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ARTICLE

# Effects of Forest Restoration Techniques on Community Diversity and Aboveground Biomass on Area Affected by Mining Tailings in Mariana, Southeastern Brazil

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ABSTRACT

Currently there is an urgent and special attention in actions to restore tropical forests. In this study, we evaluated the effect of different restoration methods on aboveground biomass (AGB) stock, tree community diversity and structure, in areas affected by the Fundão tailings dam collapse in Mariana, Minas Gerais state, Brazil. We measured and compiled data of the AGB, community diversity and structure attributes in 36 plots distributed in six restoration treatments and six replicas: planting of native tree seedlings with fertilization and pH correction (PSf) and without fertilization and pH correction (PS); seeding of native trees with fertilization and pH correction (SDf) and without fertilization and pH correction (SD); natural regeneration with fertilization and pH correction (NRf) and without fertilization and pH correction (NR). No significant differences in substrate properties and AGB between treatments. Although biomass storage between treatments was not statistically different, there is a clear pattern showing higher values active restoration method. The Pielou index ranged from 0.520 (SDf) to 0.943 (NR), except for SDf all the others treatments had values higher than 0.76. This result suggests floristic heterogeneity, without ecological dominance in the plant community. Overall, active restoration had important implications for the forest restoration where natural regeneration is limited.

## 1. Introduction

Five years after the Fundão dam collapse in Mariana, Brazil, the ecological recovery of degraded forest continues to have a high priority [1]. The collapse released almost 40 million $m^3$ of tailings in the Gualaxo do Norte, Carmo and Doce rivers. Since then, different methods of active and passive restoration have been applied throughout the affected area [1]. Nevertheless, there is still an urgent need to know the efficiency of these methods and their response to fertilization on the area affected by the taillings by monitoring ecological indicators

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of rapid assessment and direct positive effects on ecosystem stabilization, such as plant community diversity and aboveground biomass.

Passive restoration is the spontaneous forest recovery that occurs without active human intervention, nevertheless this method can also require fencing to control livestock grazing, invasive species control, and fire protection [2,3]. However, in areas with extensive deforestation, invasive grasses, isolated from seed sources and soil degradation may reduce natural regeneration [4,5]. Thus, active restoration might be more effective to speed up the recovery process (i.e. biodiversity and ecosystem functioning), accomplished through planting of nursery-grown seedlings, direct seeding, weeding, and thinning to achieve desired structural features of the vegetation [5]. Furthermore, larger mixed tree plantings may also supply significant ecosystem services in isolated landscapes, such as in situ biodiversity conservation of planted tree species and habitat refuge in highly deforested regions [6-8].

Site preparation, such as fertilization, mechanical interventions, prescribed burning, herbicides, and mulching, can remarkably influence forest restoration success, because it is designed to improve early natural regeneration, tree survival and growth [9-11]. Thus, soil nutrient content; undoubtedly play important roles in biomass recovery [12]. Soil properties variability can create conditions that allow the species colonization and growth, and that differ in their resource requirements, consequently determine species richness and ecosystem function [13-15]. In this context, forest restoration must transcend the manipulation of tree species according to their potential in the ecosystem services provision, as well as, better understand the biodiversity and ecosystem functioning pattern (i.e. biomass stock) in restoration plans [16]. Thus, studies have compared seedling growth and survival rates following different restoration methods, but few have directly compared the effects of active and passive restoration and their response to fertilization on AGB and diversity.

In this study, we evaluated the effect of different restoration methods (i.e. planting of nursery-grown seedlings, direct seeding and natural regeneration) and fertilization/pH correction on tailings substrate on AGB stock, tree community diversity and structure in areas affected by the Fundão tailings dam collapse in Mariana, Minas Gerais state, southeastern Brazil. We hypothesize that: different restoration methods and fertilization/pH correction determine changes in plant community structure and diversity, and aboveground biomass. We expect AGB stock to be higher for treatments with fertilization/correction and lower for natural regeneration plots without fertilization and pH correction.

