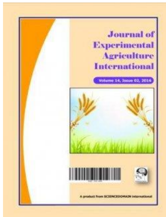

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The Effect of Different Levels of Inclusion for Forest Restoration Assessment

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Authors' contributions

This work was carried out in collaboration between all authors. Author DJOP prepared the study, collected the data and wrote the first draft of the manuscript. Authors ALPF and LCM were the guided research. Author MIOS assisted in discussing the data and writing in the manuscript. Author JNBS performed the statistical analysis. Author AVFP helped in literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this work is to compare two sampling methods (called survey A and B) with different inclusion criteria to evaluate floristic composition, species richness and horizontal structure of a five-year-old forest restoration area.
Methodology: The area with a total of 32.21 ha is located in a Tropical Rainforest region. Survey A included individuals with circumference at breast height (cbh) ≥ 6 cm while in survey B, the minimum cbh was ≥ 15 cm.
Results: Forty-six species have been found in survey A belonging to 40 genera and 21 families. In survey B, only 33 species were found, belonging to 30 genera and 15 families. Regarding species richness, method B did not reach the theoretical asymptote in relation to A, leading to

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underestimation. The number of individuals and absolute density of survey A and B, we found 891 individuals or absolute density of 1,782 ind. ha^{-1} and 166 individuals or absolute density of 332 ind. ha^{-1}, respectively, representing a difference of 81.4%. Also, significant differences have been observed for cbh, height and crown projection, where method A presented higher potential to express the data.

Conclusion: The present study confirmed the hypothesis that the inclusion limit cbh ≥ 15 cm, commonly used for assessing forest restoration processes in recently established areas, provides biased estimates about different ecological processes, such as floristic composition, species richness and forest structure. It is therefore recommended to use a lower cbh for tree measurements in assessments of low-aged tropical rainforest restoration areas.

Keywords: Monitoring; evaluation; recovery; sampling.

1. INTRODUCTION

For decades, the unsustainable use of natural resources, associated with deforestation, has resulted in extensive degraded areas, including forest fragments subject to anthropic pressures [1]. This loss of forest cover leads to habitat fragmentation and consequently results in negative impacts on biodiversity, as well as potential losses of biological processes and ecosystem services [2]. To minimize and reverse this situation while maintaining forests biologically viable, it is essential to promote ecological restoration of forest ecosystems [3].

Generally speaking, forest restoration is a set of practices aiming at gradual reconstruction of a forest ecosystem, recovering its biodiversity and contributing to the reestablishment of the ecological processes responsible for the sustainability and maintenance of the forest [4,5]. Once the restoration of these environments starts, it is necessary to evaluate and monitor the process in order to check if the proposed goals and objectives are being achieved [6]. It is therefore vital to carry out the assessments on a regular time basis so as to avoid the occurrence of unforeseen events that could jeopardize the restoration process and apply corrective measures [7], eliminating errors and higher costs and favoring efficient evolution of the process.

When monitoring natural ecosystems or assessing the ecological aspects of ecosystem restoration, ecological indicators are used [8], which should include the basic ecosystem attributes [9,10]. Information on woody species composition, diversity and structure being important indicators of the level of forest conservation and land use changes, are often used as ecological indicators in monitoring of restoration areas [11,12,13]. However, there is a tendency to use consolidated criteria in phytosociological studies and replicate them in the assessment of recently established restoration areas [14,15,16,17,18]. An example of such a criterium is the use of circumference at breast height (cbh) ≥ 15 cm, without knowing its efficiency to predict ecological processes and real vegetation development (composition and structure).

In this way, the aim of this work was to understand the effect of two sampling methods for the evaluation of forest restoration using (i) floristic composition, (ii) species richness and (iii) forest structure of a five-year-old tropical rainforest restoration area. The hypothesis of this study is that the inclusion limit of cbh ≥ 15 cm, commonly adopted in the evaluation of young forest restoration areas, provides erratic estimations of floristic composition, species richness and forest structure, leading to biased estimates of forest community structuring processes, thus negatively influencing the definition of forest management and maintenance strategies.

