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Genetic Diversity and Differentiation in *Plathymenia reticulata* Benth.: A Comparative Study of Forest and Cocoa Agroforest Systems in the Atlantic Forest Domain

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Abstract: Tropical forests, housing about two-thirds of Earth's terrestrial biodiversity, face significant deforestation and fragmentation, leading to the loss of over half their area. Conservation and restoration efforts are crucial, especially for regions like the Brazilian Atlantic Forest, which suffers with a reduction in biomass and species diversity. The Brazilian agroforest system, typical from the region of Southern Bahia called "cabruca", are helping to mitigate these effects by combining cocoa cultivation with forest management, enhancing both socio-economic and environmental benefits. In a study focusing on *Plathymenia reticulata*, a tree species native to the Atlantic Forest, we evaluated the effectiveness of the cabruca agroforest system versus the natural forest for maintaining genetic diversity and landscape connectivity. Results showed that cabruca systems, while managed, exhibit a level of genetic diversity compared to natural forests. However, forest populations remain critical for their genetic reservoirs and adaptability. The cabrucas agroforest system also showed higher inbreeding levels, emphasizing the need for effective management to reduce inbreeding risks.

Keywords: biodiversity conservation; tropical forest; agroforest; genetic variation

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1. Introduction

The tropical forests harbor approximately two-thirds of Earth's terrestrial biodiversity, but these ecosystems are under threat from extensive deforestation due to human activities [1]. While global forest conservation and restoration programs are working to change land-use practices in tropical regions, deforestation and forest fragmentation remain significant drivers of biodiversity loss and the decline of ecosystem services [2]. The pervasive destruction has already led to the loss of over half of the world's tropical forests, posing a significant conservation challenge for this century [3]. For instance, the Brazilian Atlantic Forest, known for its rich biodiversity, has only about 23% of its original forest cover remaining, with 97% of forest fragments being smaller than 50 hectares, suffering significant reductions in biomass and species diversity [4].

Brazil is a megadiverse country, yet it faces a significant loss of tropical forest cover, which requires urgent efforts to reverse the damage caused by deforestation [5]. In this context, studies in Brazil have highlighted the socioeconomic benefits of cabrucas, which hosts one of the largest forested areas in the form of an agroforest–native forest mosaic [6]. Over 26% of this forested area is covered by cabrucas, spanning 600,000 hectares [7].

Cabrucas, where cocoa is cultivated under the thinned canopy of the Atlantic Forest trees, can help mitigate the projected effects of climate change for the 2050s, emphasizing the critical role of forest conservation in Bahia Cocoa [8]. Cocoa cultivation, which began in the 18th century in Southern Bahia, continues to hold significant economic value for the region [9].

Cabruca contribute to conservation by providing habitat and biological corridors that enhance the connectivity of plant and animal populations between habitat remnants [10]. It helps in producing various products, and its products are called "bio-based" [11]. However, understanding the genetic status of populations is crucial in order to investigate the factors and implications of management practices on them.

Genetic tools play a crucial role by offering valuable insights into source populations, adaptive variations, gene flow, and other important parameters [12]. It is a fundamental element of biodiversity, as it is critical for maintaining the evolutionary potential of species [12,13]. Anthropogenic disturbances negatively impact genetic diversity, which is essential for population persistence and fitness [14]. It can also provide past and present information on species, such as their expansion or contraction, connectivity, and the identification of source or provenance [15]. Genetic diversity, connectivity, and population size are interrelated and serve as key indicators of ecosystem health [16].

A reduction in landscape connectivity between habitat patches can lead to increased inbreeding depression [17,18]. It is also crucial to select suitable species and identify populations that have suffered from low genetic diversity and inbreeding [19,20]. Therefore, genetic parameters are important indicators for landscape connectivity, influencing gene flow, population size, and genetic diversity [21]. Although gene flow can occur in many species, the effects of isolation and gene flow in most tropical trees remain poorly understood [22]. Addressing these factors is essential to ensure the adaptive potential and long-term fitness of species and to determine the best approaches for restoration projects. The aim of our study is to provide insight on the long-term persistence of species under different land-use practices, i.e., forest and cabruca, by using genetic parameters. In the present study, we used the Brazilian native species *Plathymenia reticulata* Benth. to investigate the effectiveness of forest conservation and the typical cocoa agroforest called "cabruca" in maintaining landscape connectivity. Our target species is one of the largest trees, endemic to the Atlantic Forest. We selected *P. reticulata* due to its utilization in restoration efforts within the Atlantic Forest [23], and its notable logging potential [24,25]. Therefore, studies to better understand the genetic diversity and gene flow of the species are a key strategy to its in situ or ex situ conservation. It is very important to have a better understanding of the genetic diversity and gene flow of the species to identify the species that have a high level of genetic polymorphism [26]. The focus on genetic connectivity will help to choose appropriate statistical techniques and spatial scale for successful conservation and landscape management projects.

2. Materials and Methods

2.1. Target Species

Plathymenia reticulata Benth., popularly known as *vinhático* [27], can reach up to 1.59 m in DBH (Diameter at Breast Height) (observational data). It has a stem with regular plate formation and wood with a reddish tone, which is the origin of its popular name. It is pollinated by small insects [28], especially bees, and its seeds are dispersed by anemochory.

It stands out among the species most used in the restoration of the flora of degraded areas. It is widely distributed in Brazilian biomes, being present from the Atlantic Forest to the Caatinga, [27]. *Vinhático* is highly adaptable, which makes it an excellent candidate for implementation in the restoration of degraded areas. Furthermore, it is one of the most desired species by farmers in cocoa agroforestry systems in southern Bahia [25]. Due to its high-quality and durable wood, *vinhático* is of great economic importance, being used in civil construction and the manufacture of luxury furniture, stakes, supports, and fence posts [27]. This makes it one of the most sought-after species in commercial plantations of native species. Thus, it has itself as an excellent biological model with notable ecological and economic importance.

2.2. Study Area

The study was conducted in sites based on the occurrence of *P. reticulata* in two treatments: the Atlantic Forest and Cabruca in the southern region of the state of Bahia, Brazil, specifically from the municipalities of Camacan CA(F); Ibirapitanga IB(F) IA(C) and IJ(C); Amargosa AM(F); Ilheus CC(C); Itabuna VA(C); Una UA(C) and UJ(C) (Figure 1). According to the Köppen climate classification, the prevailing climate type is "Af", characterized by a tropical rainforest climate. This climate typically receives annual rainfall ranging between 1200 to 1300 mm and maintains an average temperature around 25 °C. The predominant vegetation associated with this climate is the Dense Ombrophilous Forest, which thrives in such warm and consistently moist conditions.

2.3. Sampling

We sampled adults and juveniles in the seven study areas and collected leaves from these individuals for subsequent DNA extraction. A total of 96 adults from 4 different cabrucas, 41 juveniles from 2 cabrucas and 74 adults from 3 different native forests were sampled. The selection of sampled areas was based on the criterion of the overlap of regions within the phytogeographic domain of the Atlantic Forest, specifically in the southern part of Bahia, where the cocoa-cabruca agroforestry system occurs, the local presence of *P. reticulata,* and a high number of adult individuals (minimum of 24).

A previous study concluded that *P. reticulata* has a spatial genetic structure in forests [29], thus, in the forest areas, individuals at least 100 m apart were sampled. Individuals with CAP $\geq$ 25 cm were considered adults and those with CAP $\leq$ 10 cm were considered juveniles. We georeferenced each individual by Garmin GPS.

2.4. DNA Extraction and Quantification

The DNA extraction of each sampled individual was performed through the Protocol CTAB 2% [30]. To estimate the quality and concentration of DNA, Gel Green$^{\mathrm{TM}}$ (Biotium, Fremont, CA, USA) DNA stain was used through contrasting the samples with dilutions of bacteriophage λ DNA diluted to a known standard concentration, in an electrophoresis in 1% agarose gel.

