Original Research Article

Biodiversity in Forest Fragments Under Different Forms of Environmental Conservation, Brazil

ABSTRACT

Start Abstract with problem statement sentence – what led to this investigation for this area?
The objective of this work was to evaluate the biodiversity of forest fragments at different levels of anthropization in the municipality of Jaboticabal, State of São Paulo, Brazil. The related research was carried out in three forest fragments, an area under reforested conditions, a natural remnant area and an area under natural regeneration. Each ~~area~~ fragment was divided into three sampling units of 240 m^2, constituting the replicates. The total frequency of insects, amphibians, birds, mammals, reptiles and trees was evaluated. ~~By the c~~Cluster analysis indicated that~~,~~ there were~~as dissimilarity~~ differences between the three fragment types~~natural regeneration area and the reforestation and natural remnant areas~~. The analyzed variables were summarized in two main components, which explain 87.1% of the accumulated total variance. It was concluded that ~~there are differences regarding biodiversity in the forest fragments, highlighting~~ the areas of natural remnants and reforestation~~, which~~ presented higher frequencies~~y~~ of ~~the raised~~ species and, consequently, greater biological diversity d~~etriment~~ than~~e~~ the natural regeneration area.

Keywords: Environment; ecology; fauna; flora; frequency of species.

1. INTRODUCTION

~~In the current situation the environment makes necessary the sum of efforts to better understand the dynamic interplay between man and environment. The growing environmental awareness and the expansion of knowledge between the different knowledge areas have mobilized the scientific community and the population in favor of getting to know these interaction man/environment [1] and design strategies for the sustainable exploitation of the environment by man.~~ (This paragraph does not provide a high level of quality and important information that is vital to understand the rest of the paper)

~~Among other things, the h~~Human exploratory actions have promoted significant changes in the dynamic equilibrium of ecosystems~~. In fact, the interference of man can cause disorders to environmental factors [2]~~, notably because of the disturbances in the natural habitat of several species, such as insect eating birds [3, 4, 5], insect bioindicators [6], and~~in addition~~ to plant species [7, 8, 9]. This has led to fragmented areas that are in various states of regeneration.

~~To quantify the quality of habitat for wildlife is a task that is extremely challenging, this being essential to the development of quantitative techniques with robustness sufficient to express the real ability of the natural shelters [10].~~ (This paragraph does not provide a high level of quality and important information that is vital to understand the rest of the paper)

~~On the basis of the above, it is emphasized that the stratification of areas to study their quality, is a preponderant step to understand the peculiarities of each environment [11].~~ The focus of this investigation is NOT on the applied methodology – rather the outcome/results of the applied methodology ~~Thus,~~ Summers et al. [12] point out that areas in reforestation can be divided into three main categories: assisted natural regeneration, direct sowing, and planting of seedlings; these areas being, according to Cunningham et al. [13], essential for the maintenance of biodiversity. Already Botello et al. [14], dealing with areas of natural remnants, reported~~s~~ the contribution of these environments to the richness of fauna and flora. Fiorentin et al. [9] added that ~~areas~~ processes in natural regeneration areas are highly complex and dynamic due ~~derive from~~to the interaction of various processes that~~,~~ converge to drive ecological succession. ~~ing in an extremely important phenomenon, highly complex and dynamic~~.

It would have been much more helpful if the Introduction could have focused on the three types of areas: (1) reforested conditions, (2) natural remnant area and (3) natural regeneration area and showed how these transitional areas interact, relate and impact on their BIODIVERSITY. What are the various processes that drive them, what are the timelines and changes that we can expect in the short, medium and long term in these areas. How does conservation relate to them, what ecosystem services do they provide. Etc etc. The focus of the Introduction should be on these 3 types of area, *not methodology* - readers need to know the DYNAMICS of these three types of areas = This mean 3 separate paragraphs, one for each type of area.