2. MATERIALS AND METHODS

2.1 Study Site

The study was carried out in a forest restoration area of 32.21 ha. The site was previously used for sugarcane cultivation, located in the municipality of Cabo de Santo Agostinho, Pernambuco-Brazil (8°17'32.88" S and 35°0'27.84" W) in a landscape dominated by urban areas and nearby urban sites with productive backyards. The original vegetation of the region is tropical rainforest. The climate of the region is Am (tropical climate), according to Köppen-Geiger, with mean annual temperature of 25.1°C and mean annual rainfall of 1,991 mm [19].

The restoration has been carried out using native species in the whole area being 50% pioneer and early secondary species and 50% of late secondary and unclassified species. Plantation has been carried out in January 2012 suing 3 x 2 m spacing. It should be noted that all access roads were fenced, and the area was surrounded by fire-breaks. Adequate fertilization was provided as well as regular cleaning and ant control until January 2017. Irrigation was carried out whenever necessary.

2.2 Data Collection and Analysis

Two sampling methods (survey A and B) have been applied considering different inclusion criteria. In the method A, all individuals with circumference at breast height (cbh) ≥ 6 cm have been measured, while in method B, only individuals with cbh ≥ 15 cm. The use of the first method sought to contemplate neglected species and individuals in monitoring of restoration areas that commonly use cbh ≥ 15 cm. Data collection was carried out in January 2017 in 10 randomly distributed 500 m² (10 x 50 m) plots, where all tree and shrub individuals were measured. Species were identified by experts and using specialized literature [20,21,22,23]. The classification system adopted was according to [24], and confirmation of authors and scientific names was carried out based on the [25].

After identification, species were classified according to their ecological group (pioneers, early secondary, late secondary and without characterization), according to [26]. Additionally, they were classified following their dispersal syndrome of diaspores in zoocoric or abiotic species (anemocoric, baroque and autochiroic) according to [27].

The following phytosociological parameters were calculated using Mata Nativa 4 software ®: absolute and relative density (AD and RD), absolute and relative frequency (AF and RF), absolute and relative dominance (ADo and RDo) and importance value (IV). Crown projection was calculated using the equation $C = \sum_{i}^{n} Ci/A \cdot 100$, where C = percentage of coverage (%); Ci = crown area of tree i (π (Di) ² / 4); Di = mean crown diameter (m) of tree i; n = number of trees measured in area A; and A = plot area (m²) [28,29].

The structural parameters in the different sampling methods were estimated using circumference at breast height (cbh), that together with crown area (C) can explain better the heterogeneity of trunks and crown area of the trees, respectively. Total height (H) allows to describe the heterogeneity of the different forest layers while being associated also with the competitive capacity of the plants [30,31].

The differences between the structural parameters (cbh, H and C) for each survey (A and B) have been estimated using Wilcoxon Rank test (α = 0.05). The "paired = TRUE" argument was used in the "Wilcox.test" function [32], considered appropriate for dependent samples. The null hypothesis was that distributions of the two samples differ by a location offset in *mu*, and the alternative is that they differ from some other location offset.

For species composition analysis and in order to detect possible divergence of species and number of individuals between the sampling methods, the exploratory analysis was carried out using non-metric multidimensional scaling (NMDS) for the distance of Bray- Curtis. The significance of the groups was estimated by means of the permutational multivariate analysis of variance (PERMANOVA) with 999 permutations (α = 0.01). Bray-Curtis distances and dissimilarity matrix were calculated by the "vegdist" and "metaMDS" functions, respectively, from the "vegan" package [33]. The "metaMDS" function runs the NMDS and attempts to find a stable solution using multiple randomizations. In addition, it standardizes the scaling in the result so that configurations are easier to interpret and adds species scores to the site ordering [33].

The actual species number was calculated by the exponential of the Shannon diversity (order: q = 1) [34]. This procedure was performed with the aid of the functions of the "iNEXT" package of the R environment (iNterpolation/EXTrapolation), which provides functions for plotting species diversity curves by interpolation and extrapolation [35]. All statistical data analysis and graph construction have been performed in R environment version 3.4.0 [32].

