

**UNIVERSIDADE FEDERAL DE VIÇOSA**

**ÍTALO FAVORETO CAMPANHARO**

**EVALUATION OF FOREST RESTORATION TECHNIQUES IN AREA WITH  
FUNDÃO DAM TAILING IN MARIANA, MINAS GERAIS, BRAZIL**

**VIÇOSA - MINAS GERAIS**

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Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Ciência Florestal, para obtenção do título de *Magister Scientiae*.

Orientador: Sebastião Venâncio Martins

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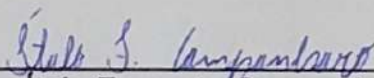
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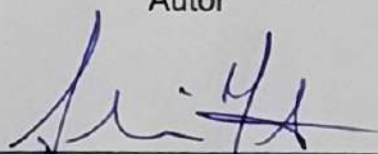
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Ítalo Favoreto Campanharo  
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Sebastião Venâncio Martins  
Orientador

To my parents, who turned my dreams  
into theirs.

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## ABSTRACT

CAMPANHARO, Ítalo Favoreto, M.Sc., Universidade Federal de Viçosa, September, 2020. **Evaluation of forest restoration techniques in area with Fundão dam tailing in Mariana, Minas Gerais, Brazil.** Advisor: Sebastião Venâncio Martins.

Forest restoration activities in the areas affected by the Fundão dam rupture, in the municipality of Mariana, MG, have been combining different methods of active and passive restoration. However, there is still a need to compare these methods and define the most efficient ones. It was measured and compiled data of the aboveground biomass (AGB), penetration resistance of tailings and fertility, community diversity, structure attributes and functional traits in 36 plots (12 × 12 m each) distributed in six 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). The data on substrate and vegetation were collected in 2019 (2 years and 4 months post-restoration interventions) and the penetration resistance measured in two contrasting periods (dry and wet). No significant differences in penetration resistance between treatments were observed, only between periods (dry and wet). The main univariate model explained the significant effects of depth and seasonality, mainly by a negatively wet effect on penetration resistance. Despite of the early stage of restoration interventions the substrate properties are not limiting the AGB recovery. The study results show how resistance can be an indicator to select the best period to manage the soil and the restoration process. The AGB stored in the plots ranged from 0.06 Mg ha<sup>-1</sup> to 10.49 Mg ha<sup>-1</sup>, following this order: PSf > PS > SDf > SD > NRf > NR. Although biomass storage between treatments was not statistically different, there is a clear pattern showing higher values for each restoration method where fertilization/correction were used compared with the same methods, but without fertilization and pH correction. No significant differences in soil fertility were found between treatments, the results were similar to the soils found in the region. The only contrasting nutrient was Fe, which demonstrated high values; however, it was

expected due to the iron tailings deposition. 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 evenness in the plant community. On the other hand, the Shannon index ranged from 0.528 (PS) to 1.705 (PSf), however it is a low value compared to those found in areas under restoration processes in Minas Gerais. As expected, at this early stage of the restoration process, the more representative successional group was the pioneer species and the anemochoric syndrome mainly represented by naturally regenerated stems. The autochoric dispersal and the nitrogen-fixing trees showed to be biomass dominant groups, driven mostly by *Senna alata*, because of its high abundance. It was found that high community-weighted mean of wood density and active restoration methods determine higher levels of AGB on mining tailings. These results support the mass ratio hypothesis, and explain how functional composition as the main predictor determines AGB during restoration. The functional dominance overrides the effects of species richness and abundance on AGB, and hence, it is important to test the mutual effects of functional composition when testing the effects of restoration methods on forest functioning. Overall, active forest restoration showed better responses in the study area, indicating important implications for the forest restoration where natural regeneration is limited.

**Keywords:** Active restoration. Passive restoration. Fertilization. Aboveground biomass. Mining tailings.



## RESUMO

CAMPANHARO, Ítalo Favoreto, M.Sc., Universidade Federal de Viçosa, setembro de 2020. **Avaliação de técnicas de restauração florestal em área com rejeito da barragem de Fundão em Mariana, Minas Gerais, Brasil.** Orientador: Sebastião Venâncio Martins.

As atividades de restauração florestal das áreas atingidas pelo rompimento da barragem de Fundão, no município de Mariana, MG, vem combinando diferentes métodos de restauração ativa e passiva. No entanto, ainda existe a necessidade de se comparar esses métodos e definir os mais eficientes. Foram medidos e analisados dados de biomassa acima do solo (AGB), resistência do solo à penetração, fertilidade do solo, diversidade da comunidade, atributos estruturais e características funcionais em 36 parcelas (12 × 12 m cada) distribuídas em seis tratamentos com seis repetições: plantio de mudas de espécies arbóreas nativas com fertilização e correção de pH (PSf) e sem fertilização e correção de pH (PS); semeadura de espécies arbóreas nativas com fertilização e correção de pH (SDf) e sem fertilização e correção de pH (SD); regeneração natural com fertilização e correção de pH (NRf) e sem fertilização e correção de pH (NR). Os dados do substrato e da vegetação foram coletados em 2019 (2 anos e 4 meses após o início do processo de restauração) e a resistência do solo à penetração medida em dois períodos contrastantes (estação seca e chuvosa). Não foram observadas diferenças significativas na resistência à penetração entre os tratamentos, apenas entre os períodos (seco e úmido). O modelo univariado explicou os efeitos significativos da profundidade e sazonalidade, principalmente por um efeito negativo da umidade na resistência à penetração. Apesar do estágio inicial das intervenções voltadas à restauração, as propriedades do substrato não estão limitando o acúmulo de AGB. Os resultados do estudo mostram como a resistência pode ser um indicador para selecionar o melhor período de manejo do solo e do processo de restauração. A AGB acumulada por parcela variou de 0,06 Mg ha<sup>-1</sup> (NR) a 10,49 Mg ha<sup>-1</sup> (PSf), seguindo a ordem: PSf > PS > SDf > SD > NRf > NR. Embora a AGB entre tratamentos não ter sido estatisticamente diferente, há um padrão claro mostrando valores mais altos para cada método de restauração onde a fertilização/calagem foi utilizada em

comparação com os mesmos métodos, mas sem fertilização e correção do pH. Não foram encontradas diferenças significativas na fertilidade do solo entre os tratamentos, os resultados foram semelhantes aos solos com pastagens da região. O índice de Pielou variou de 0,520 (SDf) a 0,943 (NR), exceto para SDf todos os demais tratamentos apresentaram valores superiores a 0,76. Este resultado sugere uniformidade na comunidade. Por outro lado, o índice de Shannon variou de 0,528 (PS) a 1,705 (PSf). Como era esperado, neste estágio inicial do processo de restauração, o grupo sucessional mais representativo foi das espécies pioneiras e a síndrome de dispersão anemocórica representada principalmente pelos indivíduos regenerantes. A dispersão autocórica e as espécies fixadoras de nitrogênio foram grupos dominantes em biomassa, impulsionadas principalmente por *Senna alata*, devido à sua alta abundância. A elevada média dos atributos funcionais ponderada pelas abundâncias nas comunidades em relação à densidade da madeira e aos métodos de restauração ativa determinaram níveis mais altos de AGB. Esses resultados apoiam a hipótese da razão de massa e explicam como a composição funcional como principal preditor determina a AGB durante a restauração. A dominância funcional sobrepõe os efeitos da riqueza e abundância de espécies sobre a AGB e, portanto, é importante testar os efeitos mútuos da composição funcional ao avaliar os métodos de restauração. De maneira geral, a restauração florestal ativa apresentou melhores respostas na área de estudo, indicando implicações importantes para a restauração nos locais onde a regeneração natural é limitada.

**Palavras-chave:** Restauração ativa. Restauração passiva. Fertilização. Biomassa acima do solo. Rejeitos de mineração.

## TABLE OF CONTENTS

<b>GENERAL INTRODUCTION</b> .....	12
<b>REFERENCES</b> .....	14
<b>1 ARTICLE 1 - RELATIONSHIPS OF FOREST RESTORATION METHODS, SEASONALITY, AND PENETRATION RESISTANCE DOES NOT AVOID ABOVEGROUND BIOMASS STOCK IN AREA WITH FUNDÃO DAM TAILING IN MARIANA, MINAS GERAIS STATE, BRAZIL</b> .....	16
<b>1.1 ABSTRACT</b> .....	16
<b>1.2 INTRODUCTION</b> .....	17
<b>1.3 MATERIAL AND METHODS</b> .....	18
1.3.1 <i>Experimental site</i> .....	18
1.3.2 <i>Experimental design</i> .....	19
1.3.3 <i>Fertilization and site preparation techniques</i> .....	20
1.3.4 <i>Substrate compaction measurement</i> .....	20
1.3.5 <i>Vegetation data collection and aboveground biomass</i> .....	21
1.3.6 <i>Data analyses</i> .....	21
<b>1.4 RESULTS</b> .....	22
1.4.1 <i>Penetration resistance of tailings between treatments and periods</i> .....	22
1.4.2 <i>Vertical penetration resistance</i> .....	24
1.4.3 <i>Effects of treatments, depth and seasonality on substrate penetration resistance</i> .....	26
1.4.4 <i>Effects of predictors on aboveground biomass</i> .....	30
<b>1.5 DISCUSSION</b> .....	31
<b>1.6 CONCLUSION</b> .....	32
<b>1.7 REFERENCES</b> .....	33
<b>2 ARTICLE 2 - COMPARATIVE EFFECTS OF FOREST RESTORATION TECHNICS ON COMMUNITY DIVERSITY AND ABOVEGROUND BIOMASS ON AREA AFFECTED BY MINING TAILINGS IN MARIANA, MINAS GERAIS STATE, BRAZIL</b> .....	35
<b>2.1 ABSTRACT</b> .....	35
<b>2.2 INTRODUCTION</b> .....	37
<b>2.3 MATERIAL AND METHODS</b> .....	39
2.3.1 <i>Experimental site</i> .....	39

