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Seed bank analysis as an indicator of environmental recovery following the fundão dam disaster in Mariana, Minas Gerais

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ABSTRACT

Background: The study aimed to assess the effectiveness of restoration practices following the Fundão disaster in the Brazilian Atlantic Forest, a key region for global biodiversity. Focusing on affected areas in Mariana, MG, the study used seed bank analysis as a key indicator to understand environmental recovery progress. The study compared active and passive restoration areas with a reference ecosystem by collecting 168 soil samples, which were subjected to germination in a greenhouse.

Results: The results revealed a high germination rate of herbaceous seeds, predominantly native species. Significant differences were found in floristic composition among the different restoration types studied. Areas undergoing active restoration showed greater similarity to the reference ecosystem, emphasizing the importance of Distance from Forest Fragment, Percentage of Forest Area in the Surroundings, and species diversity for restoration success.

Conclusion: The results highlight the crucial importance of landscape connectivity for the success of ecological restoration. Active restoration strategies play a fundamental role in accelerating environmental recovery and bringing degraded areas closer to the floristic composition of reference ecosystems.

Keywords: Atlantic Forest; Connectivity; Ecological restoration.

HIGHLIGHTS

Herbaceous species dominated the seed bank, even in mature ecosystems. Increased forested area in the surrounding matrix enhanced local diversity. Proximity to forest fragments boosts species diversity. Active restoration techniques show greater ecological effectiveness.

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INTRODUCTION

The Brazilian Atlantic Forest stands out as a critical global biodiversity hotspot (Myers et al., 2000). It is estimated to harbor around 15,000 plant species, with approximately half of them being endemic (Lima et al., 2024). Despite its original extent of about 131 million hectares, only 12.3% of its forest cover remains today (SOS Mata Atlântica and INPE, 2024). Consequently, the landscape is now comprised of forest fragments embedded within a matrix dominated by urban areas, agricultural activities, and other anthropogenic land uses (Broggio et al., 2024).

In Minas Gerais, the Atlantic Forest initially covered 49% of the state's area. However, by 2023, the remaining forest fragments of this biome represented only 10.1% of its original coverage (SOS Mata Atlântica and INPE, 2024). The collapse of the Fundão dam in 2015 significantly contributed to the loss of vegetation cover. Omachi et al. (2018) estimated that the mud from the dam's waste destroyed 457.6 hectares of native Atlantic Forest, with the greatest losses concentrated along the first 100 km from the Fundão dam. In this context, ecological restoration activities emerge as a crucial tool for mitigating the impacts on local flora (Holl, 2020).

In areas affected by the Fundão waste, different restoration techniques have been applied (Campanharo et al., 2020, 2021; Martin et al., 2020). However, despite these efforts, there remains a significant gap in understanding the effectiveness of these restoration approaches. In this context, the seed bank emerges as a crucial ecological indicator, capable of providing valuable insights into the progress of restoration (Silva et al., 2021).

The analysis of the seed bank enables a deeper understanding of the evolution of areas undergoing restoration (Martins et al., 2024). This tool provides an objective assessment of the effectiveness of restoration practices and offers a solid foundation for improving and guiding future strategies (Balestrin et al., 2019; Martins et al., 2021). In this study, we evaluated the techniques for recovering areas degraded by the Fundão waste in Mariana, Minas Gerais, Brazil, using environmental variables and seed bank analysis.

MATERIAL AND METHODS

Study Area

This study was conducted along the banks of the Gualaxo do Norte River, within the Doce River basin, located in the municipality of Mariana, Minas Gerais (MG). The predominant climate of the region, according to the Köppen international classification system, is Cwa (temperate mesothermal climate), with dry winters and rainy summers (Alvares et al., 2013). The average temperature and precipitation are 19.7°C and 1,804 mm, respectively (Climate-Data.Org, 2024).

The original vegetation was characterized by semideciduous seasonal forest, within the Atlantic Forest domain. However, the study areas were subjected to the deposition of tailings from the collapse of the Fundão dam in 2015, primarily composed of iron oxide and silica (Samarco, 2017; Esteves et al., 2020).