## 2. Material and Methods

### 2.1 Experimental Site

The study was carried out in areas affected by the Fundão tailings dam collapse in the district of Paracatu de Baixo (43°11'59.55"W, 20°16'32.91"S) municipality of Mariana, Minas Gerais, Brazil (Figure 1). The study area has a moderate humid tropical climate, with a dry season occurring from May to September and a wet season occurring between December and March. The mean annual relative humidity is ca. 80%, mean annual air temperature is 19°C and mean annual precipitation is 1340 mm. The study area is located between 505 and 515 m above sea level, and the relief is mainly flat to weakly undulating. The region is characterized by the presence of two dominant soil classes: a Dystric Red-Yellow Latosol covers hilltops and mountainsides, while a Cambic Red-Yellow Podzolic dominates the upper fluvial terraces [17].

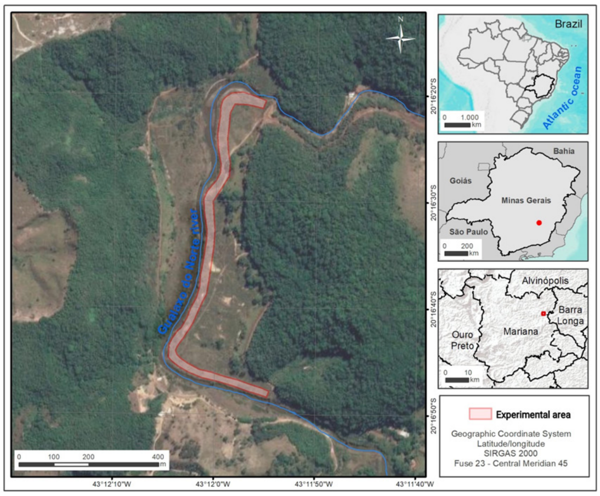

**Figure 1.** Location of the study area along the Gualaxo do Norte river, in relation to South America, the Minas Gerais State and the Mariana municipality

The tailings accumulated in study area present different depths (ca 80-100 cm) on a flat and homogeneous topography along river. The riparian vegetation along the Gualaxo do Norte river is classified as semideciduous seasonal forest [18]. Also, it is worth mentioning that the study area had a long history of land use based on pasture for livestock before the accumulation of mining tailings.

### 2.2 Experimental Design

Approximately 16 months after of the Fundão dam collapse, in March of 2017, 36 plots with different restoration treatments were established in line along the river. A randomized block design with six restoration treatments was used, consisting of six replicates plots for each treatment: planting of native tree seedlings with fertilization and pH correction (soil acidity correction by limestone) (PSf) and without fertilization and pH correction (PS); seeding of native trees with fertilization and pH correction (SDf) and without fertilization and pH correction (SD); natural regeneration with fertilization and pH correction (NRf) and without fertilization and pH correction (NR), as a control treatment. Native seedlings were planted using a spacing of 3 × 2 m while the plots where seeding were foreseen the spacing was 3 m between lines of seeding (see species list in table SI and SII from supplementary material, SM hereafter). Two years and four months post restoration an intervention was demarcated a regular area of 144 m² (12 × 12 m) in the center of each plot, where the data was collected, so that a possible edge effect would be eliminated.

## 2.3 Fertilization and Site Preparation Techniques

Calcined dolomitic limestone (100 kg ha$^{-1}$), agricultural gypsum (350 kg ha$^{-1}$), ammonium sulfate (100 kg ha$^{-1}$) and super simple phosphate (150 kg ha$^{-1}$) were applied to improve the substrate fertility in plots where correction and fertilization were foreseen. Subsoiling was used with a depth of 60 centimeters in all the treatments to mix the remaining soil (when available) and breakup the interface between the covered soil and the tailings cover, as well as, plowing to incorporate and mix the fertilizers.