2.3.2	<i>Experimental design</i> .....	40
2.3.3	<i>Fertilization and site preparation techniques</i> .....	41
2.3.4	<i>Substrate sampling and analyses of chemical and physical properties in the laboratory</i> .....	41
2.3.5	<i>Vegetation data collection and aboveground biomass estimation</i> .....	42
2.3.6	<i>Data analyses</i> .....	43
<b>2.4</b>	<b>RESULTS</b> .....	43
2.4.1	<i>Substrate properties pattern</i> .....	43
2.4.2	<i>Differences in species diversity, abundance and aboveground biomass</i> .....	46
<b>2.5</b>	<b>DISCUSSION</b> .....	48
<b>2.6</b>	<b>CONCLUSION</b> .....	51
<b>2.7</b>	<b>REFERENCES</b> .....	51
<b>3</b>	<b>ARTICLE 3 - FUNCTIONAL COMPOSITION ENHANCES PLANT ABOVEGROUND BIOMASS STORAGE UNDERGOING ACTIVE RESTORATION ON MINING TAILINGS IN MARIANA, BRAZIL</b> .....	57
<b>3.1</b>	<b>ABSTRACT</b> .....	57
<b>3.2</b>	<b>INTRODUCTION</b> .....	59
<b>3.3</b>	<b>MATERIALS AND METHODS</b> .....	61
3.3.1	<i>Experimental site</i> .....	61
3.3.2	<i>Experimental design</i> .....	63
3.3.3	<i>Site preparation techniques and fertilization</i> .....	63
3.3.4	<i>Technosol sampling and analyses of chemical and physical properties in the laboratory</i> .....	63
3.3.5	<i>Plant inventory and functional traits</i> .....	64
3.3.6	<i>Quantification of functional composition</i> .....	65
3.3.7	<i>Aboveground biomass estimation</i> .....	65
3.3.8	<i>Stem and biomass distribution between functional groups</i> .....	66
3.3.9	<i>Statistical analyses</i> .....	66
<b>3.4</b>	<b>RESULTS</b> .....	68
3.4.1	<i>Species richness and community composition</i> .....	68
3.4.2	<i>Biomass and abundance (stem count) distribution</i> .....	71
3.4.3	<i>Linear mixed-effect models</i> .....	75
<b>3.5</b>	<b>DISCUSSION</b> .....	77
3.5.1	<i>Aboveground biomass distribution between species and functional groups</i> .....	78
3.5.2	<i>Functional traits composition shape aboveground biomass</i> .....	80

3.5.3	<i>Implications for forest management and restoration</i> .....	82
<b>3.6</b>	<b>CONCLUSION</b> .....	83
<b>3.7</b>	<b>REFERENCES</b> .....	83
	<b>FINAL CONSIDERATIONS</b> .....	93
	<b>APPENDICES</b> .....	94
	APPENDIX A – ARTICLE 1.....	94
	APPENDIX B – ARTICLE 2.....	98
	APPENDIX C – ARTICLE 3.....	100

## GENERAL INTRODUCTION

Mining activities are essential for human beings, but at the same time, mining, like other anthropic activities, however well planned, may generate environmental impacts (Dongmei e Changqun, 2008; Silva-Junior et al., 2018; Quadra et al., 2019).

The collapse of the Fundão dam in Mariana, MG, released almost 40 million cubic meters of tailings in the Gualaxo do Norte, Carmo and Doce rivers. The deposition of tailings on the banks of these rivers and their tributaries was restricted to a 113 km stretch that runs from the Fundão dam to the Risoleta Neves hydroelectric plant (Candongia), called the Environmental Area 1 (Fundação Renova, 2019; Martins et al., 2020). Since then, different methods of active and passive restoration have been applied throughout the area affected with tailings deposits, as well as in compensation areas throughout the Doce River basin (Fundação Renova, 2019; Martins et al., 2020). In this sense, the restoration of these environments requires a special and urgent attention to ensure the re-establishment of biodiversity and ecosystem services (Crouzeilles et al., 2017; Stuble et al. 2017).

Currently, several restoration methods are being used in the affected areas, combining active and passive restoration techniques (Martins et al., 2020). These techniques are generally recommended for different situations. 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 (Holl e Aide, 2011; Holl, 2017). However, in areas isolated from propagule sources, extensive degradation of the vegetation and soil, the resilience of the environment is generally compromised (Holl, 2012; Martins, 2018). Thus, active restoration is more recommended in these cases to accelerate the forest recovery process, through the planting of seedlings, direct sowing or nucleation techniques such as transposition of topsoil and litter. (Martins, 2018). Taking this into account, the need arose to evaluate and monitor the effectiveness of each technique used in the regions affected by the tailing in order to provide the necessary knowledge so that the most appropriate approach can be used in each situation.

The selection of good ecological indicators that accurately reflect the trajectory of forest recovery are essential for the management of degraded areas (Holl et al., 2020; Martins, 2018). Widely studied, the above ground biomass accumulation is one of the main indicators for both passive and active restoration (Holl et al., 2020). Other important indicators related to vegetation are those that take into account species richness, diversity and functional groups (Brancalion et al., 2015). In addition to the biotic aspects, physical aspects must also be taken into account, especially those related to soil quality. For example, soil penetration resistance as an indicator of compaction is a variable that reflects the resistance offered by the soil to root growth, and is also sensitive to changes in other properties such as soil structure, texture, density, water content and organic matter (Hamza e Anderson, 2003; Singh et al., 2015). Furthermore, the assessment of soil fertility is essential, since the success of restoration in tropical forests is directly linked to this attribute. (Guariguata e Ostertag, 2001; Quesada et al., 2012; Bu et al., 2018)

In this context, the present study aimed to evaluate the response of different active and passive forest restoration (i.e. planting of nursery-grown seedlings, direct seeding and natural regeneration) and the effect of fertilization and correction of soil acidity, in an area impacted by the Fundão dam collapse, in Mariana, Minas Gerais, through six treatments: tree seedlings with fertilization/correction and without fertilization and pH correction; seeding of native trees with fertilization/correction and without fertilization and pH correction; natural regeneration with fertilization/correction and without fertilization and pH correction.

In order to elucidate and discuss the results more clearly and completely, the dissertation was organized in three articles: 1) Relationships of forest restoration methods, seasonality, and penetration resistance does not avoid aboveground biomass stock in area with Fundão dam tailing in Mariana, Minas Gerais state, Brazil; 2) Comparative effects of forest restoration technics on community diversity and aboveground biomass on area affected by mining tailings in Mariana, Minas Gerais state, Brazil; 3) Functional composition enhances plant aboveground biomass storage undergoing active restoration on mining tailings in Mariana, Brazil.

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# **1 ARTICLE 1 - RELATIONSHIPS OF FOREST RESTORATION METHODS, SEASONALITY, AND PENETRATION RESISTANCE DOES NOT AVOID ABOVEGROUND BIOMASS STOCK IN AREA WITH FUNDÃO DAM TAILING IN MARIANA, MINAS GERAIS STATE, BRAZIL**

## **1.1 ABSTRACT**

The restoration methods applied on the areas affected by the Fundão tailings dam collapse have a high priority in Mariana region. We evaluate the effect of different restoration methods and fertilization, depth and seasonality on penetration resistance of tailings, and how these predictors affect tree aboveground biomass in areas affected by the Fundão dam collapse in Mariana, Brazil. No significant differences in penetration resistance and aboveground biomass between treatments were observed, but significant differences were observed between periods. The main univariate model explained the significant effects of depth and seasonality, mainly by a negatively wet effect on penetration resistance. According to the best models (univariate and multivariate) were those that had depth as a predictor. This study showed how penetration resistance can be an indicator to select the best period for restoration process in areas affected by the collapse of the Fundão dam, but no limit to the aboveground biomass recovery on tailings.

**Key words:** Fundão dam, penetration resistance, resilient mitigation, restoration ecology, site effects.

## 1.2 INTRODUCTION

Almost five years after the collapse of the Fundão tailings dam in Marina, Brazil, active and passive restoration methods on the Atlantic forest affected continue to be applied (Martins et al. 2020). Therefore, identifying ecological indicators related to natural forest recovery is essential to improve the management criteria of different active and passive restoration methods (Martins 2018; Holl et al. 2020). For example, aboveground biomass (AGB) is one of the main ecological indicators of tropical forests recovery using either active or passive restoration methods (Holl et al. 2020). Thus, 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 (Martins 2018; Holl et al. 2020). Meanwhile, 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 recovery status (Martins 2018). The success or failure of the various methods of ecological restoration must be investigated to propose adaptations and to know the limiting factors of their success before their large-scale implementation.

In this context, the tailings management play an important role to achieve the restoration goals, such as prioritizing efficient alternatives that allow a fast forest recovery and ecosystem functioning, for example AGB recovery (Holl et al. 2020). The correct decision of initial interventions depend on environmental conditions, disturbance intensity and restoration project objectives (Stuble et al. 2017). For example, site preparation techniques, such as mulching, prescribed burning, mechanical procedures, and fertilization, can remarkably influence soil recovery and forest restoration success, which designed to improve the site conditions and promotes plant growth and ecosystem functioning recovery (Löff et al. 2016, Stuble et al. 2017; Pitz et al. 2019). However, a limited number of studies have directly compared the effects of restoration methods and fertilization on penetration resistance of tailings. Thus, it is still necessary to understand the relationship of environmental factors (i.e. climate) and restoration methods (i.e. planting of nursery-grown seedlings, direct seeding and natural regeneration) on the physical properties of the tailings (i.e. depth and penetration resistance), and how this relationship can

affect the aboveground biomass stock on areas affected by tailings in Mariana, MG, Brazil.