Five areas undergoing active or passive restoration processes and one reference ecosystem area were evaluated. These areas were divided into different groups, as detailed in Table 1 and Figure 1. In 2016, the areas under active restoration received interventions through the sowing of a mix of fast-growing species, mainly herbaceous species (Table 2). Subsequently, native tree species from the region were planted. No silvicultural practices, such as soil preparation or fertilization, were carried out in any of the areas. Lastly, it is important to note that the reference ecosystems were not impacted by the tailings and are mature forest areas with a low degree of human intervention.

Data Collection

The transects, 10 meters wide and of varying lengths, were installed, extending from the riverbank to the end of the area impacted by the tailings. The number of transects varied according to the size of the area. In total, 3 transects were allocated in G1 and G4, and 5 transects in G2, G3, and G5. Within each transect, four equally spaced plots were marked. Two soil samples were collected from each plot, totaling eight samples per transect. A metal frame of 0.25 x 0.25 meters was used to collect soil up to a depth of 5 centimeters. In total, 168 soil samples were collected.

Table 1: Description of the groups evaluated along the Gualaxo do Norte River banks, in the Doce River ba	sin, after the
deposition of tailings from the Fundão dam collapse in Mariana, MG.	

Restoration methods	Evaluated area (hectares)	Geographic coordinates
G1 - Reference ecosystem without tailings	01 ha	20°14'51.0"S 43°20'58.7"W 20°15'30.6"S 42°59'22.1"W
G2 - Passive restoration	02 ha	20°16'16.3''S 43°18'49.8''W 20°16'19.5"S 43°11'42.4"W
G3 - Active restoration with direct seeding in 2016 and total planting in 2018	02 ha	20°15'07.9"S 43°22'31.4"W 20°18'04.1''S 43°13'54.5''W
G4 - Active restoration with direct seeding in 2016 and total planting in 2019	01 ha	20°16'16.1"S 43°18'40.9"W 20°14'24.9"S 43°25'12.8"W
G5 - Active restoration with direct seeding in 2016 and total planting in 2020	02 ha	20°14'31.5"S 43°24'15.0"W 20°15'44.7"S 43°07'59.9"W



Figure 1: Location map of the study areas in the Doce River basin, along the banks of the Gualaxo do Norte River, in the state of Minas Gerais.

Table 2: Species	present in the	seed mix u	used for th	ne recover	y of areas	s impacted	by tailing	s from t	he Fundão	dam
rupture in Mariana	, MG.									

Family	Species	Common Name	Habit	
Amaranthaceae	Alternanthera tenella	Apaga fogo	Subshrub	
Asteraceae	Helianthus annuus	Girassol forrageiro	Herbaceous	
Brassicaceae	Raphanus sativus	Nabo forrageiro	Herbaceous	
Fabaceae	Cajanus cajan	Feijão guandu	Shrub	
Fabaceae	Canavalia ensiformis	Feijão de porco	Herbaceous	
Fabaceae	Crotalaria sp.	Chocalho de cascavel	Shrub	
Fabaceae	Lablab purpureus	Dolichos lab lab	Herbaceous	
Fabaceae	Neonotonia wightii	Soja perene	Herbaceous	
Fabaceae	Stylosanthes sp.	Estilosante	Herbaceous	
Fabaceae	Vicia sativa	Ervilhaca	Herbaceous	
Poaceae	Avena sp.	Aveia amarela	Herbaceous	
Poaceae	Cynodon dactylon	Grama seda	Herbaceous	
Poaceae	Lolium multiflorum	Azevém	Herbaceous	
Poaceae	Cenchrus americanus	Milheto	Herbaceous	
Poaceae	Cenchrus polystachios	Capim custódio	Herbaceous	
Poaceae	Sorghum bicolor	Sorgo formoso	Herbaceous	

The collected material was stored in labeled plastic bags and transported to the Forest Nursery at the Federal University of Lavras. The trays, measuring 50 cm x 30 cm x 10 cm, were irrigated on alternate days or as needed, and the resulting seedlings were counted, identified, and removed immediately after recording over a three-month period.