## 2.4 Substrate Sampling and Analyses of Substrate Properties

Substrate were sampled to obtain a better understanding of the different characteristics of the impacted site where the experiment was conducted, to enable comparisons with other experiences in different settings and to serve for reference for interpreting results. In order to measure the substrate properties, composite samples of 5 sub-samples were taken at the edges and center of each plot. Substrate sub-samples were collected from 0 to 10 cm depth using a Dutch auger and pooled together in labeled plastic bags. The samples were analyzed for physical (Sand_c = Coarse sand; Sand_t = Fine sand; Silt and Total clay) and chemical properties following standard protocols [17]. All substrate analyses were performed at the Soil Analysis Laboratory of the Federal University of Viçosa. We measured pH in water (1:2.5 soil-to-solution ratio), extractable phosphorous (P), potassium (K), manganese (Mn), iron (Fe), zinc (Zn) and copper (Cu) using Mehlich-1 extraction. Exchangeable Calcium (Ca) and Magnesium (Mg) were extracted with 1M KCl solution and exchangeable acidity (H + Al) with a 0.5 mol L$^{-1}$ calcium acetate solution at pH 7.0. Total carbon content was estimated by wet combustion and organic matter (OM) calculated using the conventional factor of 1.724. Sum of bases (SB), cation exchange capacity at pH 7 (T), effective cation exchange capacity (t), base saturation percentage (V), were calculated according to the following expressions: SB = $Ca^{2+} + Mg^{2+} + K^{+} + Na^{+}$; CEC pH7 (T) = SB + (H+Al); t = $Ca^{2+} + Mg^{2+} + K^{+} + Al^{3+}$;V = (SB/T) × 100, respectively . Remaining phosphorus (Prem) was analyzed by colorimetry after reacting the samples with a solution of 60 mg L$^{-1}$ of P.

## 2.5 Vegetation Data Collection and Aboveground Biomass Estimation

In each plot were measured the diameter at breast height (DBH = 1,30 m), total height and identified the shrubs and trees species DBH ≥ 2 cm. The Angiosperm Phylogeny Group IV [19] was used for taxon classification.

We calculated the AGB of individual stems using a general allometric equation, based on tree DBH (cm), height (H, m) and wood density (ρ, g cm$^{-3}$) [20]. Tree height was measured with a telescopic ruler. We used Neotropical data from the Global Wood Density Database to obtain the wood density of each species, using genus averages whenever species-level information was not available. We calculated the AGB as follows:

$$AGB = 0.0673\ (\rho \times\ DBH^{2} \times H)^{0.976}$$

The total AGB per plot was the sum of the AGB of all trees with a diameter at breast height ≥ 2 cm which was then converted into megagrams per hectare (Mg ha$^{-1}$) [21]. Species-level biomass was calculated as the sum of the biomass of all stems from a given species [21].

## 2.6 Data Analyses

All analyses were carried out in R Environment [22]. Shannon-Wiener's diversity and Pielou's evenness index were calculated for each plot and restoration treatment. For all variables, we tested normal distribution with Shapiro-Wilk test and by evaluating the Q-Q plot and homogeneity of variances by Bartlett´s test using the "*dplyr*" package [23]. To compare soil properties and aboveground biomass (normally distributed data) between treatments of site conditions, we used ANOVA one-way followed by a posterior Tukey test [22,23]. For species richness, abundance, and diversity indices (non-normally distributed data), we used Kruskal-Wallis's test followed by a posterior Dunn's test performed with the "dunn.test" package [24].

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## 3. Results

### 3.1 Substrate Properties Pattern

No significant differences in substrate properties between treatments of site conditions were observed (Figure 2). The texture properties showed that there is a higher proportion of sand; however, it presents a similar variability between clay and silt (Figure 3). Based on the United States Department of Agriculture soil textural classification, most of the plots were framed as loam texture.