Penetration resistance (or soil strength) resistance is a physical property of the soil that reflects changes in other soil properties, such as structure, texture, bulk density, water content, and soil organic matter (Hamza & Anderson 2003, Singh et al. 2015). Furthermore, soil strength predicts the resistance offered by soil to root penetration and can be used as a measure of soil compaction (Hamza & Anderson 2003, Singh et al. 2015). Compaction is a limiting factor for root growth, water supply and nutrient availability due to the reduction of the amount and size of soil pores leading to the decrease of soil infiltration and consequently to waterlogging and runoff (Hoefler et al. 2010; Al-Gaadi 2012). Moreover, soil is a fundamental ecosystem component directly linked to the erosional, biogeochemical and hydrological cycles (Keesstra et al. 2012, Brevik 2015; Smith et al. 2015). Thus, we presume that tailings properties monitoring can be a feasible tool to the restoration success.

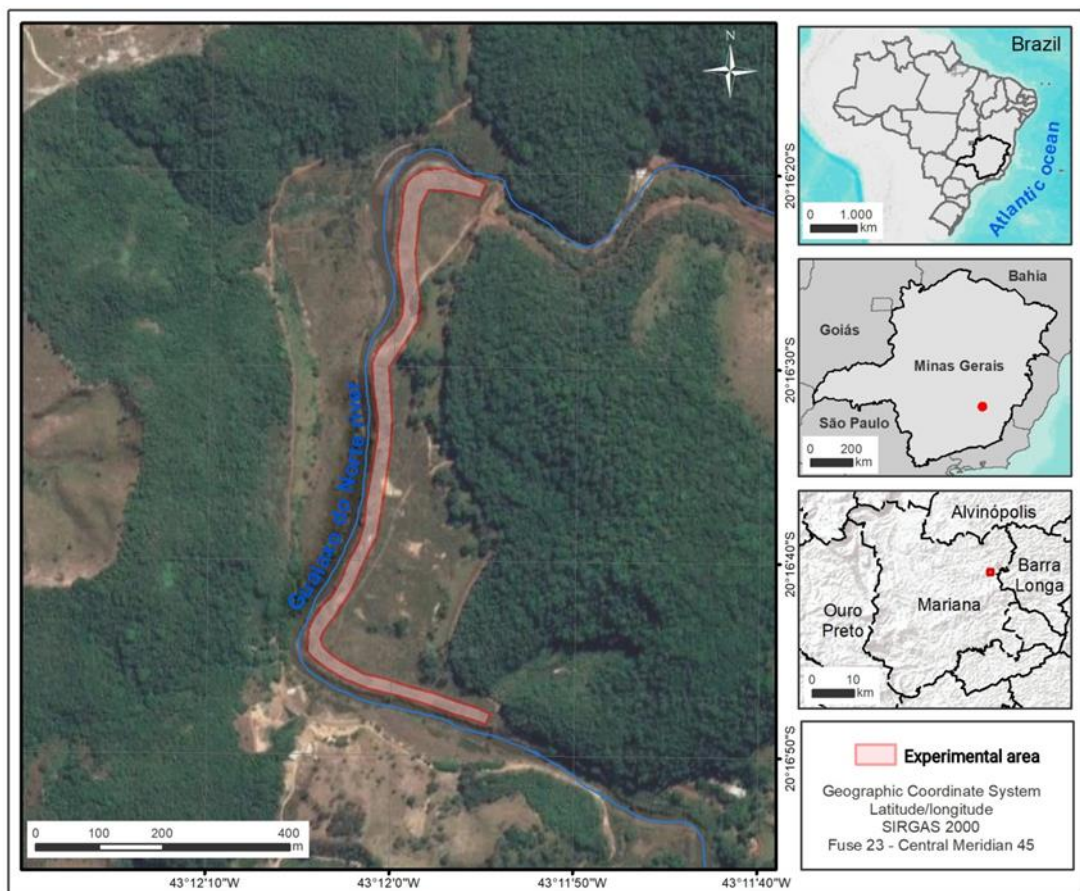
In this study, we evaluate the effect of different restoration methods (i.e. planting of nursery-grown seedlings, direct seeding and natural regeneration) and fertilization, depth and seasonality on penetration resistance of tailings, and how these predictors affect tree AGB stock in areas affected by the Fundão dam collapse in Mariana, Minas Gerais state, southeastern Brazil. Here we assessing the tailings penetration resistance post restoration interventions, based in six treatments: tree seedlings with fertilization/correction and without fertilization and pH correction; seeding of native trees with fertilization/correction and without fertilization and pH correction; natural regeneration with fertilization/correction and without fertilization and pH correction.

### **1.3 MATERIAL AND METHODS**

#### *1.3.1 Experimental site*

Active and passive restoration experiments were established 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 and Figure S1 from supplementary material, SM hereafter)). The study area

is located between 505 and 515 m above sea level, and the relief is mainly flat to weakly undulating. The climate is moderate humid and tropical, with a dry season occurring from May to September and a wet season occurring between December and March. The mean annual precipitation is 1340 mm, mean annual air temperature is 19°C and mean annual relative humidity is ca. 80%. Two dominant soil classes are found in the site: Cambic Red-Yellow Podzolic covers the upper fluvial terraces, while Dystric Red-Yellow Latosol represents hilltops and mountainsides (Martins et al. 2020).



**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.

### 1.3.2 Experimental design

Approximately 16 months after of the Fundão dam collapse, in March of 2017, different restoration treatments with plots varying from the size of 300 to 500 m<sup>2</sup> were

established in line along the Gualaxo do Norte 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/correction (SDf) and without fertilization and pH correction (SD); natural regeneration with fertilization/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 SM).

Aiming to standardize the study, two years and four months after of the Fundão dam collapse, in 2019, in the center of each original plot was demarcated a regular area of 144 m<sup>2</sup> (12 × 12 m), totaling 36 plots, where the data was collected, so that a possible edge effect would be eliminated.

### *1.3.3 Fertilization and site preparation techniques*

Calcined dolomitic limestone (100 kg ha<sup>-1</sup>), agricultural gypsum (350 kg ha<sup>-1</sup>), ammonium sulfate (100 kg ha<sup>-1</sup>) and super simple phosphate (150 kg ha<sup>-1</sup>) 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.

### *1.3.4 Substrate compaction measurement*

The substrate compaction was measured in all plots of each treatment using an electronic soil compaction meter (penetroLOG Falker), which indicates the soil penetration resistance value corresponding to each centimeter at different depths (0-20 cm). The measurements were done in two periods, during the wet season (March 2019) and the dry season (September 2019), two years and half post restoration interventions. Systematically, in the center of each plot, 5 measurements were taken

in the wet season and 3 during the dry season, totaling 30 and 18 replicates per treatment, respectively. It is worth mentioning that less replicates were taken in the dry season due to the elevated compaction of the substrate, moreover, for the same reason, in many measurement points were not possible to exceed the first eleven centimeters in depth, while in the wet season, for all the measured points, was possible to reach 20 centimeters in depth.

### 1.3.5 *Vegetation data collection and aboveground biomass*

In each plot were measured the diameter at breast height (DBH  $\geq$  2 cm at 1,30 m) and total height of the tree species. The aboveground biomass of all individual for each treatment was estimated using tree DBH (cm), height (H, m) and wood density ( $\rho$ , g cm<sup>-3</sup>) based on a general allometric equation (Chave et al. 2014). The total aboveground biomass per plot was the sum of the aboveground biomass of all trees, which was then converted into megagrams per hectare (Mg ha<sup>-1</sup>).

### 1.3.6 *Data analyses*

All analyses were run in R 3.6.0 (R-Core Team 2019). We tested the normal distribution of all variables using the Shapiro-Wilk test and the Q-Q plot, and homogeneity of variances by Bartlett's test using the "dplyr" package (Crawley 2013). To compare penetration resistance and aboveground biomass (non-normally distributed data) between treatments of site conditions, we used Kruskal-Wallis's test followed by a posterior Dunn's test performed with the 'dunn.test' package. For test compare penetration resistance (non-normally distributed data) between periods (dry and wet periods) we used Wilcoxon test (Crawley 2013, R-Core Team 2019).

