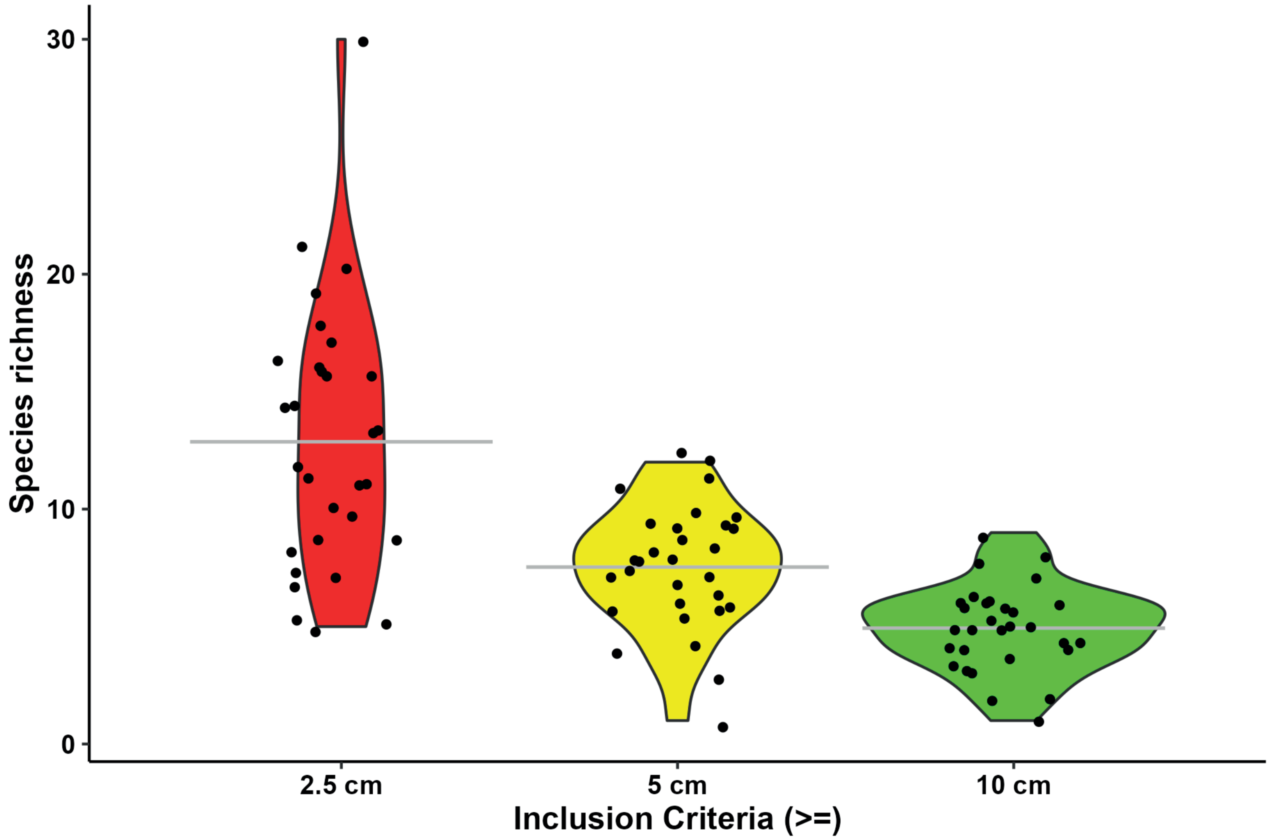

Figure 2. The means (gray cross bars) of species richness per plots for each of inclusion criteria. Dots stand for plots and their respective values of species richness.