3. RESULTS AND DISCUSSION

The survey using method A found 46 species (of which four exotic) belonging to 40 genera and 21 families. Using method B, 33 species (of which two exotic) were found, belonging to 30 genera and 15 families. In both inventories, it was not possible to identify one of the individuals and another three were identified only at the genus level (Table 1).

Differences in floristic composition between both sampling methods were sufficient to group the pairs of plots of each level in a cohesive way by NMDS ranking analysis (Fig. 1). PERMANOVA supported these findings as both axes showed consistent differences between the means of the NMDS scores for each sample method with 999 permutations (Axis 1: F (1, 18) = 1.26; P =.36; Axis 2: F (1, 18) = 0.80; P=.360).

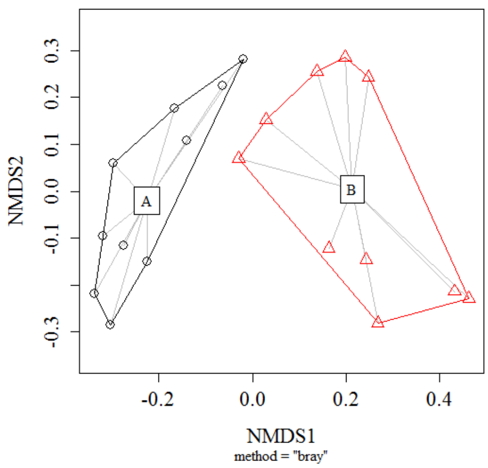

Fig. 1. NMDS ranking of tree species using different sampling methods

Circles and triangles show the distance estimates ("method" = Bray-Curtis) between the number of individuals of each species in both sampling methods (A and B)

The number of species (qD) found in survey A was 46 and in survey B 33. The integrated sampling curve allows reliable comparisons, starting from the size of a sample observed up to twice as many individuals (Fig. 2). Considering the sample intensity curve, it is noted that the real number of species in survey B is significantly lower (α=.05). So, for sample B to reach the asymptote, it would be necessary to increase the number of samples to about 27.80 plots, corresponding to a minimum increase of about 279 individuals in the sample.

Comparing surveys A and B, the richness indicates that 28.26% of the species have not yet reached tree size, since these were not included in the sample B. Therefore, the recommendation based on the cbh ≥ 6 cm, optimizes the data collection, allowing more reliable estimates to evaluate species richness in the forest restoration area, minimizing loss of time and resources.

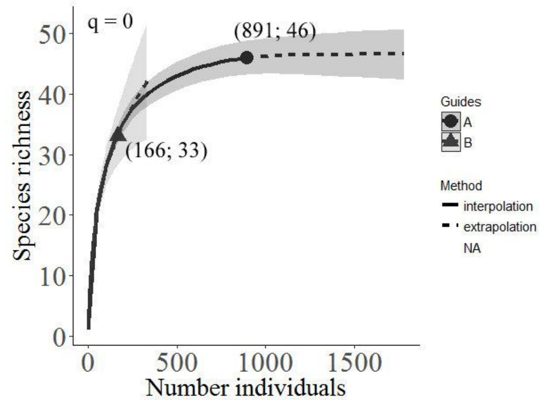

Fig. 2. Sampling curves by interpolation (-) and extrapolation (-) with 95% confidence intervals (shaded areas), obtained by the *bootstrap* method based on 50 randomizations

The data of the two sampling methods (A and B) are shown separately for ranking q = 0 (species richness). The solid point and triangle represent the limits of the reference samples. The numbers in parentheses are the number of individuals and the number of species (qd = Hill's numbers)

In recently established restoration areas, low richness is observed, according to [19,20], who found thirty-eight and forty-five species, respectively, disregarding inclusion criteria in the sampling. Using the most usual criterion (i.e., cbh ≥ 15), [22] found ninety-two species in a 50-year-old tropical forest, and [36] studying a forest fragment in Catende-PE, 100 km from our study area, found ninety-one species.