We tested generalized linear mixed-effect models (GLMM) to investigate the effect of treatments, seasonality and depth on penetration resistance (continuous response variable), and after checking the effects of these predictor on tree aboveground biomass. Thus, predictor variables were grouped into three categories, i.e. tailing depth (continuous explanatory variable), seasonality (wet and dry period) as a categorical explanatory variable, and the restoration treatments of site conditions (categorical explanatory variable). Then we evaluated the effect of these

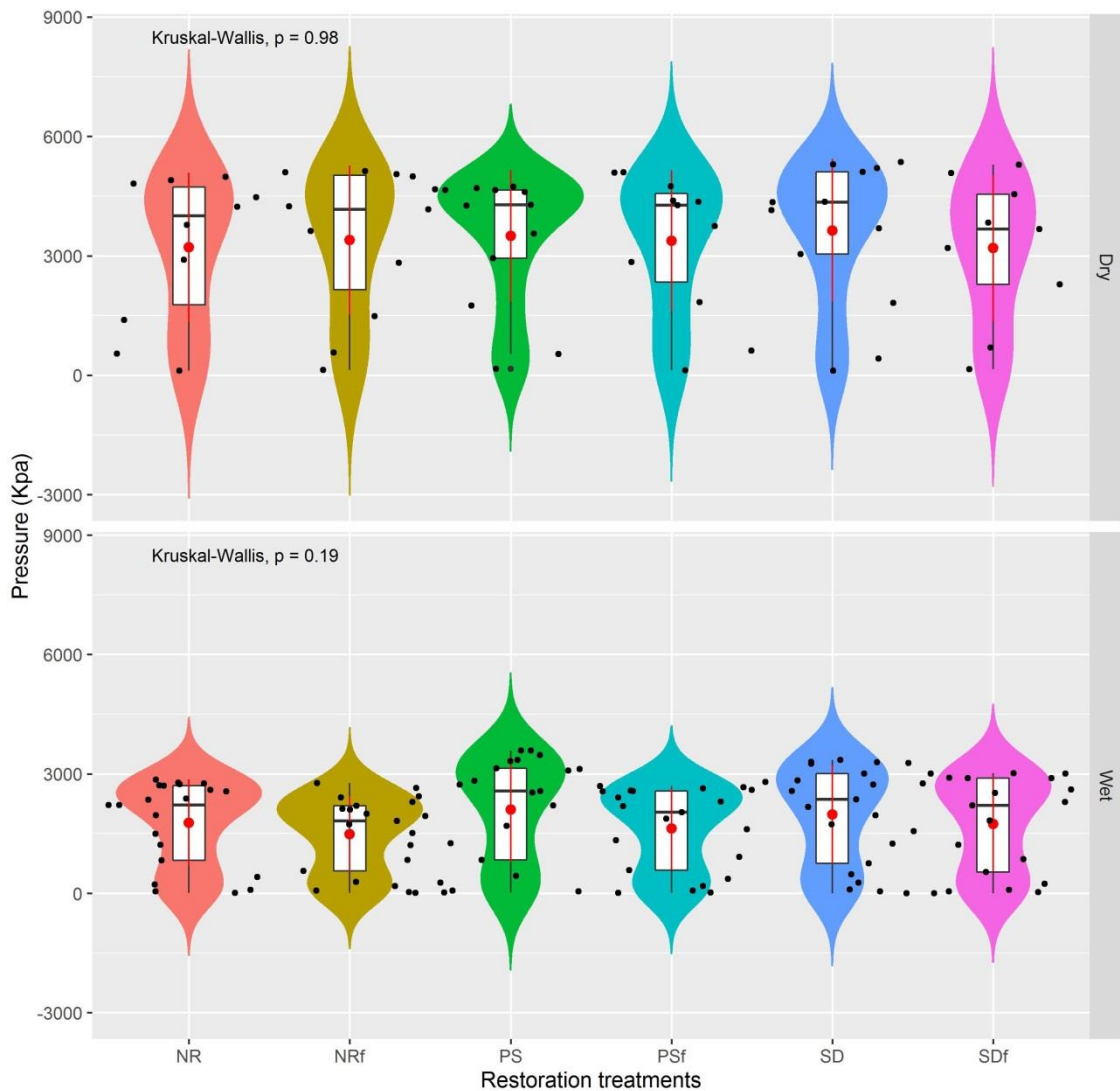
predictors on AGB. The treatments included six levels (i.e. each treatment of site condition). We tested alternative models with individual effects of predictors and different combinations of predictors with low correlation, and the patches and plots were considered as a random effect (1 | patch:plot). All models were calculated using the package 'lme4' in the platform R (Crawley 2013, R-Core Team 2019).

## 1.4 RESULTS

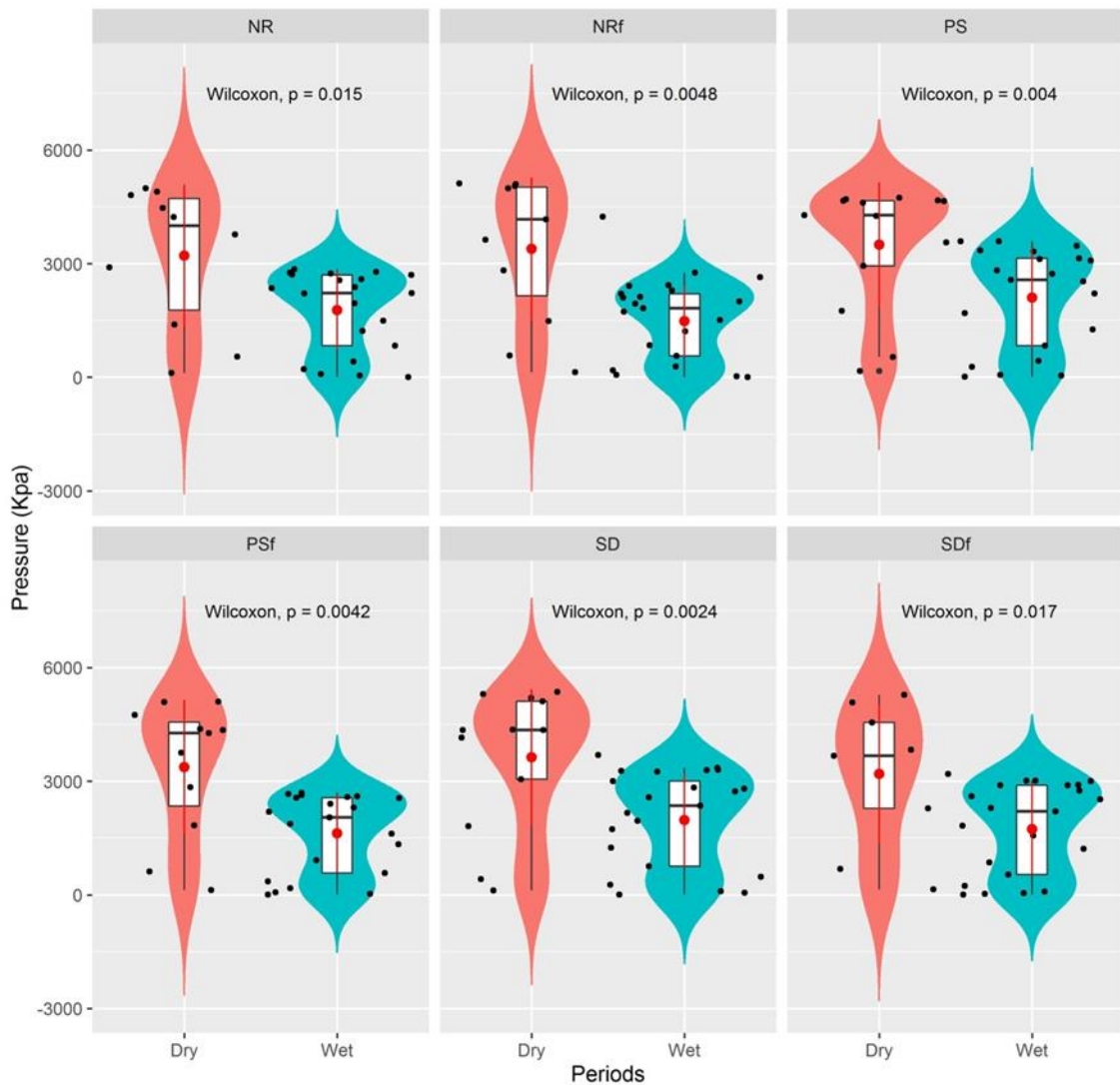
### *1.4.1 Penetration resistance of tailings between treatments and periods*

No significant differences in penetration resistance between treatments were observed (Figure 2); however significant differences were observed between periods (Figure 3). The mean per treatment followed this order in the wet period: NRf (1484.23 kPa) < PSf (1607.86 kPa) < SDf (1710.60 kPa) < NR (1758.91 kPa) < PS (1894.32 kPa) < SD (1933.22 kPa). However, in the dry period the order was: NR (3075.95 kPa) < SD (3084.63 kPa) < PS (3114.85 kPa) < NRf (3120.29 kPa) < PSf (3159.11 kPa) < SDf (3161.01 kPa). The average resistance over all observations for the treatments in the dry season was 3130.98 kPa while for the wet season was 1731.07 kPa; the mean of maximum pressures per sampling point during the dry season was 5445.31 kPa and during the wet season 3572.33 kPa.





**Figure 2.** Penetration resistance differences between treatments. A randomized block design with six restoration treatments was used, consisting of six replicates for each treatment: Seedlings with fertilization/pH correction (PSf); Seedlings without fertilization/pH correction (PS); Natural Regeneration with fertilization/pH correction (NRf); Natural Regeneration without fertilization/pH correction (NR); Seeding with fertilization/pH correction (SDf); Seeding without fertilization/ pH correction (SD).