~~Owing to the importance of the ecosystems mentioned above, [10] point out that, their quantitative analysis consists of an important strategy for the generation of local environmental quality indicators in order to subsidize the decision making on areas to be destined for legal reserve. However, it is known that the evaluation of forest fragments is usually based on a single taxonomic group, evidencing the need for rapid assessments based on multitax on indicators [15] that can be excellent tools to help conservationists and managers in the definition of environmental conservation strategies [14].~~ The focus of this investigation is NOT on the applied methodology – rather the outcome/results of the applied methodology

Indicate the level of anthropogenic influence of the three areas under study. Thus provide the ultimate problem statement (e.g. various levels of human impact in the three areas necessitate different management regimes to conserve the native biodiversity, yet promote sustainable utilization of ecosystem services) that led to the initiation of this investigation in the study area. The objective of this work was to analyze the biodiversity of three forest fragments under different forms of environmental conservation. Indicate the value of this investigation for the area under study

2. MATERIAL AND METHODS

2.1 Research Coverage Area

The research was carried out in April 2015 in three forest areas in the municipality of Jaboticabal, State of São Paulo, Brazil. The areas covered by the study, consisting of 720 m^2 each (1) how is it possible that the 3 areas are EXACTLY the same size? (2) 720 **m^2** = a area of approximately 20x36 m?? – are you sure this is 720 m^2 and not 720 km^2 – recheck the size of the various areas under investigation), were characterized according to the level of anthropization, namely: Area under reforestation conditions, denominated fragment 1 (FRA-1), located at 21°14'54.7"S and 48°17'48.5”W; area of natural remnant, fragment 2

(FRA-2), located at 21°14'47.1"S and 48°17'29.4"W; and an area under natural regeneration, fragment 3 (FRA-3), located at 21°15'02.5"S and 48°17'42.3"W. During the month (April) of conduction of the research, climate variables were monitored: relative air humidity (RH%), accumulated rainfall (R mm), mean atmospheric temperature (AT °C), and mean solar radiation (SR MJ m^2), as Illustrated in Figure 1A and B. – why do we ABSOLUTELY NEED to know these environmental conditions if no result OR discussion refer back to it – thus giving context – seems rather meaningless? Do we lose any vital connection or understanding of the results if we remove it?

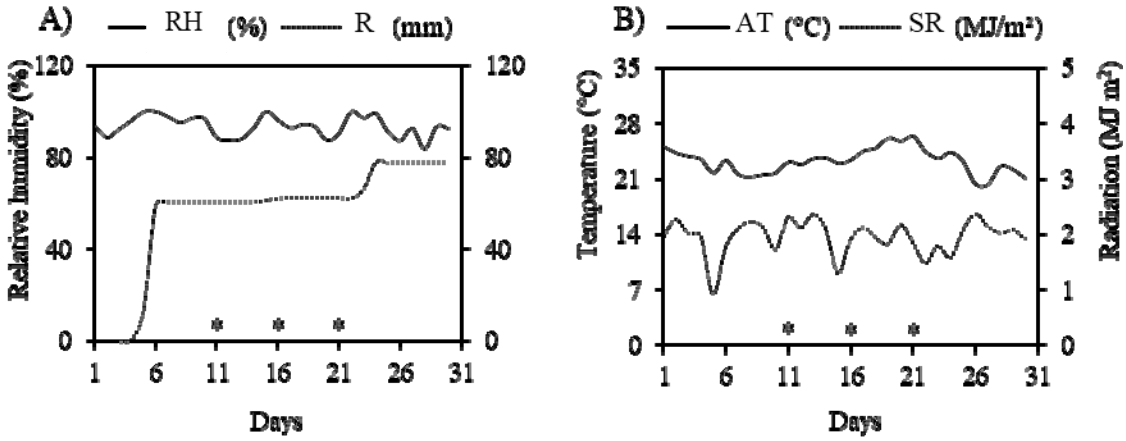

Figure 1. Climate variables. (A) relative humidity (RH) and rainfall (R), (B) atmospheric temperature (AT) and solar radiation (SR), * days the evaluations were performed. Jaboticabal, SP, 2015 – see journal guidelines as to how this should be cited.