2.5. Microsatellite Loci Amplification

The DNA of each sampled individual was amplified by 11 microsatellite marker loci (also known as SSR—simple sequence repeats) developed specifically for *P. reticulata* [29,31]. For DNA amplifications, we used a PCR mix (final volume 13 µL) containing 4.8 µL of sterile ultrapure water, 1.3 µL of Buffer(10X), 1.3 µL of dNTPs (deoxynucleotide triphosphates) (2.5 mM), 1.3 µL of BSA (Bovine Serum Albumin, Sigma-Aldrich, St. Louis, MO, USA) (2.5 mg/mL), 0.4 µL of $MgCl_2$ (25 mM), 0.4 µL of labeled M13 tail dye (10 mM), 0.3 µL of Primers (Forward and Reverse) (10 mM), 0.2 µL of Taq polymerase (1 U) (Phoneutria, Belo Horizonte, Minas Gerais, Brasil), and 3.0 µL of genomic DNA. We performed the PCR in a Life Pro thermocycler (Bioneer Technology Co., Shanghai, China), with the amplification program according to previously published protocols [29,31]. The amplicons were submitted to capillary electrophoresis in an ABI3500 automatic analyzer (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) to separate the SSR fragments in a multiload strategy. A mixture containing 0.3 µL of GeneScan™, 500 µL of Liz™ (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA), and 7.7 µL of formamide (Applied Biosystems) was prepared. Genotyping was performed with GeneMarker software version 2.5.2 (SoftGenetics, State College, PA, USA).

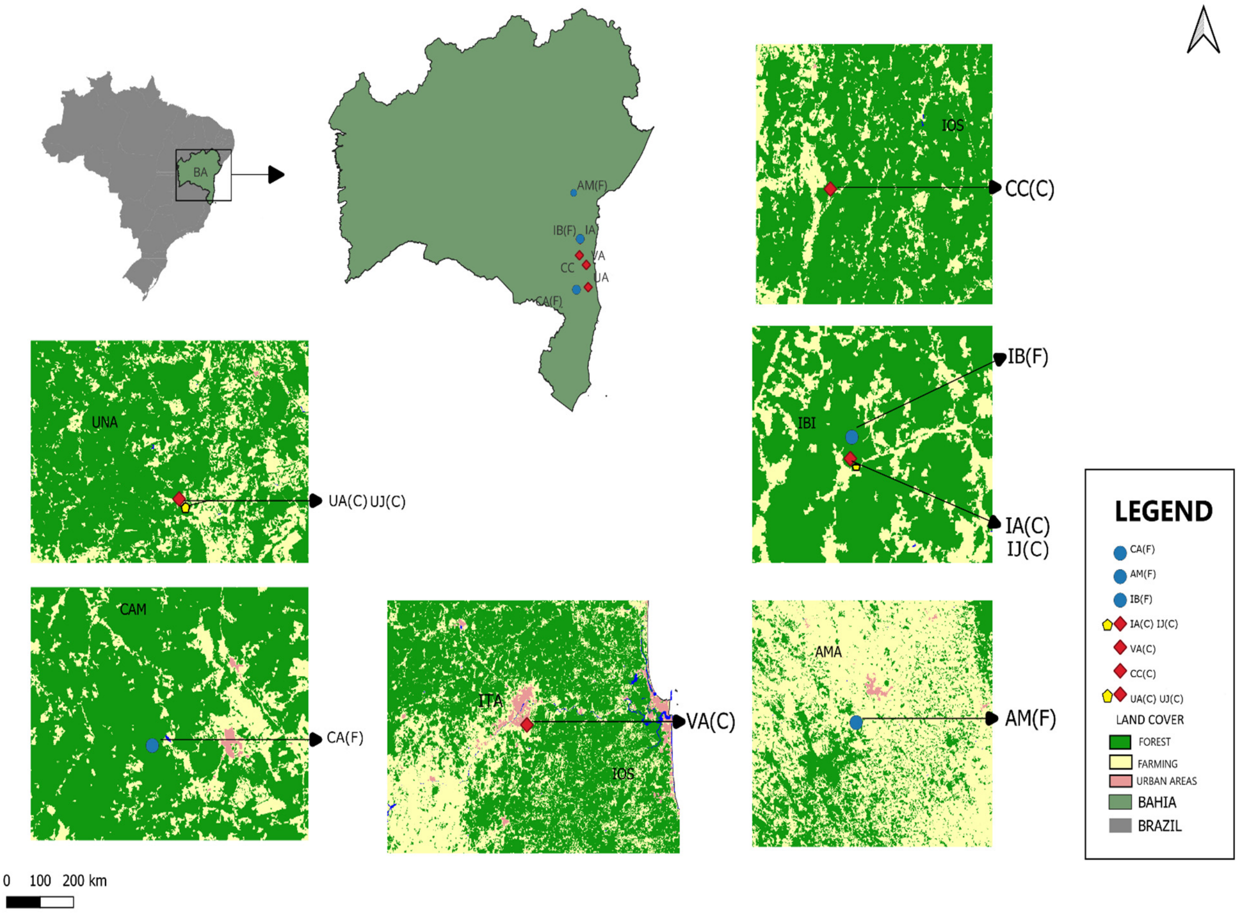

Figure 1. Map of Brazil, highlighting the Bahia state. The collection areas of Plathymenia reticulata are geographically indicated in the Bahia map, where Red Diamonds = "Cabruca" and Blue circles = "Forest". Mapbiomass highlights landcovers "Forest", "Farms", "Urban Areas". Figures represent collection areas with landcovers where, UA(C) = Cabruca near Una, CA(F) = Forest near Camacan, VA(C) = Cabruca near Itabuna, CC(C) = Cabruca near Ilheus, IA(C) and IB(F) = Cabruca and Forest near Ibirapitanga, and AM(F) = Forest near Amargosa. UJ(C) and IJ(C) are juveniles in the respective cabruca.

2.6. Genetic Parameters Estimate

To characterize the genetic diversity of all study areas, we estimated the following standard genetic parameters: Number of Alleles (N_a), Effective Number of Alleles (N_e), Shannon's Information Index (I), Observed Heterozygosity (H_O), Expected Heterozygosity (H_E), and Fixation Index (F) using GenAlEx version 6.5 software [32]. Allelic Richness (A_R) was estimated in the R4.0.1 environment using the rarefaction model as sample size is unequal between populations [33,34]. We also compared f-coefficient, such as F_{IS} (inbreeding coefficient within subpopulations), F_{IT} (inbreeding coefficient within individuals relative to the total population), F_{ST} (genetic differentiation among subpopulations), and pairwise F_{ST} between forest and cabruca using GenAIEx version 6.5 software and demonstrated them using the heatmap in the R4.0.1 environment. The Discriminant Analysis of Principal

Components (DAPCs) was performed to explore the genetic structure of populations using the adegenet package in the R4.0.1 environment [35]. The DAPCs was used to explore the genetic structure of the populations, as it is a robust technique that allows for the distinction of groups based on genetic variation. Principal Component Analysis (PCA) was applied for dimensionality reduction, followed by Discriminant Analysis to maximize the separation between predefined groups and facilitating the visualization of the structure among populations or subpopulations. The number of retained principal components (PCs) was determined based on the cumulative proportion of explained variance and the information saturation criterion. This process helps to more precisely delineate the genetic boundaries between the groups, providing a clearer understanding of the genetic variability and organization of the studied populations.

For the individual assignment analysis, the STRUCTURE program was used, running the algorithm for K values ranging from 1 to 10. The use of this approach provides a quantitative measure of confidence in the allocation of individuals, which is particularly useful and effective for identifying genetic groups, a crucial aspect for understanding issues such as the connectivity between the populations studied here. Each K was evaluated with 10 independent replicates, considering 100,000 burn-in iterations followed by 100,000 collection iterations. The results were processed in Structure Harvester to determine the most likely number of clusters (K) based on the Evanno method [36]. The clustering results were aligned across multiple independent runs using CLUMPP software (version 1.1.2) [37]. For bar plot visualization, the pophelper package was employed. To evaluate the genetic relationships among populations, a dendrogram was constructed using Nei's genetic distance matrix [38]. Hierarchical clustering was performed using the UPGMA method (Unweighted Pair Group Method with Arithmetic Mean). The analysis was conducted in R environment (version 4.0.1), utilizing the adegenet and ape packages [39]. This combination of approaches was employed with the aim of providing a clear visual representation of the genetic similarities and differences among the populations analyzed. Furthermore, we applied these methods because they allow for a robust evaluation of genetic relationships, providing important insights into the patterns of genetic diversity and the evolutionary processes that have shaped the studied populations.