A convex spherical densiometer was used to determine canopy cover. Readings were taken at the center of each plot in four directions (north, south, east, and west), approximately 1.3 meters above the ground. The canopy cover percentage was calculated as the average of the readings in the four directions (Piaia et al., 2021).

To understand the impact of the surrounding matrix on the results, we recorded the distance from each plot to the nearest forest fragment. Using a GPS, we marked the coordinates of the furthest point from each plot and drew a straight line to the forest fragment's edge using QGIS 3.16.8. We also mapped land use within a 1 km radius around each study site, determining the percentage of native forest and anthropized areas in the surrounding matrix. Sentinel-2 satellite images with bands 11, 8, and 4 were used. The images were processed and classified in QGIS 3.16.8, employing the Dzetsaka Classification Tool plugin and the supervised classification method for satellite images.

Data Analysis

The changes in species composition among the analyzed groups were investigated using Permutational Multivariate Analysis of Variance (PERMANOVA; adonis in the vegan package) (Anderson, 2001). To visualize the floristic communities in space, the Non-Metric Multidimensional Scaling (NMDS) technique was applied, utilizing the Bray-Curtis similarity metric with the vegan package (Dixon, 2003).

The data underwent multivariate principal component analysis (PCA), with variables standardized based on Z-scores. This procedure involves transforming the variables so that they have a mean of zero and a standard deviation of one. It is a procedure that eliminates scale differences between variables and prevents those with higher values from dominating statistical analysis. This multivariate approach proceeded in two phases: one to select the most explanatory variables of total variance and reduce information dimensionality, and another to identify groups using biplot visualization and confidence ellipses. Selection of the most explanatory variables in the first (PC1) and second (PC2) principal components was weighted by their eigenvalues, with a uniform minimum contribution threshold set at 14.29%, calculated as the ratio of 1 unit to the total number of indicators (7 variables) (Kassambara and Mundt, 2019).

The dimensionality reduction assessment between phases of the multivariate approach followed Kaiser's criterion (Kaiser, 1958), considering eigenvalues significant if greater than 1 (λ 1 > 1). Statistical and biological analyses of Spearman's correlation coefficient were conducted for selected variables.

A significance level of 5% was used for statistical effect diagnosis. All statistical analyses were performed

using R software version 4.3.3 (R Core Team, 2024) and packages "Stats" (R Core Team, 2024) and "factoextra" (Kassambara and Mundt, 2022).

RESULTS

A total of 138.95 seeds germinated per square meter. Of this total, 85.85% were classified as herbaceous, 8.55% as subshrubs, 4.92% as shrubs, and 0.68% as trees. Additionally, among the plants identified to the species level, 75.77% of the germinated seeds were native to the region, while 24.23% were exotic. Of the total sampled plants, 90 were determined at the species level and 7 at the genus level (Supplementary Material). Due to the difficulty of precise identification within the Poaceae family, a set of different grass species were characterized at the family level and designated as *Poaceae* spp.

The sampling encompassed a total of 37 families across the entire inventoried area, with common presence of Malvaceae, Poaceae, Phyllanthaceae, Asteraceae, Solanaceae, and Fabaceae in all environments. The highest abundances of individuals per square meter identified in the reference ecosystem were represented by Brassicaceae (30.67%), Asteraceae (28.94%), and Poaceae (14.41%). In the passive restoration environment, the most abundant families were Cyperaceae (41.89%), Poaceae (26.18%), and Asteraceae (19.07%). Conversely, in the actively restored environments, Poaceae was the most representative family, with 73.75% in G3, 68.95% in G4, and 44.31% in G5 (Figure 2).



Figure 2: Non-metric multidimensional scaling (NMDS) analysis of species floristic composition in the 5 studied groups. Where: G1 – Reference ecosystem without tailings; G2 – Passive restoration with natural regeneration; G3 – Active restoration with seed mix in 2016 and enrichment planting in 2018; G4 – Active restoration with seed mix in 2016 and enrichment planting in 2019; and G5 – Active restoration with seed mix in 2020.