2.2 Experimental Design

The research was conducted in a completely randomized design (DIC), and the treatments represented by three forest fragments [16 – WHY CITE THIS REFERENCE? DOES IT SPECIFICALLY REFER TO THE THREE FRAGMENT UNDER STUDY?] with three replicates. To define the sample unit, the fragments were divided into three parts of ≈240 m^2 (1) were these "parts/units" transects or quadrats? (2) What were lengths? (3) HOW WERE THEY PLACED (RANDOM, AT THE CORNERS?), where each one represented a repetition (you cannot say this!! You have not conclusively established if the area in the various fragments were homogenous – most probably they were not if they were variously impacted by humans or relief or soil or different ecozones or vegetation communities. In each fragment, three visits (indicate the 3 dates of April when visits were made) were carried out at different times, ~~the first being at~~ 08:00, ~~the second at~~ 12:00, and ~~the third at~~ 16:00 hours~~, in a randomized way between the sample units~~. Indicate how long was the observational time (1 hour, 2 hours?).

2.3 Survey of Data

In the analyzed fragments, variables inherent in the frequency of occurrence of fauna and flora were included in the taxonomic groups: insects (F-INS), amphibians (F-AMP), birds (F-BIR), mammals (F-MAM), reptiles (F-REP), trees (F-TRE), and these are condensed into the variable total species frequency (F-TES). The data obtained from these variables consisted of *in loco* observation. Therefore, these were considered as "clues", in order to facilitate the visualization of copies of the groups; indicators of their existence, such as the diversity of leaves, flowers and fruits that can serve as food and water; trees and soil for shelter; besides

aptitude for hunting and coexistence of populations. The research was classified as exploratory [17], of the qualitative type [18].

The collection of vegetation information was carried out based on ~~specialized literature [19]~~ This does not indicate the applied methodology on the vegetation in general - correct, and two (dominant?) tree species (1) indicate how these 2 tree species were sampled (2) motivate why other species were specifically excluded from the sampling design – especially if they have an impact on the observed fauna) are commonly found in the transition areas of the Atlantic Forest and Cerrado, mainly because they represent the vegetation of the State of São Paulo. The species chosen were araticum-de-terra-fria (indicate vernacular language) (*Annona_emarginata* (Schltdl.) H. Rainer) and dairy (*Tabernaemontana_catharinensis* A. DC.). This categorization was performed ~~aiming at~~for greater precision in the visualization and obtaining of the data [20].

2.4 Statistical Analysis

The original data of the dependent variables were transformed into a sine arc of √ (x/100), to normalize the distribution of the deviations [21], data was then subjected to analysis of variance using the 5% probability F-~~-~~test. For the significant variables, the Tukey test was applied for multiple comparisons of averages [22], in order to detect differences between the fragments. Subsequently, the original data were standardized and subjected to multivariate exploratory analysis, using cluster analysis (Ward's method) and Principal Component~~s~~ Analysis (PCA). ~~The results of the multivariate analysis were expressed through tables, dendrogram and biplot[23].~~ We never indicate how we presented the data – it can be seen in the next section

3. RESULTS AND DISCUSSION

The paper is a mixed format of short communication **(SEE SHORTENED INTRODUCTION AND THE COMBINATION OF RESULTS AND DISCUSSION)** AND FULL RESEARCH PAPER **(SEE NUMBER OF TABLES AND FIGURES PRESENTED)** – DECIDE IF YOU ARE PRESENTING A short communication OR full research paper AND ADHERE TO THAT FORMAT IN PRESENTION ONLY.

It was verified that there were significant differences (*P*<.01) between the analyzed fragments for the variables, total frequency of species (F-SPE), F-INS, F-BIR, F-MAM, and (*P*= .05), F-AMP, while for frequencies of occurrence of F-REP and F-TRE no significant differences were found (*P*= .05) as summarized in Table 1. WHERE IS THE DISCUSSION OF THESE RESULTS??