2.7. Paternity Analysis

We did paternity analysis on 47 juveniles with 96 adult trees in four cabrucas using CERVUS 3.0.7 version software [40]. All samples were georeferenced, and by using it, we calculated distance between adults and their alleged progeny. The likelihood method is used with 95% confidence to identify possible progeny, by positive LOD score.

3. Results

3.1. Genetic Parameters

The *P. reticulata* populations evaluated show, in general, low to moderate genetic diversity. The forest populations have higher values in relation to the cabruca populations for the Number of Alleles (N_a), Effective Number of Alleles (N_e), Allelic Richness (A_R), Shannon's Information Index (I), Observed Heterozygosity (H_O), and Expected Heterozygosity (H_E). The average number of alleles (N_a) was 5.00 for forest and 3.75 for cabruca, indicating higher allelic diversity in forests. CA(F) shows significant allele diversity among forest populations (Na = 5.89), while CC(C) was 5.55, showing high allelic diversity in the cabruca population. The effective number of alleles (N_e) is higher in forest populations, with a mean of 2.59, with CC(C) being only the cabruca population, which has a higher (N_e) than any population, i.e., it was 3.08, an indication of higher genetic variation. In the forest population, the Shannon Index(I) is the highest for CA(F), population I = 1.25, and for the cabruca population, CC(C) has I = 1.06. We observed that IB(F) has a negative value of fixation index (F = −0.02). Another forest population of interest is AM(F), which is close to the Hardy–Weinberg equilibrium with a slight difference between H_O and H_E (0.48, 0.49), and F_{IS} close to zero (0.010). Between the cabruca populations, we find a high fixation index, with VA(C) showing the highest value (F_{IS} = 0.80), indicating low genetic diversity and high levels of inbreeding. On average, heterozygosity among forests is higher than in cabrucas with a low fixation index, indicating that the forest population has maintained genetic diversity with lower levels of inbreeding. We compared the genetic parameters of adult cabrucas with those of juvenile cabrucas. For these comparisons, juveniles exhibited higher values for all genetic parameters compared to adults (N_a, N_e, A_R, I, H_O, and H_E) (Table 1).

Table 1. Estimation of genetic parameters of *P. reticulata* in forest and cabruca populations. Number of Alleles (N_a), Effective Number of Alleles (N_e), Allelic Richness (A_R), Shannon's Information Index (I), Observed Heterozygosity (H_O), Expected Heterozygosity (H_E), and Fixation Index (F_{IS}).

Forest	**N**	**N_a**	**N_e**	**A_R**	**I**	**H_O**	**H_E**	**F_{IS}**
CA	28	5.89	2.94	5.28	1.25	0.45	0.61	0.26
IB	22	4.22	2.39	6.12	0.96	0.51	0.50	−0.02
AM	22	4.89	2.44	4.44	0.98	0.48	0.49	0.01
Mean	**24**	**5.00**	**2.59**	**7.92**	**1.06**	**0.47**	**0.53**	**0.09**
Cabrucas (Adults)	**N**	**N_a**	**N_e**	**A_R**	**I**	**H_O**	**H_E**	**F_{IS}**
VA	20	3.55	1.96	4.06	0.76	0.09	0.41	0.80
IA	17	3.55	1.71	4.19	0.65	0.24	0.34	0.49
CC	23	5.55	3.08	6.07	1.06	0.29	0.48	0.29
UA	23	2.33	1.49	2.87	0.46	0.21	0.27	0.26
Mean	**20.8**	**3.75**	**2.06**	**4.29**	**0.73**	**0.21**	**0.37**	**0.48**
Cabrucas (Juveniles)	**N**	**N_a**	**N_e**	**A_R**	**I**	**H_O**	**H_E**	**F_{IS}**
IJ	6	4.55	3.53	5.00	1.18	0.39	0.58	0.39
UJ	29	4.44	1.78	3.83	0.68	0.18	0.33	0.51
Mean	**17.5**	**4.50**	**2.66**	**4.41**	**0.93**	**0.28**	**0.46**	**0.45**

The F_{IS} value was higher in the cabruca (0.51) than in the forest (0.11). Similarly, cabruca populations demonstrate a significantly higher inbreeding coefficient (F_{IT}) (0.63) compared to forest populations (0.21). The F_{ST} value indicates genetic differentiation among cabruca subpopulations (0.17), while the value for forest populations (0.11) remains similar (Figure 2).

The pairwise F_{ST} matrix showed high values of F_{ST} between populations located in forests and cabruca (shown in darker shades of blue) (Figure 3). The highest F_{ST} value (0.31) was observed between UA(C) and CA(F), indicating a significant degree of genetic differentiation. Conversely, the lowest F_{ST} value (0.01) was found between AM(F) and IB(F), suggesting minimal genetic differentiation.

Populations of *P. reticulata* managed in Cabrucas exhibited closer genetic relationships with each other than with forest populations (Figure 4). Moreover, individuals from forest populations formed a more homogeneous cluster, while those from cabrucas displayed greater genetic dispersion (Figure 5).

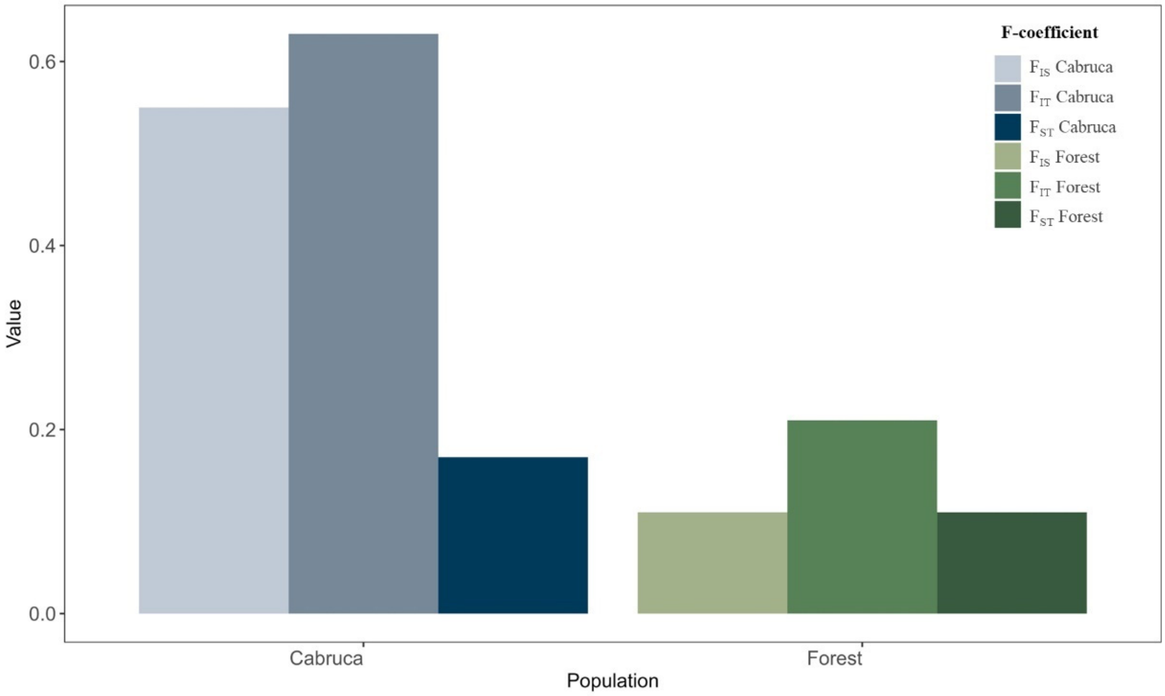

Figure 2. F-coefficient (inbreeding coefficients within populations (F_{IS}), inbreeding coefficients relative to the total population (F_{IT}), genetic differentiation among populations (F_{ST}) estimation for three forest and four cabruca populations.