The permutest analysis (F = 13.02, df = 83, p < 0.05) followed by the PERMANOVA test (F = 20.37, df = 83, p < 0.05) showed significant differences in floristic composition among the different evaluated groups. The NMDS analysis indicated that the G4 community exhibited the greatest floristic similarity to the reference ecosystem.

The mean values of the evaluated attributes after the implementation of environmental restoration strategies are described in Table 3. Statistical differences among groups were observed for all evaluated parameters, according to the Kruskal-Wallis test at a significance level of 5%: Canopy Cover ($\chi^2 = 47.27$, df = 4, p < 0.05), Distance to Nearest Fragment ($\chi^2 = 56.50$, df = 4, p < 0.05), Forested Area in the Surrounding Matrix ($\chi^2 = 83$, df = 4, p < 0.05), Density of Individuals ($\chi^2 = 18.34$, df = 4, p < 0.05), Family Richness ($\chi^2 = 23.94$, df = 4, p < 0.05), Shannon-Weaver Diversity Index ($\chi^2 = 27.84$, df = 4, p < 0.05), and Pielou Evenness Index ($\chi^2 = 23.65$, df = 4, p < 0.05).

According to the Kaiser criterion, the first phase of PCA revealed 2 principal components, explaining 64.40% of the total variance. For subsequent statistical analyses, only the variables Distance to Nearest Fragment, Forest Area in the Surrounding Matrix, Density of Individuals, and diversity indices Shannon-Weaver and Pielou (calculated per family) were selected (Table 4). In the second phase of PCA multivariate analysis, two principal components with eigenvalues greater than 1 were identified, statistically explaining 80.42% of the total variance (PC1 = 53.16% and PC2 = 27.28%).

Principal Component 1 (PC1) exhibited variables with eigenvalues of greater magnitude, expressed in absolute terms, compared to Principal Component 2 (PC2), except for the Density of Individuals variable. Both principal components had 3 variables with negative eigenvalues, with Shannon-Weaver and Pielou diversity indices being common to both. The most positive eigenvalues were found for Distance to Nearest Fragment in PC1 and Density of Individuals in PC2. The magnitude of the eigenvalues was positively associated with the contribution of the variables, with the same correlation value for each principal component (r = 0.99; $p \le 0.05$).

The biplot graphical analysis discriminated the similarity between environmental restoration strategies (Figure 3). The overlap of confidence ellipses (1 – α = 95%) revealed the following decreasing order of similarity with the reference ecosystem (G1): G5 \approx G4 > G2 > G3. The reference ecosystem was characterized especially by eigenvalues related to the percentage of forest area in the surroundings and family diversity indices (H' and J).

Higher percentages of Forested Area in the Surrounding Matrix and family diversity (H' e J) in environments under environmental restoration were associated with proximity to forest fragments (Table 5). However, Density of Individuals did not correlate significantly (p > 0.05) with forested area in the surroundings or distance to preserved fragments. Shannon-Weaver diversity indices and Pielou Evenness Index decreased with increasing Density of Individuals.

Table 3: Average values of the attributes evaluated in environmental recovery strategies following the Fundão dam rupture in Mariana, MG. CC = Canopy Cover; DNF = Distance to Nearest Fragment; FA = Forested Area; FR = Family Richness; DI = Density of Individuals; H' = Shannon-Weaver index calculated per family (H') and; J = Pielou index calculated per family.