Guideline for Reporting P values: - See Journal Guidelines

P is always italicized and capitalized.

i) Correct expression: (P = .05). Wrong Expression: (P < .05), unless P < .001.

ii) The P value should be expressed to 2 digits whether or not it is significant. If P < .01, it should be expressed to 3 digits.

iii) When rounding, 3 digits is acceptable if rounding would change the significance of a value (eg, P = .049 rounded to .05).

iv) Expressing P to more than 3 significant digits does not add useful information since precise P values with extreme results are sensitive to biases or departures from the statistical model.

v) Reporting actual P values avoids this problem of interpretation. P values should not be listed as not significant (NS) since, for meta-analysis, the actual values are important and not providing exact P values is a form of incomplete reporting.

vi) Do not use 0 before the decimal point for statistical values P, alpha, and beta because they cannot equal 1

Table 1. Summary of variance analyses for the total frequency of species (F-TSP), insects (F-INS), amphibians (F-AMF), birds (F-BIR), mammals (F-MAM), reptiles (F-REP) and trees (F-TRE). Jaboticabal, SP, 2015.

F.V.	GL	Medium Squares						
		F-TSP	F-INS	F-AMF	F-BIR	F-MAM	F-REP	F-TRE
Fragments	2	144.81**	99.74**	33.88*	57.16**	43.03**	3.66^{ns}	2.54^{ns}
Residue	6	8.80	9.10	6.45	5.16	1.99	7.32	0.63
CV (%)		17.19	32.25	68.30	26.68	32.86	212.13	10.49

**, * and ^{ns} - significant at 1 and 5% probability of error and not significant by the Fischer test, F.V. - sources of variation, GL - degrees of freedom and CV - coefficient of variation.

By analyzing the total frequency of verified species, it was possible to verify that fragment two (FRA-2), expressed superiority of 26.7 e 80.0% in relation to the fragments (FRA-1) it's three (FRA-3), though FRA-2 and FRA-3 have not differed statistically from one another, with averages of 11 and 15. The FRA-1 was superior in 72.7% when compared with FRA-3, with an average frequency of 3 (Figure 2A). The same behavior was observed when the F-INS was analyzed, having recorded averages of 4.3, 5.3 and 0.6 for FRA-1, FRA-2 and FRA-3, respectively, with percent differences of 86.0 and 88.7% when comparing FRA-1 and FRA-2 with FRA-3 (Figure 2B). WHERE IS THE DISCUSSION OF THESE RESULTS?? All results and discussion of Figures 2a, b should be placed ABOVE the figure.

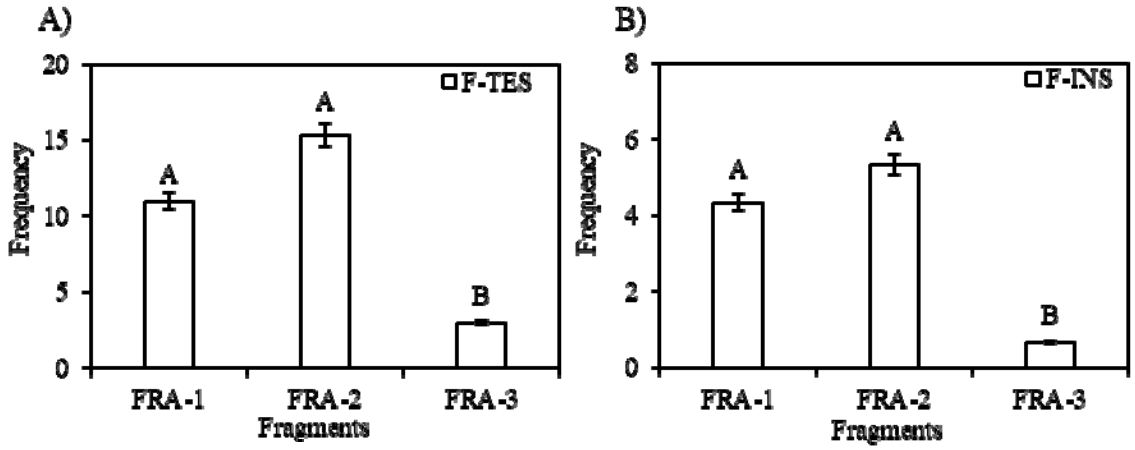

Figure 2. Total species frequency (A), with minimum significant difference (MSD) of 7.43 and frequency of insects (B), with MSD of 7.53, verified in three forest fragments. Mean data of three untransformed sine-arc replicates of the √ (x/100). Jaboticabal, SP, 2015.