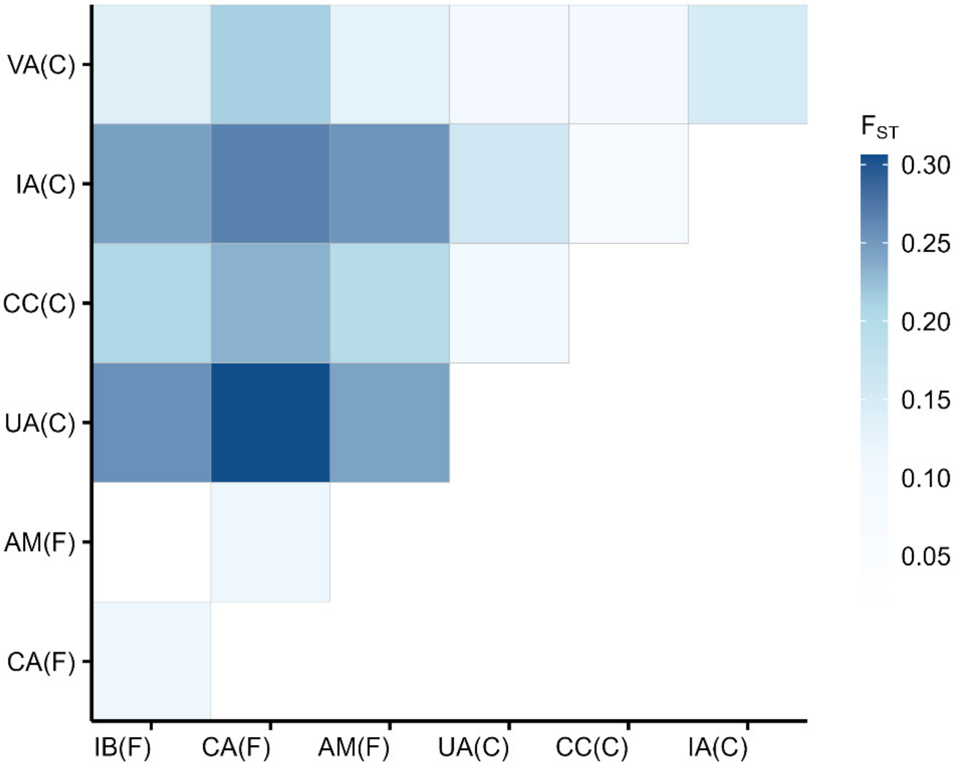

Figure 3. Pairwise F_{ST} values among seven populations of *P. reticulata* in Bahia, Brazil. Lighter blues show lower F_{ST} values, and darker tones show higher F_{ST} values. IB(F), CA(F), and AM(F) represent populations of forest. VA(C), IA(C), CC (C), and UA(C) represent populations of cabruca.

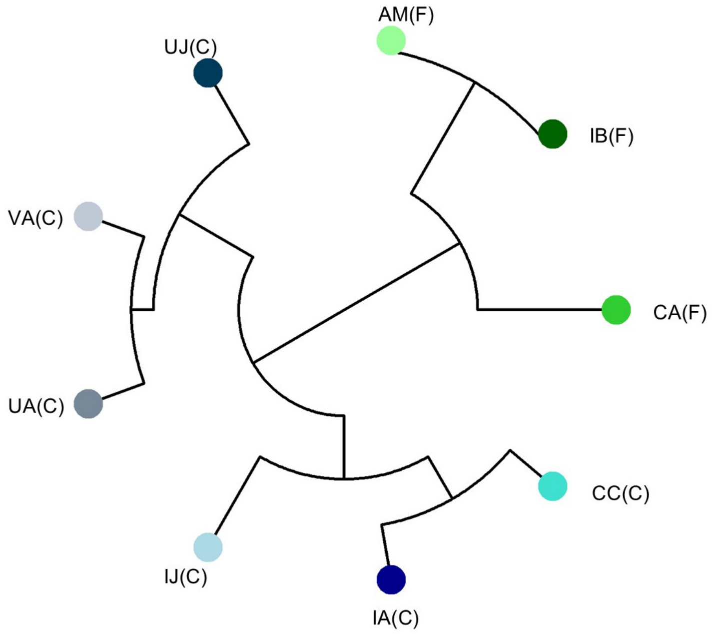

Figure 4. Graphic representation of the clustering by population analysis based on Nei's unbiased minimum genetic distance [40] for *P. reticulata* populations in both forest (green) and cabruca (blue).

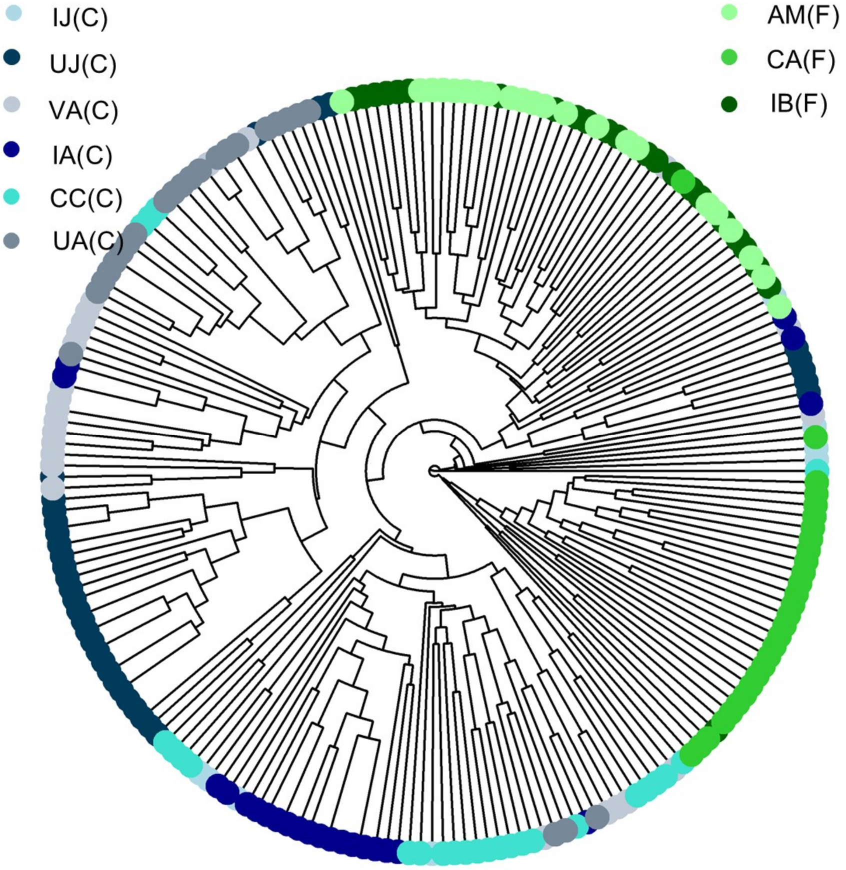

Figure 5. Graphic representation of the clustering analysis by population individuals' analysis based on Nei's unbiased minimum genetic distance [40] for *P. reticulata* populations in both forest (green) and cabruca (blue).

The scatter plot shows Discriminant Analysis of Principal Components (DAPC); it helps to visualize different sets of genetic characteristics between populations (Figure 6A).

The overlap between populations shows shared ancestry (Figure 6A). Bayesian analysis performed in STRUCTURE identified the most probable number of genetic clusters as two, revealing that individuals from the nine populations are divided into two genetic groups (Figure 6B). Most individuals are predominantly assigned to one of these two groups, with minimal evidence of genetic admixture, suggesting genetic differentiation between forest and cabruca populations. The first cluster comprises forest populations, while the second includes cabruca populations (adults and juveniles).