Variables	G1	G2	G3	G4	G5
CC (%)	73.30 ª	51.27 ^b	25.44 ^{cd}	46.74 bc	10.05 d
DNF (m)	0.00 ª	54.14 ^b	121.53 °	44.15 ^b	58.71 ^b
FA (%)	79.03 ab	74.13 °	66.48 d	80.02 ª	77.77 b
FR	9 a	5 ^b	6 ^{bc}	8 ac	7 ^{ac}
DI (ind. m ⁻²)	1083 ª	2521 ^b	1548 ab	2980 b	1421 ª
H'	1.61 a	1.03 b	0.85 b	1.00 b	1.14 b
J	0.76 ª	0.65 ab	0.47 ^b	0.51 ^b	0.66 ab

Same letters indicate no significant difference by Kruskal-Wallis (p < 0,05) according to the Kruskal-Wallis test.

Table 4: Eigenvectors and contributions of selected variables via PCA (PC = principal component 1 and PC2 = principal component 2), applied to environmental recovery strategies following the Fundão dam rupture in Mariana, MG.

Selected variables	Eigenv	vectors	Contributions (%)	
	CP1	CP2	CP1	CP2
Distance to nearest fragment (DNF) (m)	0.76	-0.49	21.88	17.75
Forested area (FA) (%)	-0.68	0.63	17.23	29.03
Density of Individuals (DI) (ind. m ⁻²)	0.41	0.74	6.45	40.21
H' calculated for families (H')	-0.85	-0.23	27.36	3.72
J calculated for families (J)	-0.85	-0.36	27.08	9.30



Figure 3: A: Biplots of principal component analysis. B: eigenvectors. Ellipses at 95% confidence level. Where: G1 – Reference ecosystem without tailings; G2 – Passive restoration with natural regeneration; G3 – Active restoration with seed mix in 2016 and enrichment planting in 2018; G4 – Active restoration with seed mix in 2016 and enrichment planting in 2018; G4 – Active restoration with seed mix in 2016 and enrichment planting in 2018; G4 – Active restoration with seed mix in 2016 and enrichment planting in 2018; G4 – Active restoration with seed mix in 2016 and enrichment planting in 2018; G4 – Active restoration with seed mix in 2016 and enrichment planting in 2018; G4 – Active restoration with seed mix in 2016 and enrichment planting in 2018; G4 – Active restoration with seed mix in 2016 and enrichment planting in 2018; G4 – Active restoration with seed mix in 2016 and enrichment planting in 2020.

Table 5: Spearman correlation matrix for the selected variables via PCA, applied to environmental recovery strategies following the Fundão dam rupture in Mariana, MG.

Selected variables	DNF	FA	DI	H'	J
Distance to nearest fragment (DNF) (m)	1	-0.67*	0.12 ^{ns}	-0.47*	-0.41*
Forested area (FA) (%)	-	1	-0.02 ^{ns}	0.36*	0.21*
Density of Individuals (DI) (ind. m ⁻²)	-	-	1	-0.32*	-0.55*
H' calculated for families (H')	-		-	1	0.77*
J calculated for families (J)	-	-	-	-	1

*, ns significant and non-significant at 5% probability by Spearman correlation, respectively.

DISCUSSION

In our study, the majority of identified species were herbaceous, a result also observed by Silva et al. (2021) in areas degraded by the Fundão tailings dam collapse in Mariana - MG. Additionally, several studies in the Atlantic Forest have concluded that herbaceous species dominate the seed bank (Figueiredo et al., 2014; Oliveira et al., 2018; Silva et al., 2019; Duarte et al., 2022; Fernandes et al., 2022).

Among the evaluated environments, the reference ecosystem exhibited the lowest density of herbaceous individuals in the seed bank, as these species are less dominant in mature forests (Mores et al., 2020). In wellestablished ecosystems, tree and shrub vegetation predominates, reducing light availability in the understory and hindering the proliferation of herbaceous species (Shah et al., 2024). However, the presence of herbaceous in this environment is due to their ability to produce large quantities of small seeds that quickly penetrate the litter layer and reach the soil faster than larger seeds (Madawala et al., 2016; Carvalho et al., 2022). Additionally, herbaceous species have rapid germination (Arnolds et al., 2015; Anju et al., 2022; Guimarães et al., 2024) and a higher likelihood of escaping granivore predation compared to larger seeds (Gómez, 2004; Zida et al., 2020).