What specific result are you discussing here from the many presented in paragraph above figure 2?

Forest fragments, given the nature of their classification, express structural differences perceptible to the animals, in order to interfere with their behavior [24]. These authors attribute these behavioral changes, above all, to changes in the natural habitat of the species, while [25] contribute to changes in predation patterns. In a natural remnant fragment, the biological richness is undoubtedly superior to that of anthropized areas [14], reducing the incidence of solar radiation and temperature, increasing the relative air humidity and, thus, favoring the development of several species of fauna and flora [26].Even in man-altered areas, some species can adapt and take advantage of this situation [27], justifying the frequency of species verified in natural regeneration areas in this work. Among the various species indicative of the quality of ecosystems, insects are often mentioned. For example, beetles [28, 29], caterpillars [30], bee species [31], and ants [32, 33, 6], are often quantified to express the level of environmental disorder based on how often they occur in environments.

Regarding the F-AMP, it was verified that FRA-2 had a higher mean (1.3), although it did not differ significantly from FRA-1 that had an average of 1, whereas FRA-3 expressed average 0, differing from FRA-2 being calculated a percentage difference of 100% between these two fragments (Figure 3A). For the variable F-BIR, this was also found to be superior to FRA-2, with an average 4.6, although this did not differ from FRA-1, with an average 2.6, with FRA-2 higher in 86.9% to FRA-3, where the mean was 0.6 (Figure 3B). DISCUSS THESE SPECIFIC RESULTS – WHAT DO THEY MEAN FOR THE AREAS AND CONSERVATION!

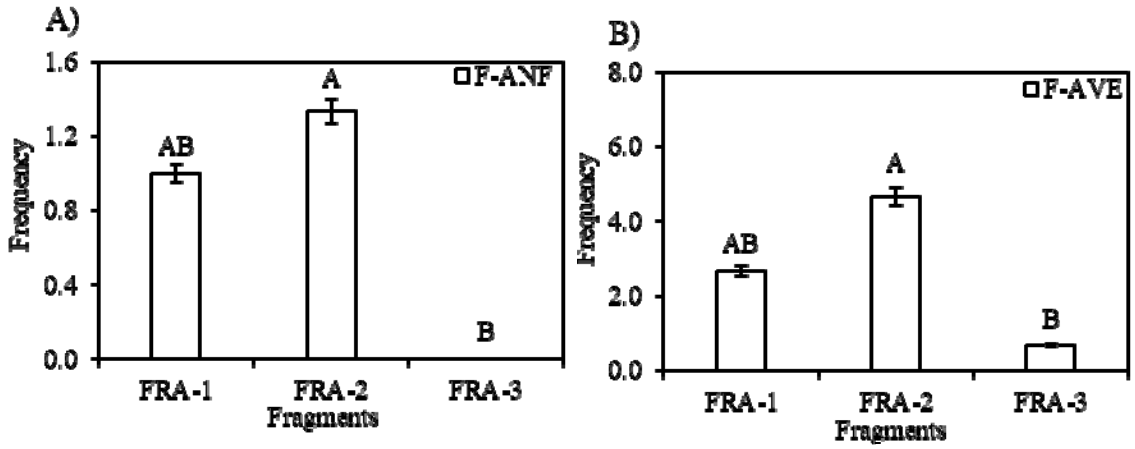

Figure 3. Frequency of amphibians (A), with MSD of 6.37 and birds (B), with MSD of 5.69, verified in three forest fragments. Mean data of three replicates untransformed in sine-arc √ (x/100). Jaboticabal, SP, 2015.

The focus is NOT ON THE METHODOLOGY!! Revisit your title!
What specific result are you discussing here from the many presented in paragraph above figure 3?

This reviewer stopped here as there are major structural problems with this paper.