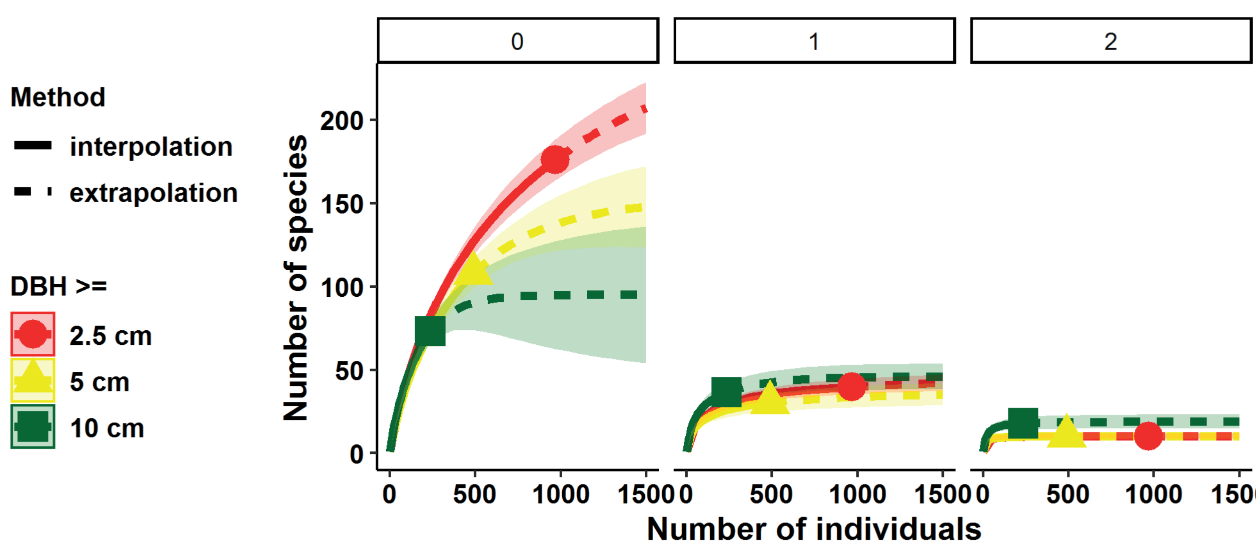

Figure 3. Interpolation and extrapolation of species using Hill numbers (q0: Species richness; q1: Shannon diversity; q2: Simpson diversity) with abundance data between different inclusion criteria (DBH ≥ 2.5 cm, DBH ≥ 5 cm and DBH ≥ 10 cm). Shades in colors stand for confidence levels.

out abundant species with statistically significant differences from the other inclusion criteria both for interpolation and extrapolation (Fig. 3; Tab. 2). The results for species diversity frustrated our predictions.

The use of different inclusion criteria also influenced the detection of threatened species present on the latest national red list (Tab. 3). While the use of DBH ≥ 5 cm detected six of them, DBH ≥ 10 cm detected four out of seven threatened species.

Basal area, mean DBH and mean tree height all presented statistically significant differences with concern to each of inclusion criteria (Fig. 4; Tab. 4). The large effect size (W) detected showed strong relationship between inclusion criteria and each of the variables (Tab. 4). The pairwise comparison (PWC) also resulted in statistically significant differences between groups (Tab. 4). The value of basal area increased and the values of mean DBH and mean tree height decreased with the decrease of inclusion criteria as expected (Fig. 4; Tab. 4).

All basal area values put Floresta da Cicuta in late successional stage regardless of inclusion criteria (Tab. 1; Tab. 4). While mean DBH ≥ 2.5 cm classified Floresta da Cicuta as early successional stage, mean DBH ≥ 5 cm and ≥ 10 cm put it in average and in late successional stages, respectively (Tab. 2; Tab. 4). The different mean height values all put Floresta da Cicuta in average successional stage particularly because of a gap between the thresholds of average and late successional stage in Resolução Conama nº 06/1994 (Tab. 1; Tab. 4).

Table 3. Threatened species detected according to inclusion criteria. DBH stands for diameter at breast height. * Stands for the actual number of individuals.

Species	DBH ≥ 2.5 cm*	DBH ≥ 5 cm	DBH ≥ 10 cm	Citation
Beilschmiedia fluminensis	1	1	0	Brasil 2022
Dimorphandra exaltata	1	1	1	
Eugenia disperma	3	1	1	
Eugenia pulcherrima	2	1	0	
Moldenhawera polysperma	21	11	6	
Urbanodendron bahiense	1	0	0	
Virola bicuhyba	1	1	1	