The groups G5 and G4 showed high similarity with the reference ecosystem (G1), indicating progress in active restoration strategies over time. The G2, representing passive restoration, exhibited moderate similarity with the reference ecosystem, suggesting some success, albeit not as much as active restoration strategies. Although G2 benefits from favorable environmental conditions, such as higher canopy cover and proximity to fragments, species planting in the G5 and G4 areas may be contributing to their greater similarity to the reference area by attracting dispersing animals that aid in local enrichment. Nevertheless, it is essential to emphasize that natural regeneration remains a valid strategy, although it may require more time to achieve effective recovery and levels of similarity comparable to the reference ecosystem (Trujillo-Miranda, 2018; Zahawi et al., 2014).

In the Spearman correlation matrix, we identified an inverse relationship between increasing density and diversity indices. This finding suggests that as individual density increases, diversity tends to decrease. This pattern may be attributed to the high dominance of certain species, particularly from the Poaceae family. Due to the ease of germination of these species, their abundant presence can suppress the occurrence of others, thus reducing overall species richness (Mantoani and Torezan, 2016; Rezende and Vieira, 2019; Truong et al., 2020; Weidlich et al., 2020).

Our data demonstrate that the percentage of forested area surrounding the study site directly influenced diversity indices H' and J. According to the Theory of Island Biogeography, as the surrounding forest area increases, species diversity also tends to increase (Anyango et al., 2024; Martensen et al., 2012). This consistency between theory and observed data reinforces the importance of the surrounding matrix in the restoration of natural areas, highlighting how the size of the surrounding area significantly influences local biodiversity (Santos et al., 2018).

Fan et al. (2024) emphasize that according to the Theory of Island Biogeography, fragment size and distance are the main determinants of species diversity in habitats. In this study, we observed that greater distances from forest fragments result in lower area diversity (Liu et al., 2019; Marcantonio et al., 2013). As fragmentation advances, finding areas for restoration near forested areas becomes challenging. Often, these areas are surrounded by lands allocated for agricultural activities, limiting the success of ecological restoration practices (Santos et al., 2018).

Distances greater than 100 meters represent a significant barrier to seed dispersal from the surroundings towards areas under restoration (Bakker et al., 1996; Cain et al., 2000; Kettle, 2012; Wunderle-Jr, 1997). This negatively impacts the restoration process and its long-term persistence due to reduced interaction with the surrounding matrix. Thus, the implementation of ecological corridors emerges as a crucial strategy to enhance landscape connectivity, contributing to the conservation and restoration of ecosystems (Dudley et al., 2024).

CONCLUSION

In this study, active restoration techniques proved to be more effective than passive restoration methods. The results indicated that herbaceous species dominate the seed bank, playing a crucial initial role in ground cover and soil protection. However, to ensure long-term ecological resilience, it is vital for woody species to also establish successfully in these restored areas. The practical implications of this study are clear: for mining-affected areas, such as those impacted by the rupture of the Fundão dam, to recover effectively, it is essential to adopt active restoration strategies that focus on species diversification and strengthening ecological connectivity. In general, ecological restoration managers tend to focus their efforts at local scales, often without adequately considering the broader landscape context of the restoration areas. In this regard, landscape connectivity analysis emerges as a critical criterion. This means identifying and prioritizing areas that contribute to landscape connectivity in the region, creating ecological corridors that support restoration efforts. Although natural regeneration is an important process, it may not be sufficient to fully restore biodiversity in severely impacted areas, such as those affected by mining tailings. Therefore, it is crucial to integrate natural regeneration with active interventions to ensure a more robust and sustainable recovery. It is recommended that studies on natural regeneration be conducted to assess how ecosystem perpetuation will unfold over time.

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AUTHORSHIP CONTRIBUTION

Project Idea: SAB; CVGR Funding: SAB Database: CVGR; BOF; AFR; ACGS Processing: CVGR; BOF Analysis: CVGR; BOF Writing: CVGR; SAB; BOF Review: CVGR; AFR; BOF; SAB; LAM

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