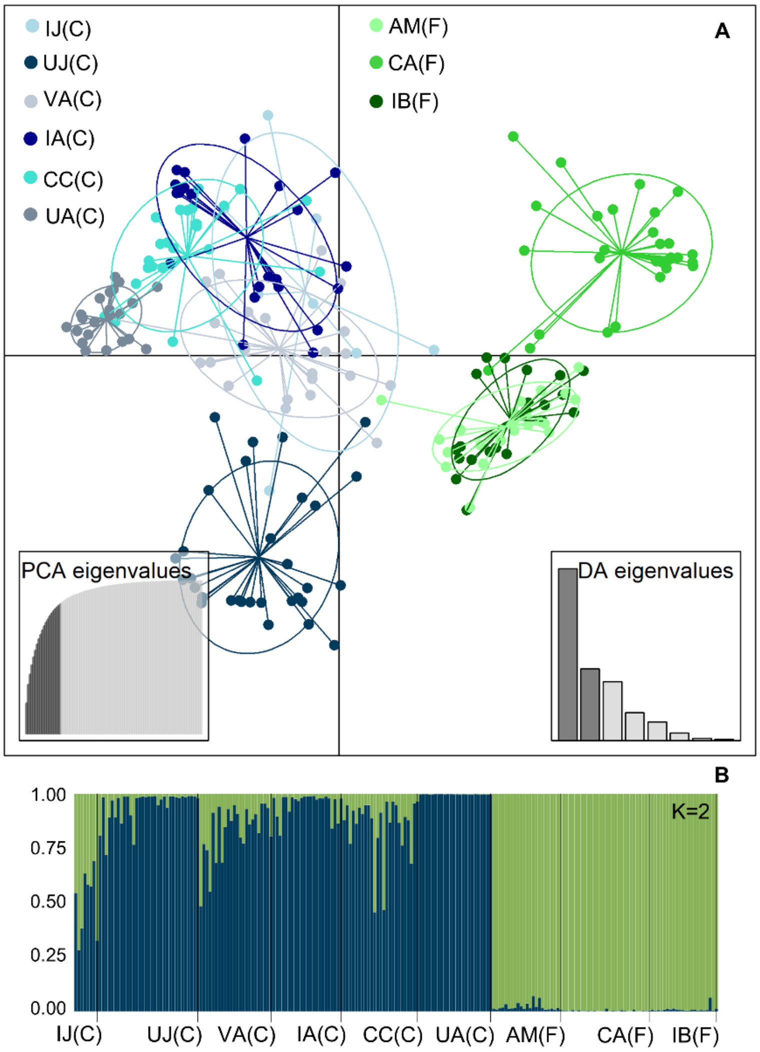

Figure 6. (**A**) Discriminant Analysis of Principal Component (DAPC) of genetic diversity indices for two treatments: Native Forest and cabruca Agroforest Systems, labeled as Forest(F) = "AM, CA, IB", Cabruca(C) = "CC, IA, IJ, UA, UJ and VA". Each point represents a population with colors. Shades of blue are Cabruca and shades of green are Forest. Principal Component analysis eigenvalues (PCA) at bottom left corner and Discriminant Analysis eigenvalues (DA) at the bottom right corner illustrate genetic variance. (**B**) Structure bar plot (K = 2). Each sampled individual of *P. reticulata* is represented by a single vertical line, with different colors (green or blue) indicating the assignment probabilities to the two inferred genetic clusters.

3.2. Paternity

Paternity analysis revealed that 22 juveniles whose parents are detected with 95% confidence have positive LOD scores. The remaining have a low probability of paternity. We also observed that the average distance between individuals of the same areas is 82m (Table 2).

Table 2. Paternity analysis of *P. reticulata* considering all sampled individuals in four cabruca in Southern Bahia, Brazil. Rows show offspring–mother–father trio relationships, LOD scores, and distances. High-confidence paternity scores are indicated with asterisk symbols.

Offsprings	Mother ID	Candidate Father ID	Trio LOD Score	Trio Confidence	Distance (m)
UJC6	UA20	UA20	5.99E+00	*	165
UJC4	UA20	UA20	5.41E+00	*	84
UJC18	UA2	UA2	5.74E+00	*	60
UJC1	UA16	UA15	4.45E+00	*	47
UJC5	UA20	UA20	2.12E+00	*	56
IJ18	IA36	IA36	6.62E+00	*	9
UJC3	UA23	UA15	3.83E+00	*	95
IJ20	IA35	IA35	2.53E+00	*	150
UJC20	UA2	UA2	5.78E+00	*	67
UJC22	UA2	UA2	5.78E+00	*	77
UJC9	UA2	UA2	5.78E+00	*	82
UJC17	UA2	UA2	5.06E+00	*	62
UJC13	UA18	UA18	4.86E+00	*	144
UJC29	UA11	UA11	4.53E+00	*	38
UJC7	UA13	UA13	4.42E+00	*	29
UJC10	UA16	UA16	3.73E+00	*	13
IJ24	IA33	IA33	6.31E+00	*	130
UJC11	UA16	UA16	2.27E+00	*	13
IJ31	IA33	IA33	2.08E+00	*	125
IJ26	IA35	IA35	4.71E+00	*	152
UJC24	UA18	UA18	4.18E+00	*	134

An asterisk (*) indicates that the confidence level is 95% using the "strict" criterion implemented in CERVUS. LOD = logarithm of odds. A = Adults and J = Juveniles.

4. Discussion

In this study, we used genetic estimates on one of the threatened species in the Atlantic Forest, *Plathymenia reticulata*, to reveal significant insights into the biodiversity of forests and cabruca. The results highlight the future of this species in an agroforest system in comparison to forests.

These findings are significant as they highlight the importance of the cabruca system in conserving the genetic diversity of *P. reticulata*, mitigating the impacts of climate change and supporting the species' bioeconomic potential. Prioritizing bio-products derived from timber trees like *P. reticulata* requires not only research and policy development to prevent illegal logging, but also providing incentives for farmers to promote conservation. Additionally, encouraging well-managed mixed cropping systems and increasing research on native forests are crucial steps to ensure the sustainability of tree populations.

With regard to specific genetic diversity indices in *P. reticulata*, the mean Number of Alleles, Effective Number of Alleles, Allelic Richness, Shannon's Information Index, Observed Heterozygosity, and Expected Heterozygosity are higher in forests as compared to cabrucas. These indices provide an accurate reflection of the genetic variation in species grown in different landscapes and environmental conditions. Cabruca populations showed a decrease in these indices but despite being an agroforestry system, they still maintained a level of genetic diversity compared to the natural forest environment. There are some studies that demonstrate that Southern Bahia cabruca has the capacity to sustain species richness and demographic health [25]. Research on another tropical species, *Inga edulis*, indicated that agroforestry systems can have a high level of allelic richness if influenced by a number of factors, including population size and the contribution of neighboring natural remnants [41]. The observed variation between agroforestry systems is dependent on the management practices employed by the farmer [42].

P. reticulata exhibits considerable genetic diversity when cultivated in forests than in agroforestry settings, particularly regarding heterozygosity. This is of critical importance for the adaptability and resilience of the species to environmental stresses. Both study areas are affected by fragmentation, yet the natural forest remains a crucial reserve for *P. reticulata*, in comparison to agroforests. These findings lend support to the hypothesis that human intervention affects the genetic diversity of species [41]. The elevated heterozygosity levels in forests are linked to cross-pollination facilitated by wasps and bees, as well as wind-dispersed seeds [43–45].

The geographical proximity of cabruca to forests can be a factor which may influence the genetic diversity present in cabrucas. This hypothesis is reinforced especially by the values observed in cabruca VA, which is located in an urban context and presented the lowest diversity ($H_O = 0.09/H_E = 0.21$). Another factor that may help explain the reduction in heterozygosity in trees grown in an agroforestry system is associated with a decrease in population size due to selective logging, which is a common practice in agroforestry systems [46]. Leal (2014) [46] explained a similar phenomena in another forest tree whose hardwood is used for construction purposes, i.e., *Cariniana legalis* trees, which occurred in small fragments and cabrucas, as it suffered logging in cocoa areas and ultimately, the loss of heterozygosity, as the number of adults decreased due to the fragmentation and expansion of cocoa in the 1960s and 1970s.