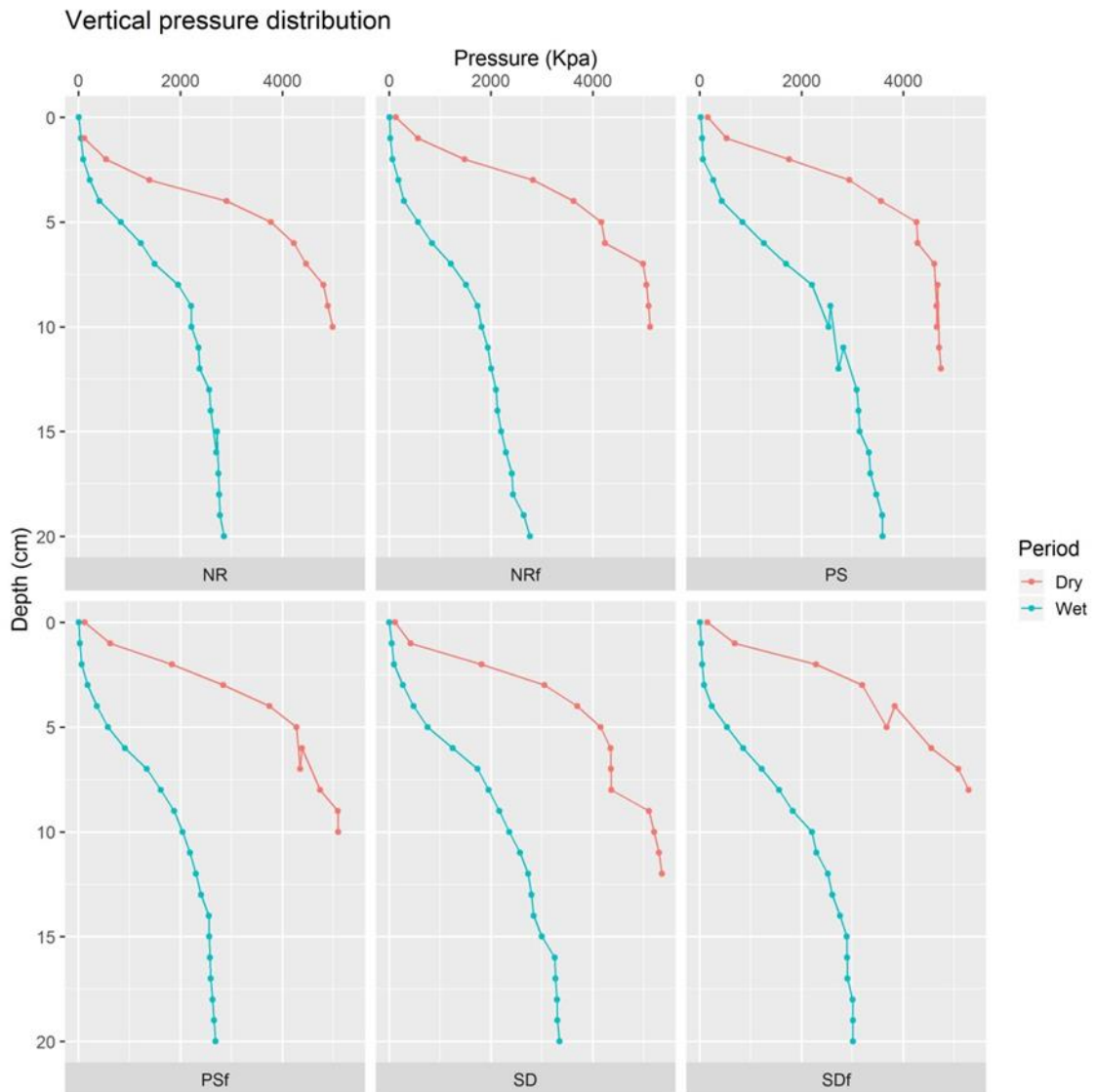


**Figure 3.** Penetration resistance differences between periods. A randomized block design with six restoration treatments was used, consisting of six replicates for each treatment: Seedlings with fertilization/pH correction (PSf); Seedlings without fertilization/pH correction (PS); Natural Regeneration with fertilization/pH correction (NRf); Natural Regeneration without fertilization/pH correction (NR); Seeding with fertilization/pH correction (SDf); Seeding without fertilization/ pH correction (SD).

#### 1.4.2 Vertical penetration resistance

The vertical substrate penetration resistance distribution maintains a similar pattern between treatments (Figure 4 and Figure S2 from SM). For both periods, the deeper the substrate layer the greater was the penetration resistance. However, in

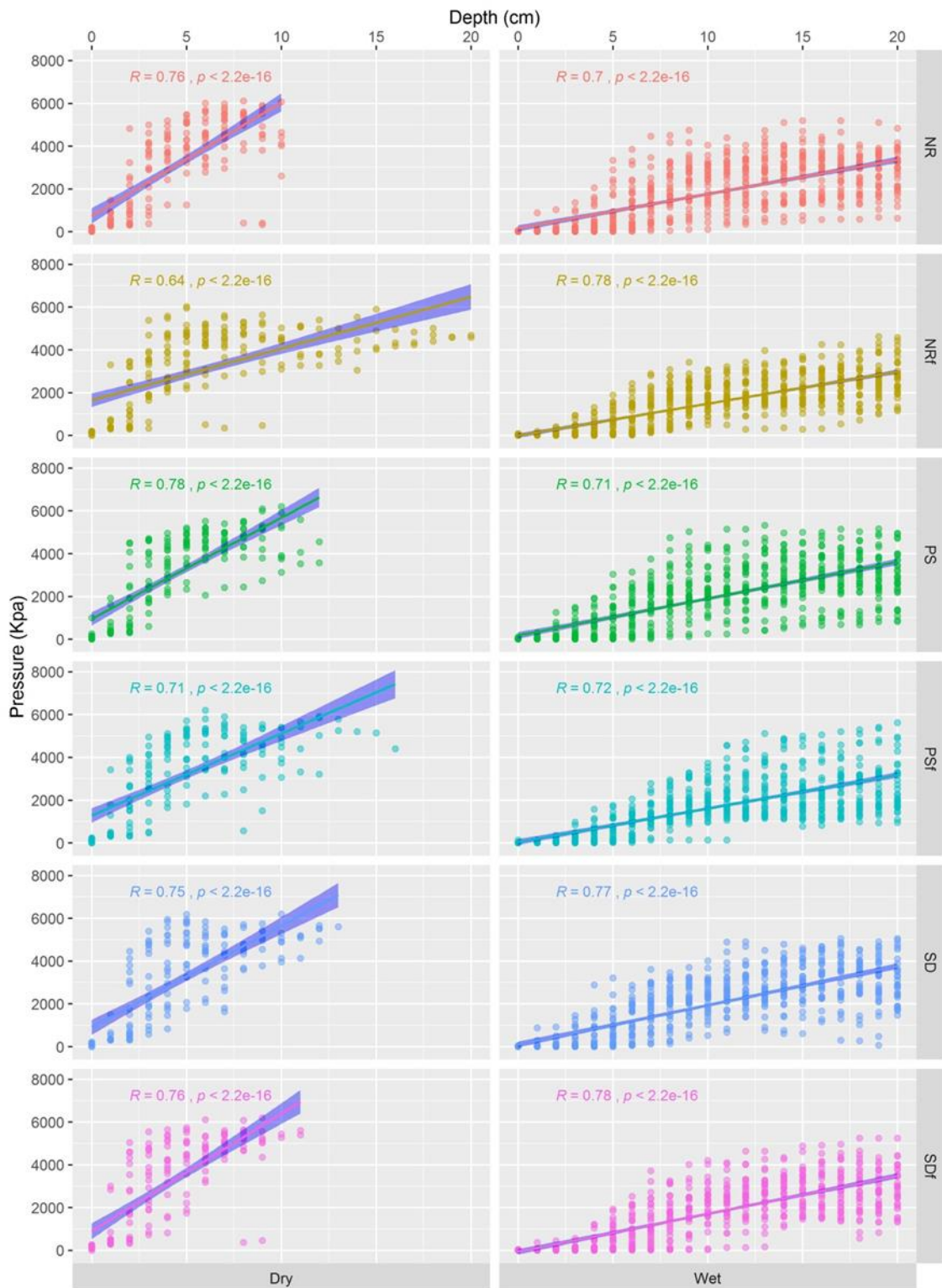
only 3 out of 108 sampling points were possible to reach 20 cm deep during the dry period and the pressure commonly exceeded 5000 kPa. On the other hand, during the wet season was possible to reach 20 centimeters in depth for all the 180 sampling points. The penetration resistance at this depth, for all the treatments, was around 3000 kPa, but did not exceed 4000 kPa (Figure 4 and Figure S2 from SM).



**Figure 4.** Vertical substrate penetration resistance distribution. Treatments: Seedlings with fertilization/pH correction (PSf); Seedlings without fertilization/pH correction (PS); Natural Regeneration with fertilization/pH correction (NRf); Natural Regeneration without fertilization/pH correction (NR); Seeding with fertilization/pH correction (SDf); Seeding without fertilization/ pH correction (SD).

### *1.4.3 Effects of treatments, depth and seasonality on substrate penetration resistance*

The main univariate model explained the significant effects of depth (GLMM: estimate = 0.11,  $t = 71.96$ ,  $p < 0.001$ , Figure 5) and seasonality, mainly by negatively wet effect (GLMM: estimate = -0.85,  $t = -19.33$ ,  $p < 0.001$ ) on penetration resistance of the substrate (Table I, Figure 5). According to multivariate model selecting depth and seasonality as predictors, had negatively influences on penetration resistance (GLMM: estimate = -0.17,  $t = -32.43$ ,  $p < 0.001$ , Figure 5). According to the best models (univariate and multivariate) were those that had depth as a predictor (Table I, Figure 6 and Figure S3 from SM).



**Figure 5.** Penetration resistance and the main predictors' relationship according with GLMM approach. The effect of depth on pressure between periods. Color fill circles indicate data per treatments. Solid lines represent the fitted values (prediction) of the models, and the shaded area the 95 % confidence interval of the predicted values of

each model. Treatments: Seedlings with fertilization/pH correction (PSf); Seedlings without fertilization/pH correction (PS); Natural Regeneration with fertilization/pH correction (NRf); Natural Regeneration without fertilization/pH correction (NR); Seeding with fertilization/pH correction (SDf); Seeding without fertilization/ pH correction (SD).