Rapid assessments and biotic integrity indexes, although generally based on a single taxonomic group, are effective methods for assessing biodiversity conservation [15]. These researchers confirm the longing for this research by suggesting that multiparameter-based assessments, for example, the frequency of occurrence of amphibians and birds, provide a more robust assessment of environmentally disturbed forest fragments. Studying the distribution of amphibians, reptiles, birds and mammals, [14] mentions that anthropic habitats are unsuitable for these species, while natural remnants and reforested fragments are potentially habitable, explaining the high frequency of amphibians and birds in FRA-2, followed by intermediate frequencies in FRA-1 and critical values evidenced in FRA-3 in this research. It should be noted that amphibians are one of the most endangered animal classes, mainly because of their sensitivity to environmental changes (for example, habitat destruction, climate change, as well as the reduction of air humidity, or the emergence of new pathogens, such as the quitrídio fungus, *Batrachochytrium_dendrobatidis*) due to its dependence on water and its permeability of the skin [34]. The distribution results of birds verified by [15] corroborate the findings of this research, certainly due to the characteristics of a particular reduction of the presence of ornithopters in places of intense antropic activity. [24], studying bird species, reported that, although the vast majority of bird species are classified as highly and moderately sensitive to environmental disturbances, there are, although in a smaller number, less sensitive species, justifying the occurrence of birds in the FRA-3 of this study.

No significant difference was recorded between the means 1.0 and 1.6 of the FRA-1 and FRA-2, respectively, when analyzed against the variable F-MAM, however, these two fragments differed significantly from the FRA-3, where there was no presence of mammals, characterizing the superiority of 100% of FRA-1 and FRA-2 in relation to FRA-3 (Figure 4A). For the variables F-REP and F-TRE, no significant differences were found (Figure 4B), which can be justified by the high variation of the original data, reflecting a high coefficient of variation of 212.13% for F-REP absence of normal distribution of data F-REP and F-TRE.

Journal guidelines:
There is no strict page limit for a Short Communication; however, we advise a length of 2500-3500 words, plus 2-3 figures and/or tables

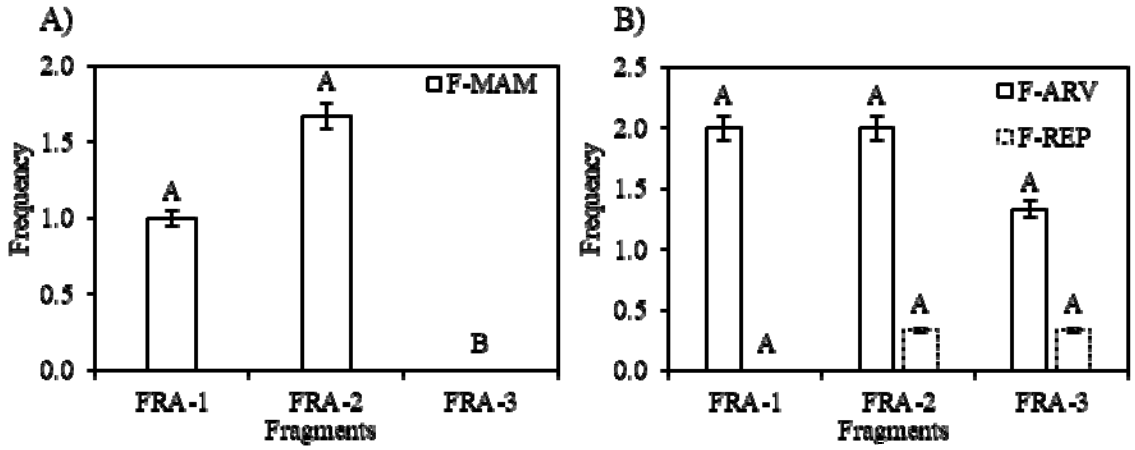

Figure 4. Frequency of mammals (A), with MSD of 3.54, reptiles and trees (B), with MSDs of 6.78 and 1.99, respectively, verified in three forest fragments. Mean data of three untransformed sine-arc replicates of the √ (x/100). Jaboticabal, SP, 2015.