We also observed an almost zero fixation index(F) in the forest population. The fixation index explains patterns of inbreeding in the population. The low levels of inbreeding and moderate value of heterozygosity are expected as the species is a hermaphrodite, and cross-pollination helps its adaptability [47]. The pollinators also help in gene flow over long distances [44], making inbreeding close to zero or negative in the continuous forest. The inbreeding coefficient is high in the agroforest, possibly due to isolation ($F_{IS} = 0.26–0.80$). Trees are in low density in agroforestry systems [48] and like in the case of cabruca VA(C) that has the highest value of inbreeding coefficient, it is vulnerable in the absence of a nearby forest that could help in pollination [49].

Regarding the comparison between genetic diversity indices in adults and juveniles of the cabruca population, we found an increase in the mean genetic diversity in juveniles. Although, we collected a limited number of juvenile individuals, these results may lead to the misinterpretation of the mean results. Specifically, when comparing only UJ (C) population with UA(C) from the same area, our results suggests that juvenile UJ exhibits higher allelic richness and overall genetic variation as compared to adult UA. Despite this, the inbreeding coefficient is elevated in juveniles, suggesting the occurrence of mating between related individuals. Similar results have been found in another tropical tree, *Manilkara Multifida* Penn. [50]. These findings highlight the importance of understanding genetic diversity across generations. Despite limitations in our sampling method, due to a lack of the presence of juveniles in well-maintained cabruca, our findings emphasize the importance of including both adults and juveniles to fully capture the genetic diversity of species, as it helps in determining adaptive potential and long-term survival of populations [51].

We also note that the use of microsatellite markers provided valuable information about the species' diversity patterns and structure. However, for a more in-depth understanding of the species' dynamics, we suggest that future research also explore additional genetic data through approaches such as next-generation sequencing (NGS), SNPs, or whole-genome analysis. These techniques could expand upon the biological questions addressed in this study and provide a broader understanding of genetic variation patterns, particularly regarding the impact of anthropogenic management in cabruca systems as a potential selective pressure, an aspect that was not fully explored due to the limitations of the technique used.

Cabruca populations show significant inbreeding, potentially leading to reduced fitness and adaptability. The higher F_{ST} value indicates distinct genetic structuring, possibly due to isolated or management practices. The higher differentiation is due to limited gene flow, genetic drift, and inbreeding [52]. CA(F) is the only forest population in our results that shows a high level of inbreeding, but it maintains some genetic diversity. This genetic differentiation could be due to the small and fragmented population which can limit gene flow [53,54].

Although the VA(C) cabruca population has the highest inbreeding coefficient and the lowest heterozygosity, it does not exhibit the highest genetic differentiation from other populations. This could be a result of the population originating from a small number of individuals (a limited population size). However, if this population came from a genetically similar source population, the differentiation (FST) between it and other populations may not be high [55,56].

The pairwise F_{ST} analysis revealed high genetic differentiation between populations of forest and cabruca. This outcome may be attributed to varying selection pressures, the loss of alleles resulting from reduced population sizes, and shifts in gene flow patterns influenced by human activities in agroforests. This pattern was consistent with the results from the Discriminant Analysis of Principal Components (DAPCs) plot, which was used to supplement the population genetic differentiation (Figures 2 and 6A). The dendrograms also indicated a well-defined genetic structure, with a pattern of two main clusters. Greater homogeneity was observed in the forests, with these populations being genetically closer to each other, while the genetic pool of cabrucas is contrasting. These patterns offered valuable insights into the evolutionary processes shaped by colonization and isolation, which indicate that the forest and cabruca populations form distinct genetic units, with implications for the management and genetic conservation of these populations. The ellipses and arrow sizes indicate genetic variability. The three forest populations AM(F), IB(F), and CA(F) are separated from other groups and confirm genetic variability in forest populations [57].

Finally, paternity analysis revealed that almost all juveniles whose parents were identified are descended from adults grown in the same cabruca, indicating that gene flow occurs mainly over a short distance. Anthropogenic activities lead to a reduction in population and limited gene flow [58]. Small and isolated populations can also lead to limited gene flow [59]. Also, in agroforest trees, distribution is over short distances and low population density, and both factors reduce the connectivity between the forest and agroforest. When connectivity depends on bees as pollinators, as in the case of *P. reticulata*, which can only travel to short distances, the low gene flow effect is even more threatening [60–62]. This can be prevented by maintaining an effective number of populations of native trees in the area and a cacao agroforest system can act as a stepping stone or a corridor to improve connectivity with natural forest fragments to improve pollen-mediated gene flow [61]. An example for this is a timber tree mahogany (*Swietenia humilis*), grown as a lone individual in pastureland, and participated in gene flow with forest trees for more than 4.5 km [63]. Another reason for distance gene flow could be anemochory properties that *P. reticulata* exhibits, and the recent climate change can modify the species dispersal ability over moderate to long distances [28,64]. The knowledge of our work can help *P. reticulata* to sustain its genetic diversity in a human-modified landscape.

5. Conclusions

This study highlights the potential importance of cabruca agroforestry, despite being a human-modified landscape, in maintaining the genetic diversity of *Plathymenia reticulata*, comparable to natural forests in the Atlantic Forest. However, higher levels of inbreeding genetic differentiation in cabruca populations indicate that management practices and reduced gene flow may impact genetic structure.

Greater genetic diversity and heterozygosity within continuous forest areas highlight their potential as genetic reservoirs for the long-term sustainability of the ecosystems. To further improve genetic connectivity and reduce the risks of inbreeding in cabruca systems, conservation measures should focus on maintaining native tree populations, developing mixed cropping systems, and pollinators and seed dispersers.

Overall, our results highlight the importance of conserving both natural forests and agroforestry systems for the long-term survival of *P. reticulata*. It is possible to conserve genetic diversity, support biodiversity, and mitigate climate change by integrating sustainable land-use practices with conservation efforts. The study offers insights for policymakers, conservationists, and farmers about the importance of genetic considerations in restoration and conservation strategies.

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References

1. Shimamoto, C.Y.; Padial, A.A.; da Rosa, C.M.; Marques, M.C.M. Restoration of ecosystem services in tropical forests: A global meta-analysis. *PLoS ONE* **2018**, *13*, e0208523. [CrossRef] [PubMed]
2. Faria, D.; Morante-Filho, J.C.; Baumgarten, J.; Bovendorp, R.S.; Cazetta, E.; Gaiotto, F.A.; Mariano-Neto, E.; Mielke, M.S.; Pessoa, M.S.; Rocha-Santos, L.; et al. The breakdown of ecosystem functionality driven by deforestation in a global biodiversity hotspot. *Biol. Conserv.* **2023**, *283*, 110126. [CrossRef]
3. Aronson, J.; Goodwin, N.; Orlando, L.; Eisenberg, C.; Cross, A.T. A world of possibilities: Six restoration strategies to support the United Nation's Decade on Ecosystem Restoration. *Restor. Ecol.* **2020**, *28*, 730–736. [CrossRef]
4. Ribeiro, M.C.; Metzger, J.P.; Martensen, A.C.; Ponzoni, F.J.; Hirota, M.M. The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. *Biol. Conserv.* **2009**, *142*, 1141–1153. [CrossRef]
5. Ditt, E.H.; Mourato, S.; Ghazoul, J.; Knight, J. Forest conversion and provision of ecosystem services in the Brazilian Atlantic Forest. *Land Degrad. Dev.* **2010**, *21*, 591–603. [CrossRef]
6. Brancalion, P.H.; de Siqueira, L.P.; Amazonas, N.T.; Rizek, M.B.; Mendes, A.F.; Santiami, E.L.; Rodrigues, R.R.; Calmon, M.; Benini, R.; Tymus, J.R.C.; et al. Ecosystem restoration job creation potential in Brazil. *People Nat.* **2022**, *4*, 1426–1434. [CrossRef]
7. Sagastuy, M.; Krause, T. Agroforestry as a Biodiversity Conservation Tool in the Atlantic Forest? Motivations and Limitations for Small-Scale Farmers to Implement Agroforestry Systems in North-Eastern Brazil. *Sustainability* **2019**, *11*, 6932. [CrossRef]
8. Heming, N.M.; Schroth, G.; Talora, D.C.; Faria, D. Cabruca agroforestry systems reduce vulnerability of cocoa plantations to climate change in southern Bahia. *Agron. Sustain. Dev.* **2022**, *42*, 48. [CrossRef]