Comparing different aged restoration areas, belonging to the same phytophysiognomy, an increase of species richness over time is expected, as observed by [18], who analyzed a restoration project with initial plantation of forty-one species and presenting ninety-five native species after 40 years. However, this could be ignored in the surveys due to the selected inclusion criterion, as observed when comparing surveys A and B. So, it is expected that older areas present more species than younger ones due to the establishment of seedlings and progress of ecological succession, even if the number of planted species was different.

In the same line of reasoning, this also occurs when observing exotic species in an area. In the present study, four exotic species were found in survey A while only two were found in survey B. Among them, *Myracrodruon urundeuva* presented 33 individuals in A and only four in B (an increase of 82.5%). This would be considered an aggravating factor if the species were invasive. Regarding species richness, although survey B presented unreliable estimates, this could be overcome by increasing the number of plots. However, this again would increase inventory costs and time. According [37] it is important to develop techniques that minimize forest restoration costs. Similarly, [38] point out that simplifying procedures and reducing costs is a priority incentive measure.

In relation to the dispersion syndrome of the species identified in survey A, twenty-two presented abiotic dispersion (anemocoric, barocoric and autochromatic), twenty zoocoric dispersion and four without classification as they had not been identified. Regarding the ecological group, fourteen were considered pioneer species, sixteen early secondary species, ten late secondary and five uncharacterized (Table 1).

While in survey B, thirteen species presented an abiotic dispersion syndrome, sixteen zoocoric and four were also unidentified. In relation to the ecological group, ten were considered pioneer species, another eleven early secondary, six late secondary and five uncharacterized (Table 1).

Table 1. List of tree species and number of individuals found in each survey

Species	N (A)	N (B)	EG	DS
Anacardiaceae				
Anacardium occidentale L.	21	5	Pi	Zoo
Myracrodruon urundeuva Allemão*	33	4	Ls	Abio
Schinopsis brasiliensis Engl.	5		Es	Abio
Schinus terebinthifolia Raddi	43	1	Pi	Zoo
Spondias mombin L.	10	5	Pi	Zoo
Tapirira guianensis Aubl.	75	25	Pi	Zoo
Annonaceae				
Xylopia frutescens Aubl.	26	14	Pi	Zoo
Apocynaceae				
Aspidosperma spruceanum Benth. ex Müll.Arg.	1		Ls	Abio
Araliaceae				
Schefflera sp.	2	1	Un	-
Bignoniaceae				
Handroanthus chrysotrichus (Mart. ex DC.) Mattos	1	1	Es	Abio
Handroanthus ochraceus (Cham.) Mattos	12	3	Es	Abio
Handroanthus sp.	68	17	Un	-
Jacaranda brasiliana (Lam.) Pers.	55	6	Ls	Abio
Tabebuia aurea (Silva Manso) Benth. & Hook.f. ex S.Moore	11		Es	Abio
Bixaceae				
Bixa orellana L.	2		Pi	Abio
Boraginaceae				
Cordia superba Cham.	2	1	Es	Abio
Calophyllaceae				
Calophyllum brasiliense Cambess.	4		Ls	Zoo
Chrysobalanaceae				
Licania tomentosa (Benth.) Fritsch	63	6	Es	Zoo
Fabaceae				
Bowdichia virgilioides Kunth	9	4	Ls	Abio
Chamaecrista ensiformis (Vell.) H.S.Irwin & Barneby	12	2	Es	Abio
Dipteryx alata Vogel*	1		Un	Zoo
Inga edulis Mart.	126	12	Pi	Zoo
Inga laurina (Sw.) Willd.	103	13	Es	Zoo
Inga sp.	14	1	Un	-
Libidibia ferrea (Mart. ex Tul.) L.P.Queiroz	2		Es	Abio
Lonchocarpus sericeus (Poir.) Kunth ex DC.	23	3	Un	Abio