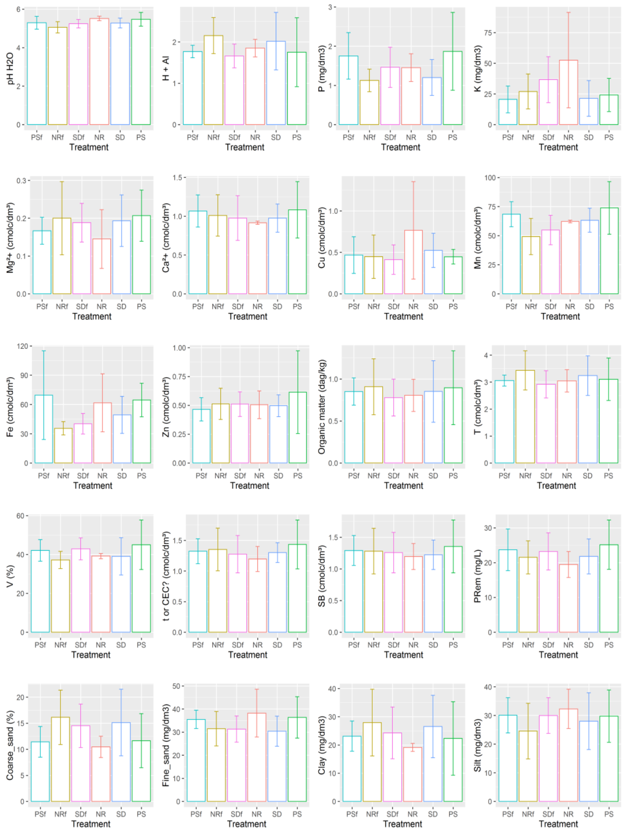

**Figure 2.** Substrate properties pattern in different treatment. For analysis, available: P, K, Ca, Mg, exchangeable acidity (H + Al), pH ($H_2O$), organic matter (OM), sum of bases (SB), effective cation exchange capacity (t), potential cation exchange capacity (T), P-Rem, percentage of bases saturation (V), and the soil texture as coarse sand, fine sand, clay and silt contents were included. Treatments: planting of native tree seedlings with fertilization and pH correction (PSf); planting of native tree seedlings without fertilization and pH correction (PS); seeding of native trees with fertilization and pH correction (SDf); seeding of native trees without fertilization and pH correction (SD); natural regeneration with fertilization and pH correction (NRf); natural regeneration without fertilization and pH correction (NR)

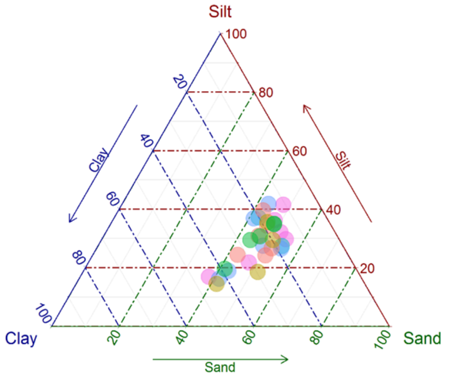

**Figure 3.** Ternary plots showing the relationships between clay, silt and sand proportions in different treatments: planting of native tree seedlings with fertilization and pH correction (PSf); planting of native tree seedlings without fertilization and pH correction (PS); seeding of native trees with fertilization and pH correction (SDf); seeding of native trees without fertilization and pH correction (SD); natural regeneration with fertilization and pH correction (NRf); natural regeneration without fertilization and pH correction (NR)

According to the classification of soils for the state of Minas Gerais [25] the substrate was classified as medium acid (pH), the base saturation varied from low to medium (34.7 - 44.95 %) and the sum of bases as low. Both the effective and potential cation exchange capacity was also framed as low, as well as, the organic matter content (varying from 0.76 to 0.96 dag kg$^{-1}$). K content was classified as low and P as very low, not reaching values above 2.35 mg dm$^{-3}$. Furthermore, all the other nutrients analyzed followed the same classification. Thus, the substrate demonstrates a low fertility. The only contrasting nutrient was Fe, which demonstrated high values (~ 55.16 mg dm-$^{3}$); however, it was expected due to the iron tailings deposition.