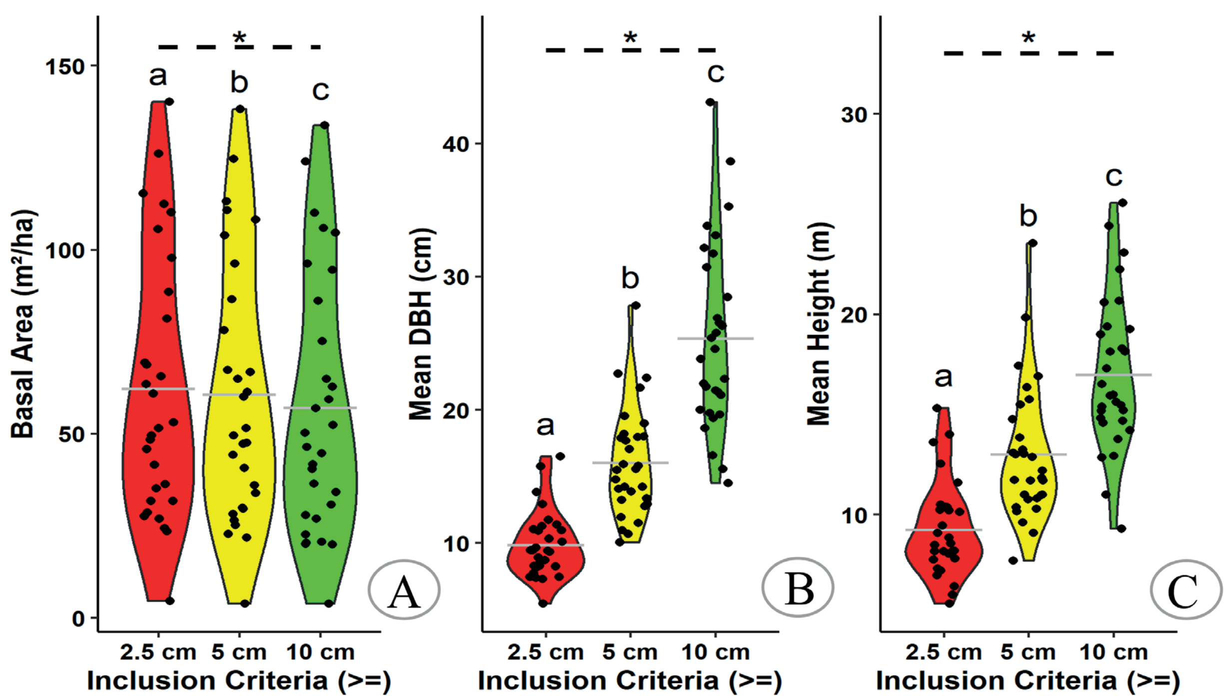

Figure 4. Structure parameters analyzed in concern to inclusion criteria (diameter at breast height) based on 30 plots of 10 x 10 m. A. Basal area; B. Mean DBH; C. Mean height. * stands for statistically significant differences on Friedman's test; a, b, c, stands for statistically significant differences on Wilcox signed rank test for multiple pairwise comparison; Gray crossbars stand for general mean values of basal area, mean DBH and mean height. Dots stand for plots and their respective values of total basal area (m^2/hectare), mean DBH and mean height.

Table 4. Reports of Friedman test between inclusion criteria (groups) and variables using Wilcoxon signed rank test for multiple pairwise comparison between groups. DBH stands for diameter at breast height, SD for standard deviation, X^2 for chi-squared, p for probability, W for effect size, PWC for pairwise comparisons and p.adj for probability adjustment.

DBH ≥	Variable	Mean	SD	X^2 (2)	p	W	PWC	p.adj (PWC)
2.5 cm	Basal Area (m^2/ha)	62.2	35.5	59.5	1.1e-13	0.99	5 cm	3.9e-8
5 cm		60.6	35.2				10 cm	7.6e-7
10 cm		57.1	35.1				2.5 cm	5.4e-6
2.5 cm	Mean DBH (cm)	9.87	2.48	52.5	4.0e-12	0.87	5 cm	4.9e-5
5 cm		16.0	4.05				10 cm	7.6e-7
10 cm		25.3	6.95				2.5 cm	5.4e-6
2.5 cm	Mean Height (m)	9.24	2.35	59.5	1.1e-13	0.99	5 cm	5.5e-9
5 cm		13.0	3.36				10 cm	8.1e-6
10 cm		17.0	3.79				2.5 cm	5.4e-6

Discussion

The results above led to the question: what is the actual successional stage of Floresta da Cicuta? The Resolução Conama nº 06/1994 did not inform the expected species richness for average and late successional stage. Such gap jeopardizes the actual classification of successional stages of secondary forests.

Although these forests may differ widely on species richness (Martin *et al.* 2013; Derroire *et al.* 2016; Rozendaal *et al.* 2019) there is enough scientific information on species richness of secondary ombrophilous and semideciduous forests of Rio de Janeiro available in databases of scholarly literature (e.g. Google Scholar, Scopus etc) that would calibrate this parameter in Resolução Conama nº 06/1994. Thus, ignoring species richness in such an important legal instrument must be considered a throwback in protecting one of the world's hotspots of biodiversity (Myers *et al.* 2000). Such gap of species richness for the average and late successional stages must be properly filled in an expected review of Resolução Conama nº 06/1994.