Species	N (A)	N (B)	EG	DS
Parkia pendula (Willd.) Benth. Ex Walp.	6	3	Ls	Abio
Paubrasilia echinata (Lam.) E. Gagnon, H.C.Lima & G.P.Lewis	3		Ls	Abio
Pterogyne nitens Tul.	12	4	Es	Abio
Senna siamea (Lam.) H.S.Irwin & Barneby	2		Pi	Abio
Lecythidaceae				
Escheweilera ovata (Cambess.) Mart. Ex Miers	6	2	Ls	Zoo
Gustavia augusta L.	2		Es	Abio
Malpighiaceae				
Byrsonima sericea DC.	13	1	Es	Zoo
Malvaceae				
Apeiba tibourbou Aubl.	2	1	Pi	Zoo
Guazuma ulmifolia Lam.	32	10	Pi	Zoo
Hibiscus pernambucensis Arruda	6	1	Pi	Abio
Pachira aquatica Aubl.*	12	4	Un	Abio
Myrtaceae				
Eugenia florida DC.	6	1	Ls	Zoo
Psidium cattleianum Sabine	12		Es	Zoo
Polygonaceae				
Triplaris americana L.	15	4	Es	Abio
Rubiaceae				
Genipa americana L.	7		Ls	Zoo
Salicaceae				
Salix humboldtiana Willd.*	2		Pi	Abio
Sapotaceae				
Pouteria peduncularis (Mart. & Eichler ex Miq.)	8	1	Es	Zoo
Simaroubaceae				
Simarouba amara Aubl.	9	3	Es	Zoo
Urticaceae				
Cecropia pachystachya Trécul	7	6	Pi	Zoo
Unknown				
Unknown	5	1	-	-

**Exotic species. N (A) – number of individuals in the sampling A; N (B) - number of individuals in the sampling B. EG - ecological group; Pi - pioneer; Es - early secondary; Ls - late secondary; Un – unclassified. DS - dispersal syndrome; Abio – abiotic; Zoo - zoocoric)*

The dispersion syndrome, in survey A, a higher number of abiotic than zoocorical species was found, which differed from observed in survey B. Species with zoocoric dispersion syndrome predominate in the region's phytophysiognomy, [39,18,20]. This allows to infer two important aspects: 1) it is necessary to take measures that encourage the entry of zoocorical species in the restoration area; 2) a more restricted sampling method leads to deficient interpretation of the restoration process in recently restored areas.

In both surveys, early succession species (pioneer and early secondary) were dominant, as expected as restoration occurred only five years ago and where conditions favor the establishment of this kind of species. In other studies using the inclusion criterion of cbh ≥ 15, the same pattern was found [18,19,20].

Regarding the number of individuals and absolute density of survey A and B, we found 891 individuals or absolute density of 1,782 ind. ha^{-1} and 166 individuals or absolute density of 332 ind. ha^{-1}, respectively, representing a difference of 81.4%.

The tree density in thirteen-year-old planted tropical forest areas, [21] found absolute density of 1,452 ind. ha^{-1}, [36,22] recorded 1,049 ind. ha^{-1} and 1,244 ind. ha^{-1}, respectively, results quite higher than 332 ind. ha^{-1} in survey B. Considering these studies, the density in our study area could be considered low. However, considering that there are still developing seedlings, as shown in survey A, the density could be considered satisfactory (1,782 ind.ha^{-1}).

The basal area found in survey A and B was 1.98 and 1.55 m^2 ha^{-1}, respectively. This represents that a 25% difference was observed, demonstrating once again the efficiency of a less restricted inclusion criterion for monitoring restoration areas.

According to Table 2, the average treetop projection in the plots was 73.3% in survey A, varying from 21.2% to 183.1%. This may indicate the occurrence of phenomena such as stratification, clearings or species concentration, associated or isolated. With an average of 58.6%, the values found in survey B follow the same trend. Thus, analyzing crown projection considering only method B, the average would be underestimated in approximately 15%.