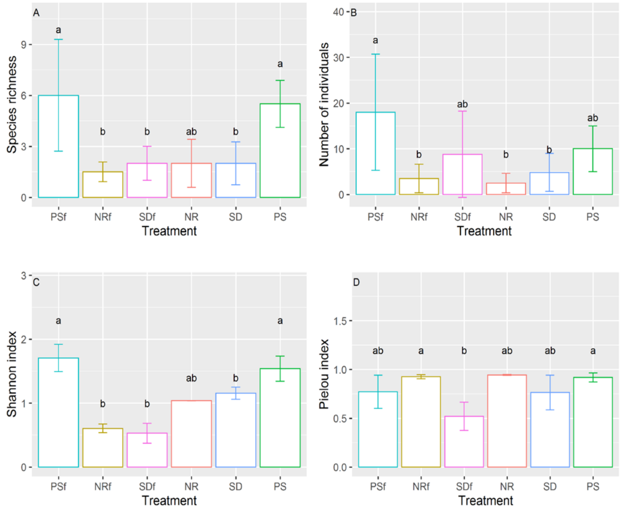

**Figure 4.** Differences in tree species richness, number of individuals, and Shannon-Wiener's diversity and

Pielou's evenness index**.** A randomized block design with six restoration treatments was used, consisting of six replicates for each treatment: planting of native tree seedlings with fertilization and pH correction (PSf); planting of native tree seedlings without fertilization and pH correction (PS); seeding of native trees with fertilization and pH correction (SDf); seeding of native trees without fertilization and pH correction (SD); natural regeneration with fertilization and pH correction (NRf); natural regeneration without fertilization and pH correction (NR)

### 3.2 Differences in Diversity, Abundance and Aboveground Biomass

Species diversity and abundance showed differences between treatments (Figure 4). Species richness ($\chi2 = 16.08$, df = 5, $p < 0.01$), abundance ($\chi2 = 11.31$, df = 5, $p < 0.01$), Shannon index ($\chi2 = 14.80$, df = 5, $p < 0.01$), and Pielou index ($\chi2 = 9.72$, df = 5, $p < 0.01$) showed differences between treatments. However, there are no differences in aboveground biomass ($p = 0.11$).

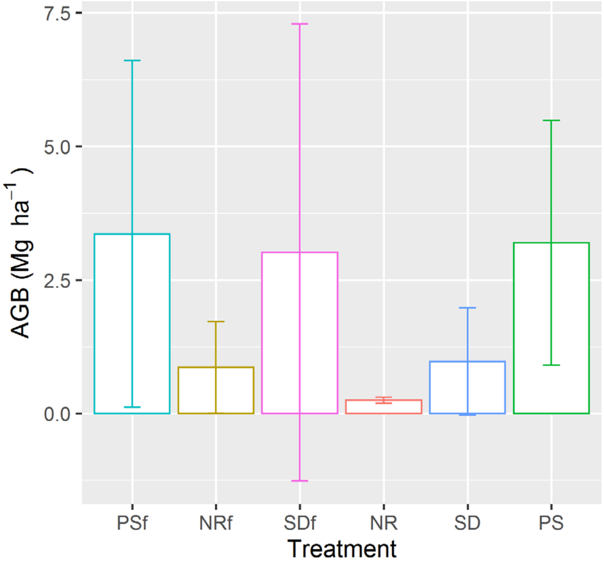

**Figure 5.** Differences in aboveground biomass under a randomized block design with six restoration treatments was used, consisting of six replicates for each treatment: planting of native tree seedlings with fertilization and pH correction (PSf); planting of native tree seedlings without fertilization and pH correction (PS); seeding of native trees with fertilization and pH correction (SDf); seeding of native trees without fertilization and pH correction (SD); natural regeneration with fertilization and pH correction (NRf); natural regeneration without fertilization and pH correction (NR)

The AGB stored in the plots ranged from 0.06 Mg ha$^{-1}$ (NR) to 10.49 Mg ha$^{-1}$ (PSf). On average, the highest AGB storage was found for PSf (3.36 Mg ha$^{-1}$), PS (3.19 Mg ha$^{-1}$), SDf (3.01 Mg ha$^{-1}$) and SD (0.97 Mg ha$^{-1}$). Despite the high variability in AGB storage, it is observed that the treatments of natural regeneration have the lowest values, NRf (0.86 Mg ha$^{-1}$) and NR (0.25 Mg ha$^{-1}$) (Figure 5).