**Table I.** The subset of models predicting the main effect of treatment, depth, and seasonality on pressure (Generalized linear mixed-effect model). The result of information-theoretic-based model selection is indicated. We present only the models with values of  $\Delta AICc < 2$ . The Akaike information criterion corrected for small samples (AICc).

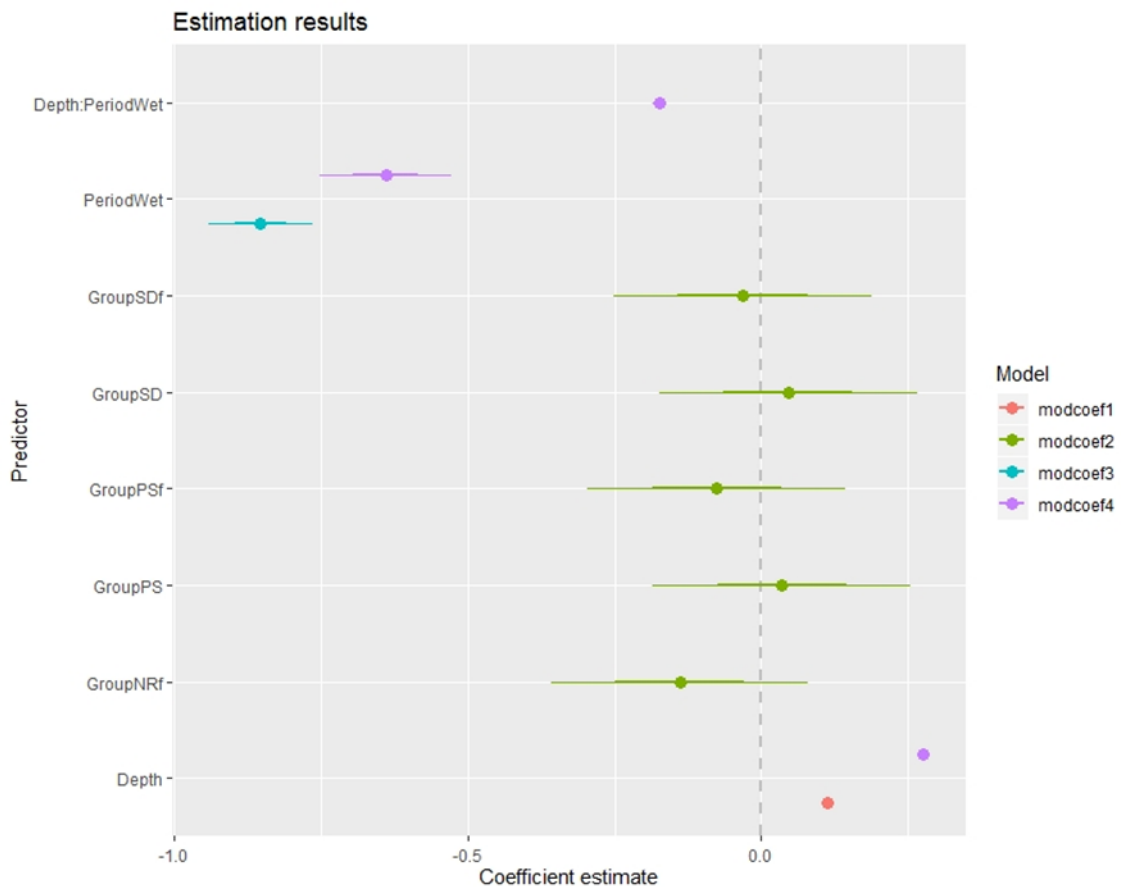
	Model 1	Model 2	Model 3	Model 4
<b>(Intercept)</b>	0.24 *** (0.05)	0.16 * (0.07)	0.66 *** (0.04)	1.87 *** (0.05)
<b>Depth</b>	0.68 *** (0.01)			1.66 (0.03) ***
<b>GroupNR</b>		-0.04 (0.11)		
<b>Group SD</b>		0.01 (0.11)		
<b>Group PSf</b>		-0.11 (0.11)		
<b>Group NRf</b>		-0.17 (0.11)		
<b>Group SDf</b>		-0.07 (0.11)		
<b>Period</b>			-0.85 (0.04) ***	-2.18 (0.05) ***
<b>Depth:Period</b>				-1.05 (0.03) ***
<b>N</b>	4835	4835	4835	4835
<b>N (Replicate)</b>	281	281	281	281
<b>AIC</b>	917.04	1028.28	963.88	926.79

<b>BIC</b>	942.97	1280.15	989.82	965.69
<b>R<sup>2</sup> (fixed)</b>	0.32	0.00	0.12	0.61
<b>R<sup>2</sup> (total)</b>	0.74	0.21	0.19	0.73

All continuous predictors are mean-centered and scaled by 1 standard deviation.

\*\*\*  $p < 0.001$ ; \*\*  $p < 0.01$ ; \*  $p < 0.05$ .

Model1 <- glmer(Pressure ~ Depth + (1|Plot), data=dados); Model2 <- glmer(Pressure ~ Treatment + (1|Plot), data=dados); Model3 <- glmer(Pressure ~ Period + (1|Plot), data=dados); Model4 <- glmer(Pressure ~ Depth \* Period + (1|Plot), data=dados).



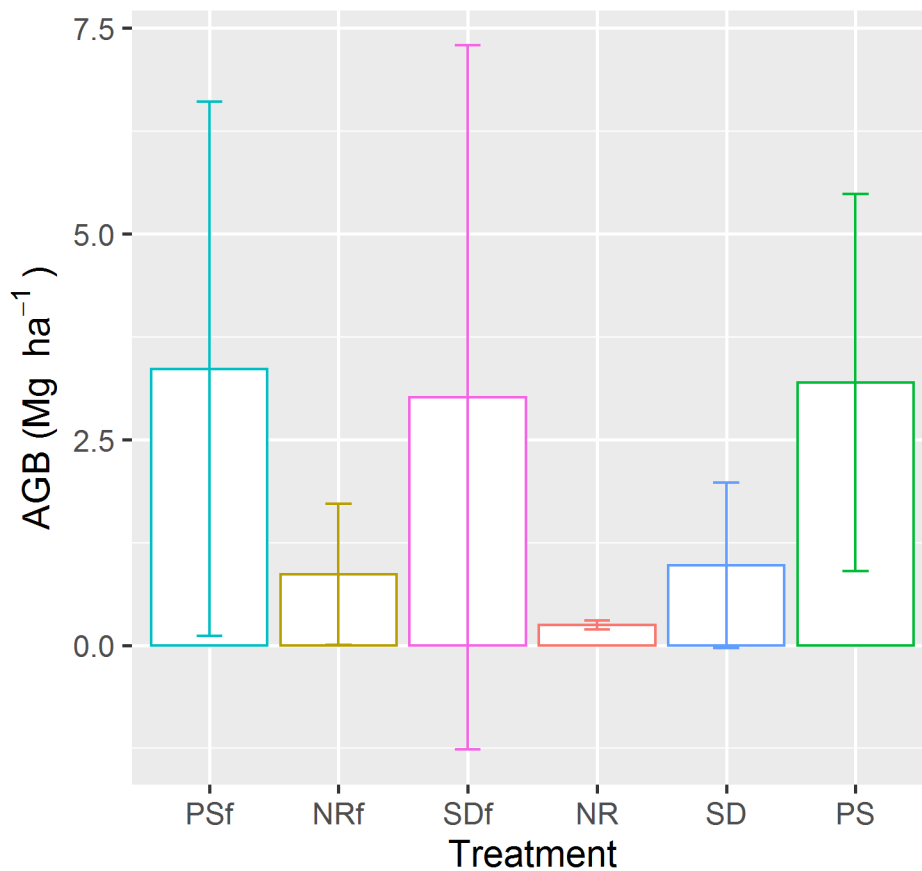
```
mod1 <- lmer(Pressure ~ Depth + (1|Plot), data=dados)
mod2 <- lmer(Pressure ~ Treatment + (1|Plot), data=dados)
mod3 <- lmer(Pressure ~ Period + (1|Plot), data=dados)
mod4 <- lmer(Pressure ~ Depth * Period + (1|Plot), data=dados)
```

**Figure 6.** Effects of multiple predictors on penetration resistance in Mariana, Brazil. Results are presented for the mean distributions. We show the averaged parameter

estimates (standardized regression coefficients) of model predictors, the associated 95% confidence intervals and the relative importance of each factor, expressed as the percentage of explained variance. The tested models show that there are no significant effects of tailing depth and penetration resistance, and seasonality on aboveground biomass, thus the coefficients were not presented.

#### 1.4.4 Effects of predictors on aboveground biomass

Although there are no differences in the substrate penetration resistance, the results show that the aboveground biomass stock is maintained without differences between treatments (Figure 7). Thus, the tested models show that there are no significant effects of tailing depth and penetration resistance, and seasonality on aboveground biomass.



**Figure 7.** Differences in aboveground biomass under a randomized block design with six restoration treatments, consisting of six replicates for each treatment:



Seedlings with fertilization/pH correction (PSf); Seedlings without fertilization/pH correction (PS); Seeding with fertilization/pH correction (SDf); Seeding without fertilization/pH correction (SD); Natural Regeneration with fertilization/pH correction (NRf); Natural Regeneration without fertilization/pH correction (NR), as a control treatment.