9. Rolim, S.G.; Chiarello, A.G. Slow death of Atlantic Forest trees in cocoa agroforestry in southeastern Brazil. *Biodivers. Conserv.* **2004**, *13*, 2679–2694. [CrossRef]
10. Casanova-Lugo, F.; Ramírez-Avilés, L.; Parsons, D.; Caamal-Maldonado, A.; Piñeiro-Vázquez, A.T.; Díaz-Echeverría, V. Environmental services from tropical agroforestry systems. *Rev. Chapingo Ser. Cienc. For. Y Del. Ambiente* **2016**, *22*, 269–284. [CrossRef]
11. Chojnacka, K. Innovative bio-products for agriculture. *Open Chem.* **2015**, *13*, 000010151520150111. [CrossRef]
12. Mijangos, J.; Pacioni, C.; Spencer, P.B.S.; Craig, M.D. Contribution of genetics to ecological restoration. *Mol. Ecol.* **2014**, *24*, 22–37. [CrossRef] [PubMed]
13. Almeida-Rocha, J.M.; Soares, L.A.; Andrade, E.R.; Gaiotto, F.A.; Cazetta, E. The impact of anthropogenic disturbances on the genetic diversity of terrestrial species: A global meta-analysis. *Mol. Ecol.* **2020**, *29*, 4812–4822. [CrossRef] [PubMed]
14. Reynolds, L.K.; McGlathery, K.J.; Waycott, M. Genetic diversity enhances restoration success by augmenting ecosystem services. *PLoS ONE* **2012**, *7*, e38397. [CrossRef]
15. Diekmann, O.E.; Gouveia, L.; Perez, J.A.; Gil-Rodriguez, C.; Serrão, E.A. The possible origin of Zostera noltii in the Canary Islands and guidelines for restoration. *Mar. Biol.* **2010**, *157*, 2109–2115. [CrossRef]
16. Thomas, E.; Jalonen, R.; Loo, J.; Boshier, D.; Gallo, L.; Cavers, S.; Bordács, S.; Smith, P.; Bozzano, M. Genetic considerations in ecosystem restoration using native tree species. *For. Ecol. Manag.* **2014**, *333*, 66–75. [CrossRef]
17. Baguette, M.; Blanchet, S.; Legrand, D.; Stevens, V.M.; Turlure, C. Individual dispersal, landscape connectivity and ecological networks. *Biol. Rev. Camb. Philos. Soc.* **2013**, *88*, 310–326. [CrossRef]
18. Herrera, L.P.; Sabatino, M.C.; Jaimes, F.R.; Saura, S. Landscape connectivity and the role of small habitat patches as stepping stones: An assessment of the grassland biome in South America. *Biodivers. Conserv.* **2017**, *26*, 3465–3479. [CrossRef]
19. Maloney, P.E.; Vogler, D.R.; Eckert, A.J.; Jensen, C.E.; Neale, D.B. Population biology of sugar pine (*Pinus lambertiana* Dougl.) with reference to historical disturbanc. *Fuel Energy Abstr.* **2011**, *262*, 770–779.
20. Weeks, A.R.; Sgro, C.M.; Young, A.G.; Frankham, R.; Mitchell, N.J.; Miller, K.A.; Byrne, M.; Coates, D.J.; Eldridge, M.D.B.; Sunnucks, P.; et al. Assessing the benefits and risks of translocations in changing environments: A genetic perspective. *Evol. Appl.* **2011**, *4*, 709–725. [CrossRef]
21. Proft, K.M.; Jones, M.E.; Johnson, C.N.; Burridge, C.P. Making the connection: Expanding the role of restoration genetics in restoring and evaluating connectivity. *Restor. Ecol.* **2018**, *26*, 411–418. [CrossRef]
22. Dick, C.W.; Hardy, O.J.; Jones, F.A.; Petit, R.J. Spatial scales of pollen and seed-mediated gene flow in tropical rain forest trees. *Trop. Plant Biol.* **2008**, *1*, 20–33. [CrossRef]
23. Ribeiro, J.; Kuhlmann, M.; Santos, D.; Sampaio, A.; Ogata, R.; Souza, R.; Oliveira, M.; Durigan, G.; Munhoz, C.; Valls, J.; et al. *Espécies Vegetais Nativas Recomendadas Para Recomposição Ambiental no Bioma Cerrado*; Brazilian Agricultural Research Corporation: Brasília, Brazil, 2018.
24. Almeida, S.P.; Proença, C.E.B.; Sano, S.M.; Ribeiro, J.F. *Cerrado: Espécies Vegetais Úteis*; Empresa Brasileira de Pesquisa Agropecuária do Brasil (EMBRAPA): Brasilia, Brasil, 1994; pp. 48–335.
25. Sambuichi, R.; Vidal, D.B.; Piasentin, F.B.; Jardim, J.G.; Viana, T.G.; Menezes, A.A.; Mello, D.L.N.; Ahnert, D.; Baligar, V.C. Cabruca agroforests in southern Bahia, Brazil: Tree component, management practices and tree species conservation. *Biodivers. Conserv.* **2012**, *21*, 1055–1077. [CrossRef]
26. Tambarussi, E.V.; Boshier, D.; Vencovsky, R.; Freitas, M.L.M.; Sebbenn, A.M. Inbreeding depression from selfing and mating between relatives in the Neotropical tree Cariniana legalis Mart. *Kuntze. Conserv. Genet.* **2017**, *18*, 225–234. [CrossRef]
27. Carvalho, P.E.R. *Espécies arbóreas brasileiras. Brasília, DF: Embrapa Informação Tecnológica*; Embrapa Florestas: Colombo, Brazil, 2008; pp. 527–535. Available online: http://www.alice.cnptia.embrapa.br/alice/handle/doc/1139362 (accessed on 6 February 2025).
28. Goulart, M.F.; Filho, J.P.L.; Lovato, M.B. Phenological Variation Within and Among Populations of *Plathymenia reticulata* in Brazilian Cerrado, the Atlantic Forest and Transitional Sites. *Ann. Bot.* **2005**, *96*, 445–455. [CrossRef]
29. Oliveira, F.; Tarazi, R.; Menezes, I.P.; Van Den Berg, C.; Tsai, S.M.; Gaiotto, F.A. Microsatellite markers for *Plathymenia reticulata* (Leguminosae). *Am. J. Bot.* **2012**, *99*, e391–e393. [CrossRef]
30. Doyle, J. *DNA Protocols for Plants, in Molecular Techniques in Taxonomy*; Hewitt, G.M., Johnston, A.W.B., Young, J.P.W., Eds.; Springer: Berlin/Heidelberg, Germany, 1991; pp. 283–293.
31. Cruz, M.V.; Rodrigues, J.G.; Souza, H.A.V.; Lovato, M.B.; Duong, T.A.; de Beer, Z.W.; Zanzot, J.W.; Wingfield, M.J.; Wingfield, B.D. Isolation and characterization of microsatellite markers for *Plathymenia reticulata* (Fabaceae). *Am. J. Bot.* **2012**, *99*, e210–e212. [CrossRef]
32. Peakall, R.; Smouse, P.E. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes* **2006**, *6*, 288–295. [CrossRef]
33. Hurlbert, S.H. The Nonconcept of Species Diversity: A Critique and Alternative Parameters. *Ecology* **1971**, *52*, 577–586. [CrossRef]
34. Jean-Louis, F.; Louis, O. Estimating allelic richness and its diversity. *Livest. Sci.* **2006**, *101*, 150–158.