## 4. Discussion

The results showed that there are not contrasting differences in soil properties between treatments, finding stable soil fertility values (i.e. effective cation exchange capacity, base sum, base saturation index, organic matter), probably due to losses by leaching, plant biomass accumulation and nutrients adsorption by oxides since the establishment of the experiment [26,27]. Thus, despite not having detected significant effects of soil properties on diversity and AGB, we presume that initial restoration methods and treatments with fertilization and pH correction (soil acidity correction by limestone) explained significant differences in plant community diversity and structure. These results demonstrate the hypotheses established in this research, that different restoration methods and fertilization/ pH correction determine changes in plant community structure and diversity, but not AGB, probably, due to the early stages of restoration.

Our study demonstrates the importance of active restoration where restoration by natural regeneration is probably limited by different environmental filters. For example, as soil properties are not important predictors to explain AGB, we presumed that invasive grasses (*Urochloa* sp. and *Cynodon dactylon*) observed in the study area might be a biotic filter for natural regeneration [28,29]. Thus, in an area affected by the Fundão dam collapse in the municipality of Mariana, has found high diversity and a fast natural regeneration where invasive grasses were controlled or not present [30]. Accordingly, our results allow to establish management criteria to ensure the establishment of potential native species and the number of individuals appropriated during the initial restoration in areas affected by the Fundão tailings dam collapse in Mariana. This study allows the elucidation of biotic factors (i.e. tree species richness and abundance) that should be considered to establish criteria for active restoration and management in tropical forest. Therefore, the fertilization of planted trees leading to increased aboveground biomass where natural regeneration is limited.

Although biomass storage between treatments was not statistically different, there is a clear pattern where fertilization and pH were used. For example, the AGB followed this order: PSf > PS > SDf > SD > NRf > NR, showing higher values for each restoration method (planting of seedlings, seeding and natural regeneration) where

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fertilization/ pH correction were applied compared with the same methods, but without fertilization and pH correction (Figure 5). Also, the number of individuals has the same pattern between treatments, suggesting that the fertilization might have been the reason for a higher abundance and biomass accumulation along regeneration time. Some reserachers found similar results, where in the first ten years after planting of seedlings the AGB accumulation was 0.95 Mg $ha^{-1}$ $y^{-1}$ and the natural regeneration was hinder by elephant grass (*Pennisetum purpureum*) [31]. In our study site, weed control seems to be particularly important, as the native tree species suffer strong competition from invasive grasses [28,29]. An study compared the effects of two contrasting silvicultural systems at a degraded riparian area in São Paulo, one based on low input (fertilization and weed control) and the other based on high input (intense weed control and fertilization), they found that the aboveground carbon stock was more than three times higher for the high input system than the low input after six years of the restoration interventions (18.2 and 5.2 Mg C $ha^{-1}$, respectively) [32]. During early restoration stages are expected lower AGB and diversity for passive restoration compared with active restoration [31]. For example, the planting of seedlings is important for the restoration of forest degraded because induce higher AGB accumulation rate than non-planted species [31]. The shade created by planted trees generates more appropriate soil quality and favorable conditions for the establishment of old-growth forest species, including suppression of grasses [31,33].

Several studies have shown that soil properties have important effects on plant communities in early forest succession [10,34], mainly, soil nutrients content which can favor the biomass accumulation and shape plant diversity in secondary tropical forests [35,36]. For instance, fertilization with N and P increased seedling richness, reduced mortality [37], accelerated plant growth [38] and also induce changes in plant species composition [39]. Researchers evaluated 72 direct-seeded sites between one and 10 years old, three seedling planting sites and six natural regeneration sites aged from seven to nine years old in Mato Grosso, Brasil [40]. They found that sites with higher P content had more tree density, basal area, and biomass compared to the others. Also, initial conditions regarding to biomass storage are expected to alter the rates of change of forest structure and composition [41]. Moreover, a more diverse community is expected to use more efficiently the available resources due to their complementary needs [42,43]. Our results indicated that AGB as ecological indicator might thus be expected to be particularly sensitive to environmental conditions during restoration ecology by different site effects treatments. Commonly, areas with different levels of initial conditions undergo succession at distinct rates, resulting in long-term divergent trajectories among communities [10,44].