## 1.5 DISCUSSION

The penetration resistance was significantly higher on the dry substrate, almost the double comparing all the observations for the dry (3130.98 kPa) with the wet period (1731.07 kPa). Moreover, we have to consider that the means for the dry period are underestimated when compared to the wet period because the penetrometer did not reach 20 cm, according with GLMM approach, the pressure needed to reach this depth would be much higher. Probably, this can be explained because the mud dynamic presents similarly to a common soil, which normally shows higher compaction when dry. Previous studies that have been conducted using penetrometer readings found similar differences comparing the same sites but with variations in soil moisture (Bécel et al. 2012, Silva et al. 2016, Singh et al. 2015). For example, a study carried out by Assis et al. (2009) compared the penetration resistance of four soil types under four moisture conditions and the means ranged from 1130 kPa to 5830 kPa. Additionally, the region, as well as, the study area before the Fundão tailings dam collapse, is traditionally used for livestock (cattle), which has caused soil compaction due to overgrazing (Curtinhas 2010).

As expected, substrate water correlated negatively with penetration resistance, explaining 85 % of its variation while depth was positively correlated with penetration resistance, explaining 11 % of the pressure variation. Penetration resistance increases naturally with depth due to shaft friction and overburden pressure of the weight of the soil above, as well as, changes in soil texture, structure and anthropogenic causes such as agricultural traffic (Manuwa 2013). The determination of the penetration resistance in dry soil conditions results in high levels of compaction (Morais et al. 2014). Thus, soil water content affects negatively the penetration resistance (Hamza & Anderson 2003, Assis et al. 2009; Singh et al.

2015). Tree root development is significantly impeded at penetration resistance values of between 2000 and 3000 kPa (Sinnott et al. 2018) and its growth is diminished in compacted soils because both root growth rate and elongation are reduced (Vocanson et al. 2006; Bécel et al. 2015, Singh et al. 2015). Furthermore, the branching and the root system shape can be affected (Bécel et al. 2012). However, the soil structure commonly provides alternative ways for the roots explore the profile through fissures or cracks, which might overestimate the real resistance that a root is submitted once the penetrometer is inserted vertically into the soil (Moraes et al. 2014, Sinnott et al. 2018).

The AGB stored in restored forest treatments ranged from 0.06 Mg ha<sup>-1</sup> to 10.49 Mg ha<sup>-1</sup>, indicating that despite of the early stage of restoration interventions the substrate properties are not impeding the AGB recovery. In addition, in the plots where active restoration was used the trees presented a considerable development, as noticed during the fieldwork. Overall, we presumed that the active restoration treatments induce a decrease of herbaceous cover due to the shade created by the trees coverage. In addition, this shade condition provides a more suitable microenvironment for the establishment of late secondary tree species (Elgar et al. 2014). Furthermore, probably the root growth in these treatments can contribute to the soil restoration through technosol structuring and nutrient cycling, as well as, the improvement of the soil properties through the incorporation of organic matter, nutrients and preventing erosion processes (Brevik et al. 2015). Thus, contributing to the technosols formation in the areas affected by the collapse of the Fundão dam can provide valuable ecosystem services during forest recovery, for example soil carbon stock (Ruiz et al. 2020).

## **1.6 CONCLUSION**

We concluded that penetration resistance showed differences between seasons, but there were no significant differences between treatments. However, despite of the early stage of restoration interventions the substrate properties are not limiting the AGB recovery. Therefore, the study results show how resistance can be an indicator to select the best period to manage the soil and the restoration process in areas affected by the collapse of the Fundão tailings dam. The relationship

between penetration resistance and water content might play a great importance in the forest restoration success.

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## **2 ARTICLE 2 - COMPARATIVE EFFECTS OF FOREST RESTORATION TECHNIQS ON COMMUNITY DIVERSITY AND ABOVEGROUND BIOMASS ON AREA AFFECTED BY MINING TAILINGS IN MARIANA, MINAS GERAIS STATE, BRAZIL**

### **2.1 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 and fertilization/pH correction 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 (12 × 12 m each) 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). The data on substrate and vegetation were collected in 2019 (2 years and 4 months post-restoration interventions). No significant differences in substrate properties between treatments of site conditions were observed. The AGB stored in the plots ranged from 0.06 Mg ha<sup>-1</sup> (NR) to 10.49 Mg ha<sup>-1</sup> (SDf), following this order: PSf > PS > SDf > SD > NRf > NR. Although biomass storage between treatments was not statistically different, there is a clear pattern showing higher values for each restoration method where fertilization/correction were used compared with the same methods, but without fertilization and pH correction. 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, similar to what is observed in other forests in the region. The Shannon index ranged from 0.528 (PS) to 1.705 (PSf), these values are generally found in areas in early stages of regeneration. Overall, active forest restoration methods showed better responses in the study area, indicating important implications for the forest restoration where natural regeneration is limited or reduced.

**Key words:** Planting of seedlings, direct seeding, natural regeneration, forest restoration, Fundão dam.

## 2.2 INTRODUCTION

Five years after the Fundão dam collapse in Marina, Brazil, the ecological recovery of degraded forest continues to have a high priority with different restoration methods (Martins et al. 2020). The collapse released almost 40 million cubic meters of tailings in the Gualaxo do Norte, Carmo and Doce rivers. The deposition of tailings on the banks of these rivers and their tributaries was restricted to a 113 km stretch that runs from the Fundão dam to the Risoleta Neves hydroelectric plant (Candongá), called Environmental Area 1 (Fundação Renova 2019; Martins et al. 2020). Since then, different methods of active and passive restoration have been applied throughout the area affected with tailings deposits, as well as in compensation areas throughout the Doce River basin (Fundação Renova 2019; Martins et al. 2020). Active and passive restoration methods are used to recover biodiversity and ecosystem services in tropical forest (Crouzeilles et al. 2017; Stuble et al. 2017; Martins 2020). 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 tailings by monitoring ecological indicators 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 (Holl and Aide 2011; Holl 2017). However, in areas with extensive deforestation, invasive grasses, isolated from seed sources and soil degradation may reduce natural regeneration (Holl 2012; Martins 2018). 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 (Martins 2018). 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 (Benayas et al. 2008; Chazdon and Guariguata 2016; César et al. 2018).

The choice of site preparation techniques may depend on restoration goals, environmental conditions, and disturbance intensity (Löff et al. 2016). Focusing on specific soil management can be a way to maximize restoration objectives, as well as prioritizing efficient site preparation that allows a rapid recovery of diversity and ecosystem functioning. (Abella et al. 2015). 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 (Löff et al. 2016; Stuble et al. 2017; Pitz et al. 2018). Thus, soil nutrient content, undoubtedly play important roles in biomass recovery (Chazdon et al. 2007). However, studies have indicated that above-ground biomass and soil nutrient content is lower in secondary tropical forests after several shifting cultivation cycles (Lawrence 2005; Eaton and Lawrence 2009; Villa et al. 2018). 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 (Mesquita et al. 2001; Jager et al. 2015; Zuo et al. 2016). 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 (Laughlin 2014).

During early restoration stages are expected lower AGB and diversity for passive restoration compared with active restoration (e.g. Wheeler et al. 2016). For example, the planting of seedlings is important for the restoration of forest degraded because induce higher AGB accumulation rate than non-planted species (Wheeler et al. 2016). 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 (Elgar et al. 2014; Wheeler et al. 2016). 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

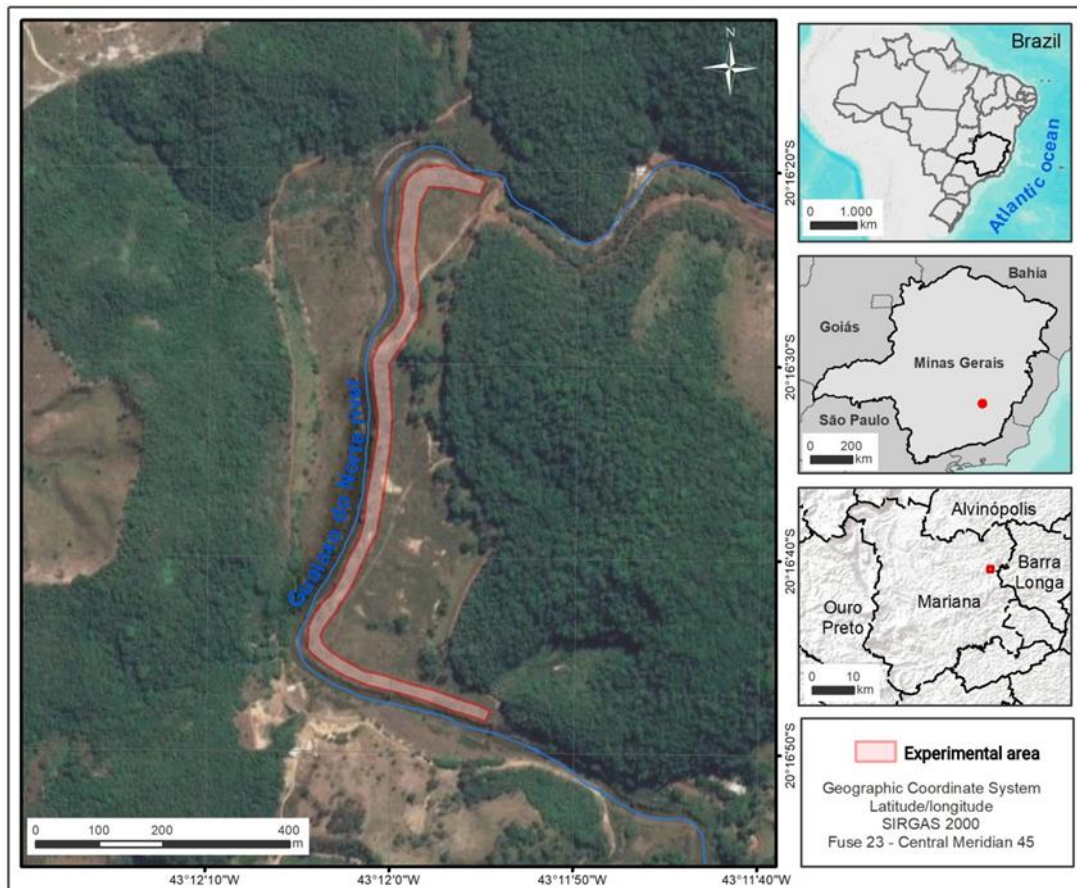


diversity and structure in areas affected by the Fundão tailings dam collapse in Mariana, Minas Gerais state, southeastern Brazil. Here we estimate AGB and tree species richness post restoration interventions, based in six treatments (tree seedlings with fertilization/correction and without fertilization and pH correction; seeding of native trees with fertilization/correction and without fertilization and pH correction; natural regeneration with fertilization/correction and without fertilization and pH correction) in areas affected by the Fundão tailings dam collapse. We hypothesize that: 1) 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.