Economic interests, to meet the demands of the growing population, have motivated predatory hunting, animal trafficking, forest deforestation, and expansion of arable land [35]. These researchers add that, fragmented forests tend to harbor fewer mammals compared

with intact areas. It should be noted that the distribution dynamics of mammals in fragmented areas is also associated with their size. In fact, [36] report that the population of small rodent mammals can be increased in areas where the frequency of large mammals is reduced. In a complementary sense, [37] explain that changes in the distribution of mammals can be influenced by increasing land occupation for agriculture and livestock, as well as suppression of part of vegetation, alteration of hydrological cycles, burning regime and nutrient cycling in ecosystems. The nonoccurrence of differences between the fragments for the frequency of trees and reptiles can be justified by the fact that local climatic conditions favor the propagation and development of the trees, providing an adequate ecosystem for the occurrence of reptiles in the area of the three fragments studied [38].

On the basis of the Euclidean Distance used to summarize the homogeneity between the experimental units within the groups and heterogeneities between the groups, there were two main groups, the first group being represented by fragment three (FRA-3) and the second group by fragments one (FRA-1) and two (FRA-2), denoting the dissimilarities between the groups based on hierarchical grouping (Figure 5).

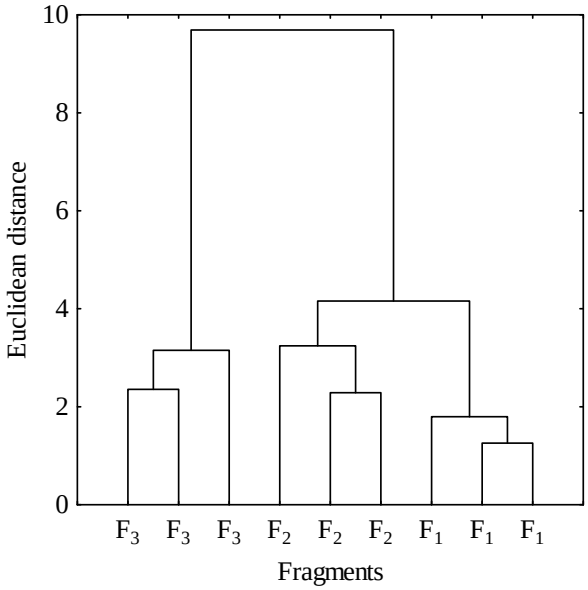

Figure 5. Dendrogram of dissimilarities between three forest fragments (F). Jaboticabal, SP, 2015.

In a research to compare two multivariate methodologies in the study of similarities between fragments of Atlantic forest, [39] point out that there is dissimilarity between groups of forest fragments, emphasizing that fragment groupings are due to the similarities of their variables, justifying these similarities due to their geographical proximity. [40]also observed that floristic similarity decreased with increasing distance between areas, in agreement with the ideas of [41] and [42], according to which geographical proximity would be the only reliable factor to predict the similarity between areas. These evidences allow us to infer that, due to the high geographic proximity of the fragments investigated in this research, the differences observed are due to the particular characteristics of these fragments, mainly anthropization in fragments of natural regeneration and reforestation.

It is observed in Table 2, that the set of seven categories (variables) analyzed was summarized in two latent variables (constructs), called Principal Components 1 (PC_1) and 2

(PC_2), which were selected based on eigenvalues, 4.83 and 1.26 because they were ≥1, satisfying the criterion of Kaiser-Meyer-Olkin (KMO).

Table 2. Eigenvalues (AV), relative variance (S^2_r) and absolute (S^2_a) and variable loads. Jaboticabal, SP, 2015.

PCs	AV	S^2_r (%)	S^2_a (%)	Variable Loads F-TES	F-INS	F-ANF	F-AVE	F-MAM	F-REP	F-ARV
PC_1	4.83	69.07	69.07	-0.98	-0.93	-0.91	-0.96	-0.85	0.09	-0.73
PC_2	1.26	18.03	87.11	0.17	0.25	-0.06	0.19	-0.18	0.97	-0.38

PCs: Principal Components.