The Pielou index ranged from 0.520 (SDf) to 0.943 (NR), except for SDf all the others treatments had values higher than 0.76 (Figure 4). This result suggests floristic heterogeneity, without ecological dominance in the plant community during restoration. Therefore, the lower Pielou index for PS is explained due to the high dominance of few species, for example *Senna alata* which represented 83.6% of the total of individuals measured. Perhaps this species was favored over others during seeding by the fertilization by its fast growth and allelopathic activity, which can cause inhibition of seed germination and radicle elongation of other species [45,46]. Studies have shown similar Pielou index values in sites under restoration processes in Brazil, for example, 0.60 [47], 0.73 [48] and 0.811 [49].

The Shannon index ranged from 0.528 (PS) to 1.705 (PSf) (Figure 4). However, it's a low value compared to those found in areas under restoration processes in Minas Gerais, 3.258 [50] and 3.103 [49]. In the state of Minas Gerais, found 2.35 for the natural regeneration after 18 months of restoration [51]. Likewise, the Shannon index normally vary from 1.3 to 3.5, but can reach values above 4.70 for tropical forests [52]; nevertheless, it is worth mentioning again that the data was collected at an early stage, after less than two and half years after the restoration interventions. The values were higher for PSf and PS due to the number of tree species used during planting (higher richness), as well as, the control of the proportion of number individuals per specie, which corroborate with the advantages of active restoration. On the other hand, although it showed the same species richness of some treatments, the lowest values for SDf can be explained due to the dominance of *Senna alata*. This result also shows that with active restoration it is possible to select key species that promote higher evenness and, consequently, maintain high diversity and biomass stock.

## 5. Conclusion

We concluded that active restorations methods are showing better responses in the study area affected by the Fundão tailings dam collapse in Mariana and natural regeneration is probably been hinder by environmental filters, mainly by invasive grasses. Although AGB was not statistically different between treatments there is a clear pattern where fertilization and pH correction were used, suggesting that the fertilization and pH correction might have been the reason for a higher biomass accumulation.

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Also, the planting of seedlings allows the selection of key species and the balancing of the proportion of individuals per specie, which promote higher evenness and, consequently, maintain higher diversity. Furthermore, this has important implications for the forest restoration of sites which faced similar disturbances; consequently, the active restoration may be the best alternative where natural regeneration is limited.

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## Appendixes

**Table SI.** Species of native seedlings planted

| Family | Species |
|---|---|
| Anacardiaceae | *Schinus terebinthifolius* |
| Euphorbiaceae | *Croton floribundus* |
| Euphorbiaceae | *Croton urucurana* |
| Fabaceae | *Senna alata* |
| Fabaceae | *Inga vera* |
| Fabaceae | *Senna pendula* |
| Fabaceae | *Senna multijuga* |
| Fabaceae | *Bauhinia forficata* |
| Malvaceae | *Guazuma ulmifolia* |
| Malvaceae | *Heliocarpus popayanensis* |
| Solanaceae | *Solanum granulosoleprosum* |

**Table SII.** Native tree species seeded, seed quantity ($g\ ha^{-1}$)

| Family | Species | $g\ ha^{-1}$ |
|---|---|---|
| Cannabaceae | *Trema micrantha* | 4 |
| Euphorbiaceae | *Croton floribundus* | 20 |
| Euphorbiaceae | *Croton urucurana* | 6 |
| Fabaceae | *Enterolobium contortisiliquum* | 78 |
| Fabaceae | *Guazuma ulmifolia* | 4 |
| Fabaceae | *Senna alata* | 10 |
| Fabaceae | *Senegalia polyphylla* | 30 |
| Fabaceae | *Senna macranthera* | 16 |
| Fabaceae | *Senna multijuga* | 8 |
| Malvaceae | *Heliocarpus popayanensis* | 4 |
| Solanaceae | *Solanum granulosoleprosum* | 6 |

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