The use of DBH ≥ 2.5 cm in forest surveys highlights species richness (Gentry & Dodson 1987; Caiafa & Martins 2007; Moro & Martins 2011; Arellano *et al.* 2016; DRYFLOR 2020; Souza *et al.* 2021). Another advantage of using DBH ≥ 2.5 cm is the optimized detection of threatened species if existent in a given forest patch, providing a crucial information in deforestation requests to government decision-makers. Thus, while evaluating the deforestation request on areas with threatened species present in the official national red list (Brasil 2022), authorities must follow strictly the commands in Federal Law nº 11.428/2006 (Atlantic Forest Law) in order to protect those species.

Although sampling effort was not included as a topic in Resolução Conama nº 06/1994, neither was evaluated in the present article, it is an important matter in forest surveys as it increases as the inclusion criterion decreases, which would be a disadvantage of using DBH ≥ 2.5 cm. A way out to such problem would be the use of the well-accepted Gentry's 0.1 ha transect (Gentry & Dodson 1987; Arellano *et al.* 2016) or an adapted version of it in forest surveys for deforestation requests.

Although Shannon diversity and Simpson diversity have no regards to Resolução Conama nº 06/1994, we used them to evaluate the influence of species diversity on inclusion criteria. The first showed that different inclusion criteria highlight frequent species equally, but the latter showed that DBH ≥ 10 cm highlights dominant species much more than the other inclusion criteria, underestimating species richness.

Concerning to the values of basal area in Resolução Conama nº 06/1994, they had been well driven since neotropical secondary forests may present basal area values around 25 m^2/ha after 2-3 decades since last disturbance, which are relatively close to basal area of old growth forests (Guariguata & Ostertag 2001; Chazdon *et al.* 2007). Siminski & Fantini (2004) highlighted basal area as the most accurate structure parameter to identify the successional stage of southern Atlantic Forests in concern to Resolução Conama n° 04/1994 (Santa Catarina State).

The most critical structure parameter in Resolução Conama nº 06/1994 is mean DBH. As DBH ≥ 5 cm (or 4.8 cm) is widely used in the Atlantic Forest (Caiafa & Martins 2007; Moro & Martins 2011) it is likely that its use has caused bias in a great number of forest inventories related to deforestation requests. Assuming that Floresta da Cicuta is in late successional stage (Souza *et al.* 2007; Faria 2017; Alves *et al.* 2021), using DBH ≥ 5 cm would match it in the average successional stage. Siminski & Fantini (2004) also pointed out that the DBH ≥ 5 cm causes bias to the actual classification of successional stage of secondary southern forests, underestimating mean DBH. One way out should be reporting mean DBH with DBH ≥ 10 cm, computed from raw data of forest inventory that used DBH ≥ 2.5 cm for instance. This way Floresta da Cicuta would be properly classified in the late successional stage.

Mean tree height is the most confusing parameter in Resolução Conama nº 06/1994 especially between the average to late successional stage. Again, the present case study agrees with Siminski & Fantini (2004) since mean tree height thresholds in the legal instrument cause bias to the actual successional stage of secondary forests. It is likely that many forest fragments surveyed to deforestation requests had been classified as average succession stage regardless of using any inclusion criteria.

In fact, important references on structure parameters did not detail specifically mean height of the Atlantic Forest trees (Caiafa & Martins 2007; IBGE 2012; Moro & Martins 2011; Souza *et al.* 2021). For instance, the measurement of tree height is not always performed in forest inventories so that modelling above ground biomass depends on tools to reduce tree height-related uncertainties (Réjou-Méchain *et al*. 2017). Besides, land use history has a role in driving successional pathways with implications to secondary forests (Jakovac *et al*. 2021) influencing not only tree height but also other structure parameters. Hence, such gap needs more than a calibration in Resolução Conama nº 06/1994: it needs scientific effort in order to provide mean height values for secondary fragments of the Atlantic Forest.

Based on this case study, we strongly advise that environmental authorities of Rio de Janeiro State and municipalities demand the use of DBH ≥ 2.5 cm in forest surveys for deforestation requests. Considering the current format of Resolução Conama nº 06/1994, the use of DBH ≥ 2.5 cm should report species richness and basal area. Mean DBH and height should be reported with DBH ≥ 10 cm computed from raw data of forest survey sampled with DBH ≥ 2.5 cm.

We invite researchers to replicate our methodology in other subformations of Mata Atlântica within Rio de Janeiro State (e.g. restinga) and in other states where this biome occurs in order to evaluate their respectives Resoluções Conama. Finally, we strongly encourage the review of Resolução Conama nº 06/1994 by environmental authorities to reduce the risk of mistaken classification of successional stage of secondary forests.

Supplementary material

The following online material is available for this article: https://doi.org/10.6084/m9.figshare.21960824

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