35. Jombart, T.; Devillard, S.; Balloux, F. Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. *BMC Genet.* **2010**, *11*, 94. [CrossRef] [PubMed]
36. Evanno, G.; Regnaut, S.; Goudet, J. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Mol. Ecol.* **2005**, *14*, 2611–2620. [CrossRef] [PubMed]
37. Jakobsson, M.; Rosenberg, N.A. CLUMPP: A cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* **2007**, *23*, 1801–1806. [CrossRef] [PubMed]
38. Nei, M. Genetic distance between populations. *Am. Nat.* **1972**, *106*, 283–292. [CrossRef]
39. Sokal, R.R.; Michener, C.D. *A Statistical Method for Evaluating Systematic Relationships*; Harvard University: Cambridge, MA, USA, 1958.
40. Marshall, T.C.; Slate, J.; Kruuk, L.E.B.; Pemberton, J.M. Statistical confidence for likelihood-based paternity inference in natural populations. *Mol. Ecol.* **1998**, *7*, 639–655. [CrossRef]
41. Hollingsworth, P.; Dawson, I.K.; Goodall-Copestake, W.P.; Richardson, J.E.; Weber, J.C.; Montes, C.S.; Pennington, R.T. SHORT COMMUNICATION: Do farmers reduce genetic diversity when they domesticate tropical trees? A case study from Amazonia. *Mol. Ecol.* **2005**, *14*, 497–501. [CrossRef]
42. Molla, A.; Asfaw, Z. Woody species diversity under natural forest patches and adjacent enset-coffee based Agroforestry in the Midland of Sidama Zone, Ethiopia. *Int. J. Biodivers. Conserv.* **2014**, *6*, 708–723.
43. Lacerda, D.R.; Lemos, J.P.; Lovato, M.B. Molecular differentiation of two vicariant neotropical tree species, *Plathymenia foliolosa* and *P. reticulata* (Mimosoideae), inferred using RAPD markers. *Plant Syst. Evol.* **2002**, *235*, 67–77. [CrossRef]
44. de Souza, L.; Júnior, A.L.S.; Souza, M.; Kunz, S.; Miranda, F.D. Genetic diversity of *Plathymenia reticulata* Benth. in fragments of Atlantic Forest in southeastern Brazil. *Genet. Mol. Res.* **2017**, *16*, 1–13. [CrossRef]
45. Conceição, T.A.; Santos, A.S.; Fernandes, A.K.C.; Meireles, G.N.; de Oliveira, F.A.; Barbosa, R.M.; Gaiotto, F.A. Guiding seed movement: Environmental heterogeneity drives genetic differentiation in *Plathymenia reticulata*, providing insights for restoration. *AoB Plants* **2024**, *16*, plae032. [CrossRef]
46. Leal, J.; Santos, R.; Gaiotto, F. Effect of selective logging on genetic diversity and gene flow in Cariniana legalis sampled from a cocoa agroforestry system. *Genet. Mol. Res.* **2014**, *13*, 626–635. [CrossRef] [PubMed]
47. Liu, X.; Dong, T.; Zhang, S. Adaptation Responses of Dioecious and Hermaphroditic Tree Species to Abiotic Stress. *Forests* **2023**, *14*, 383. [CrossRef]
48. Lowe, A.; Boshier, D.; Ward, M.; E Bacles, C.F.; Navarro, C. Genetic resource impacts of habitat loss and degradation; reconciling empirical evidence and predicted theory for neotropical trees. *Heredity* **2005**, *95*, 255–273. [CrossRef]
49. Dawson, I.K.; Leakey, R.; Clement, C.R.; Weber, J.C.; Cornelius, J.P.; Roshetko, J.M.; Vinceti, B.; Kalinganire, A.; Tchoundjeu, Z.; Masters, E.; et al. The management of tree genetic resources and the livelihoods of rural communities in the tropics: Non-timber forest products, smallholder agroforestry practices and tree commodity crops. *For. Ecol. Manag.* **2014**, *333*, 9–21. [CrossRef]
50. Waqar, Z.; Moraes, R.C.S.; Benchimol, M.; Morante-Filho, J.C.; Mariano-Neto, E.; Gaiotto, F.A. Gene Flow and Genetic Structure Reveal Reduced Diversity between Generations of a Tropical Tree, Manilkara multifida Penn., in Atlantic Forest Fragments. *Genes* **2021**, *12*, 2025. [CrossRef]
51. Phang, A.; Niissalo, M.A.; Ruhsam, M.; Pezzini, F.F.; Neo, W.L.; Burslem, D.F.R.P.; Middleton, D.J.; Wilkie, P.; Khew, G.S. Genetic erosion in a tropical tree species demonstrates the need to conserve wide-ranging germplasm amid extreme habitat fragmentation. *Biodivers. Conserv.* **2024**, *33*, 2527–2548. [CrossRef]
52. Barreto, J.R.; Leakey, R.; Clement, C.R.; Weber, J.C.; Cornelius, J.P.; Roshetko, J.M.; Jamnadass, R. When forest loss leads to biodiversity gain: Insights from the Brazilian Atlantic Forest. *Biol. Conserv.* **2023**, *279*, 109957. [CrossRef]
53. Ksiazek-Mikenas, K.; Fant, J.B.; Skogen, K.A. Pollinator-mediated gene flow connects green roof populations across the urban matrix: A paternity analysis of the self-compatible forb Penstemon hirsutus. *Front. Ecol. Evol.* **2019**, *7*, 299. [CrossRef]
54. Duminil, J.; Hardy, O.J.; Petit, R.J. Plant traits correlated with generation time directly affect inbreeding depression and mating system and indirectly genetic structure. *BMC Evol. Biol.* **2009**, *9*, 177. [CrossRef]
55. Ratnam, W.; Rajora, O.P.; Finkeldey, R.; Aravanopoulos, F.; Bouvet, J.-M.; Vaillancourt, R.E.; Kanashiro, M.; Fady, B.; Tomita, M.; Vinson, C. Genetic effects of forest management practices: Global synthesis and perspectives. *For. Ecol. Manag.* **2014**, *333*, 52–65. [CrossRef]
56. Klimova, A.; Mondragón, K.Y.R.; Aguirre-Planter, E.; Valiente, A.; Lira, R.; Eguiarte, L.E. Genomic analysis unveils reduced genetic variability but increased proportion of heterozygotic genotypes of the intensively managed mezcal agave, Agave angustifolia. *Am. J. Bot.* **2023**, *110*, e16216. [CrossRef] [PubMed]
57. Kithure, R.K.; Muchugi, A.; Jamnadass, R.; Njoka, F.M.; Mwaura, L. Genetic diversity of Faidherbia albida (Del.) A. Chev accessions held at the World Agroforestry Centre. *For. Trees Livelihoods* **2015**, *24*, 219–230. [CrossRef]
58. Bem, E.A.; Bittencourt, J.V.M.; Moraes, M.D.; Sebbenn, A. Scenarios of selective logging on genetic diversity and basal area of Araucaria angustifolia populations, with base in Ecogene modeling. *Sci. For. For. Sci.* **2015**, *43*, 453–466.
59. Wang, R.; Compton, S.G.; Chen, X.-Y. Fragmentation can increase spatial genetic structure without decreasing pollen-mediated gene flow in a wind-pollinated tree. *Mol. Ecol.* **2011**, *20*, 4421–4432. [CrossRef]

60. Kindt, R.; Van Damme, P.; Simons, A.J.; Beeckman, H. Planning tree species diversification in Kenya based on differences in tree species composition between farms. I. Analysis of tree uses. *Agrofor. Syst.* **2006**, *67*, 215–228. [CrossRef]
61. Dawson, I.K.; Guariguata, M.R.; Loo, J.; Weber, J.C.; Lengkeek, A.; Bush, D.; Jamnadass, R. What is the relevance of smallholders' agroforestry systems for conserving tropical tree species and genetic diversity in circa situm, in situ and ex situ settings? A review. *Biodivers. Conserv.* **2013**, *22*, 301–324. [CrossRef]
62. Ward, M.; Dick, C.W.; Gribel, R.; Lowe, A.J. To self, or not to self... A review of outcrossing and pollen-mediated gene flow in neotropical trees. *Heredity* **2005**, *95*, 246–254. [CrossRef]
63. White, G.M.; Boshier, D.; Powell, W. Increased pollen flow counteracts fragmentation in a tropical dry forest: An example from Swietenia humilis Zuccarini. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 2038–2042. [CrossRef]
64. Lemos Filho, J.P.D.; Goulart, M.F.; Lovato, M.B. Populational approach in ecophysiological studies: The case of *Plathymenia reticulata*, a tree from Cerrado and Atlantic Forest. *Braz. J. Plant Physiol.* **2008**, *20*, 205–216. [CrossRef]