The PC_1 and PC_2 account for 87.1% of the total cumulative variance, with PC_1 accounting for 69.07% of this total, with PC_2 accounting for 18.04%. It is observed that the FRA-3 presented greater dissimilarities compared with the FRA-2, with the FRA-1 occupying intermediate position (Figure 6).

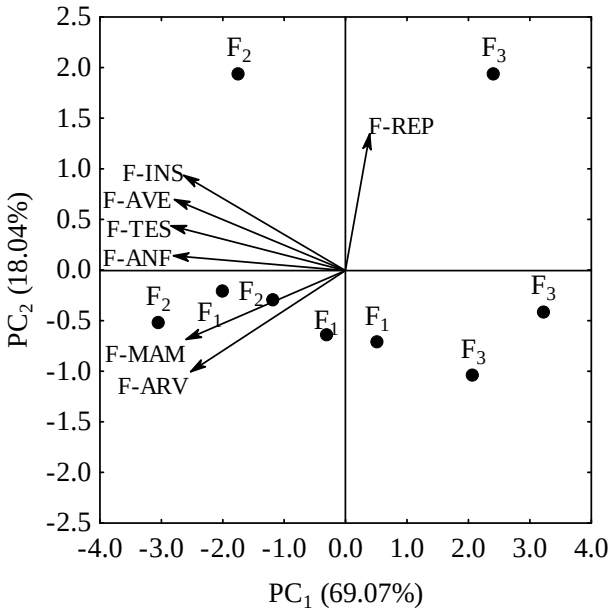

Fig. 6. Two-dimensional projection (Biplot) of three forest fragments (F) and representatives of fauna and flora in two Principal Components (PC_1 and PC_2). Jaboticabal, SP, 2015.

~~Analyzing forest fragments through Principal Component Analysis, [35] report that the first two components account for 56% of the total variation in mammalian distribution among the sampled sites. These researchers point out that the lower incidence of mammalian species in altered areas can be explained by the hunter's pursuit of animals, especially game animals and those that cause damage to agricultural crops, while trees and nontarget species of hunting tend to be seen more frequently in anthropized areas.~~

You are not establishing a NEW methodology for this type of forest areas – focus according to the title should be on the biodiversity under Different Forms of Environmental Conservation

~~On the basis of the results, the use of Principal Component Analysis is justified, since it provides a structural simplification of the original data. In fact, in the research carried out by [39], 462 dimensions were reduced in 10 Principal Components resulting from linear combinations between original variables. Therefore, these authors report that using the first 10 PCs is as efficient as the use of the 462 initial variables with regard to the explanation of the variance. Thus, the use of two PCs in this research was sufficient enough to explain the variance under study.~~

On the basis of this information, it is believed that the divergences evidenced between the fragments of this research can be a reflection of the anthropic actions, especially of the illegal hunting that can occur in these places. For [39], each fragment exhibits a species composition that appears to result from a series of factors that varied differently over time and space. Perhaps that is why it is so difficult to establish these areas. This difficulty, however, indicates that each fragment presents a set of its own characteristics, which emphasizes its importance in terms of conservation.

~~4. CONCLUSION~~

SHORT COMMUNICATIONS DO NOT HAVE CONCLUSION SECTION – THIS INFORMATION SHOULD BE WORKED INTO THE VARIOUS SECTIONS.

~~There are differences in the biodiversity of the forest fragments analyzed, highlighting the areas of natural remnants and reforestation with greater biological diversity, to the detriment of the natural regeneration areas with insufficient biological indicators, denoting adequacy of the first two fragments and inadequacy of the latter with respect to the potential of use as a legal reserve.~~

~~Two groups of environments were evidenced according to the potential hierarchy for use as legal reserve, the first group being characterized as inadequate and the second as adequate. Of the seven analyzed variables, six were considered essential to the correct evaluation of the environments.~~

COMPETING INTERESTS

The authors have not declared any conflict of interests.

AUTHORS' CONTRIBUTIONS

This work was carried out with the collaboration of all authors. All authors read and approved the final manuscript.

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