Table 2. Crown projection and number of individuals per plot in each survey

Plot	Survey A		Survey B	
	C %	N	C %	N
1	50.18	99	35.7	19
2	27.45	59	11.78	4
3	68.06	85	42.84	15
4	24.44	58	7.03	4
5	128.63	126	111.81	31
6	21.18	49	6.13	3
7	67.01	132	54.62	16
8	71.5	103	64.26	22
9	183.1	51	172.68	19
10	91.38	129	78.64	33
Mean	**73.29**		**58.55**	
Total		**891**		**166**

C - Crown projection in percentage. N - Number of individuals

Using the same methodology for crown projection, [29] found 109.3% and 35.7% in two seven-year-old restoration areas and they might have found higher values if a more restricted inclusion criterion was used. This accuracy is important when the purpose is to determine management and maintenance activities.

It must be emphasized that the methodology used for crown projection considers individual trees, without accounting for overlapping crowns. This leads to a certain overestimation of the real crown projection per plot. Nevertheless, for the purposes of this study, it was considered adequate and efficient as it allowed to compare the different inclusion criteria.

Regarding the number of individuals, it should be considered that initially (2012) 1,667 seedlings have been planted per hectare. So, in several plots (1, 3, 5, 7, 8 and 10) with more than 84 individuals, natural regeneration is happening, and this ecological process could only be revealed in sampling method A.

Sampling using the limit of cbh ≥ 15 cm leads to different results of structural parameters of the forest community in restoration process. The hypothesis on the mean displacements by the Wilcoxon Rank test at 95% probability was confirmed for all structural parameters, depending on the sampling method (Fig. 3). For example, survey B captured only the highest values for cbh (x = 14.1 and 22.5 cm, survey A and B, respectively) and H (x = 3.5 and 4.8 cm, respectively), corresponding only to plants with higher above-ground tree biomass and ignoring growing seedlings. Likewise, significant differences were also found for crown projection, where results show that 14.7% of it in survey A come from individuals with cbh <15 cm (x = 73.3 and 58.6%, respectively).

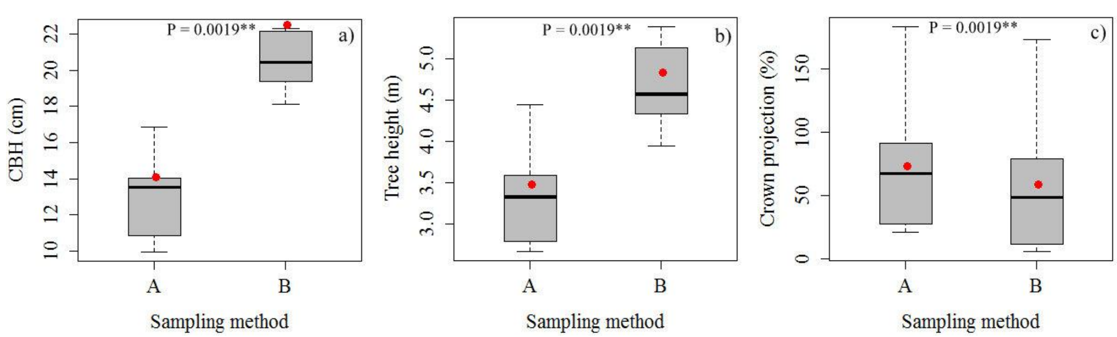

Fig. 3. Box plots on the differences in the change of the mean of structural components: cbh (a), H (b) and C% (c)

The boxes show the interquartile ranges with the median (center line) and mean (red dot); the lower and upper limits indicate the minimum and maximum values. Significant differences between the means of the different sampling methods by the Wilcoxon Rank test (α=.05) are indicated by asterisks (**)

4. CONCLUSION

The present study confirmed the hypothesis that the inclusion limit cbh ≥ 15 cm, commonly used for assessing forest restoration processes in recently established areas, provides biased estimates about different ecological processes, such as floristic composition, species richness and forest structure.

For monitoring of restoration areas with growing seedlings in tropical rainforest, it is recommended to use cbh ≥ 6 cm as criterion for inclusion of tree individuals. It is plausible that lower cbh limits can be more inclusive and more accurate in predicting structural ecological processes in young plantations, as well as reducing forest inventory costs and time.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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