## **2.3 MATERIAL AND METHODS**

### *2.3.1 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 (Fig. 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 (EMBRAPA 1997).



**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 this 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 (Carmo et al. 2017). 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 by the collapse of the Fundão dam.

### 2.3.2 *Experimental design*

Approximately 16 months after of the Fundão dam collapse, in March of 2017, 36 plots varying from the size of 300 to 500 m<sup>2</sup> 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 4 months post restoration interventions was demarcated a regular area of 144 m<sup>2</sup> (12 × 12 m) in the center of each plot, where the data was collected, so that a possible edge effect would be eliminated.

### *2.3.3 Fertilization and site preparation techniques*

Calcined dolomitic limestone (100 kg ha<sup>-1</sup>), agricultural gypsum (350 kg ha<sup>-1</sup>), ammonium sulfate (100 kg ha<sup>-1</sup>) and super simple phosphate (150 kg ha<sup>-1</sup>) 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.

### *2.3.4 Substrate sampling and analyses of chemical and physical properties in the laboratory*

Substrate were sampled to obtain a better understanding of the different characteristics of the impacted site where the experiment were 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 (EMBRAPA 1997). 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<sup>-1</sup> 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 (Walkley and Black 1934). 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:  $SB = Ca^{2+} + Mg^{2+} + K^{+} + Na^{+}$ ;  $CEC\ pH7\ (T) = SB + (H+Al)$ ;  $t = Ca^{2+} + Mg^{2+} + K^{+} + Al^{3+}$ ;  $V = (SB/T) \times 100$ , respectively. Remaining phosphorus (Prem) was analyzed by colorimetry after reacting the samples with a solution of 60 mg L<sup>-1</sup> of P.

### 2.3.5 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. All individuals were identified using specialised literature, through consultation with the Herbarium of the *Universidade Federal de Viçosa* or by taxonomists. The Angiosperm Phylogeny Group IV (APG IV 2016) 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 ( $\rho$ , g cm<sup>-3</sup>) (Chave et al. 2014). 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 or family 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<sup>-1</sup>) (Rodrigues et al. 2019). Species-level biomass was calculated as the sum of the biomass of all stems from a given species (Rodrigues et al. 2019).

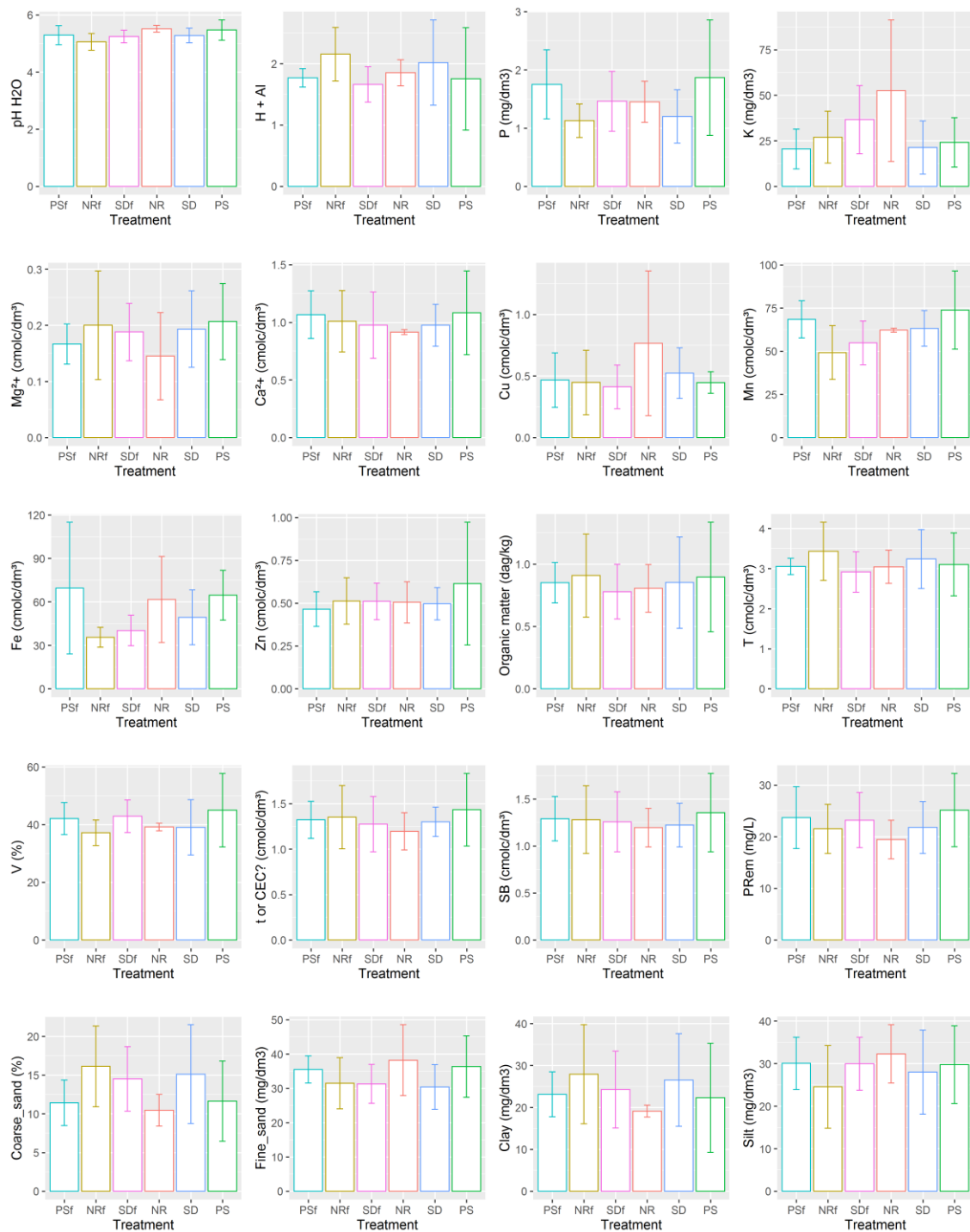
### 2.3.6 Data analyses

All analyses were carried out in R Environment (R Core Team 2018). 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 (Crawley 2012). 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 (Crawley 2012; R Core Team, 2018). 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 (Dinno 2017).

## 2.4 RESULTS

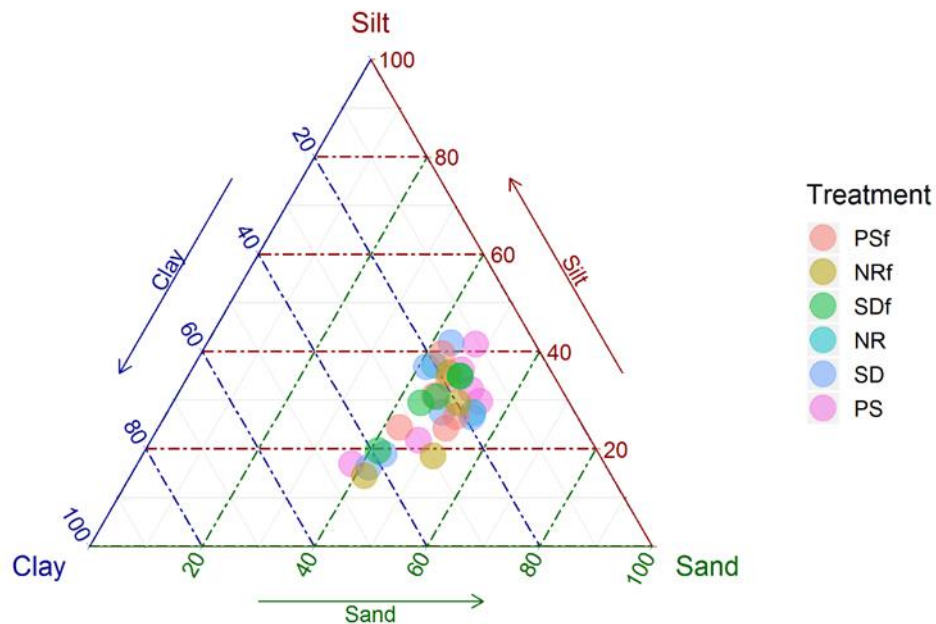
### 2.4.1 Substrate properties pattern

No significant differences in substrate properties between treatments of site conditions were observed (Fig. 2). The texture properties showed that there is a higher proportion of sand; however, it presents a similar variability between clay and silt (Fig. 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<sub>2</sub>O), 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).



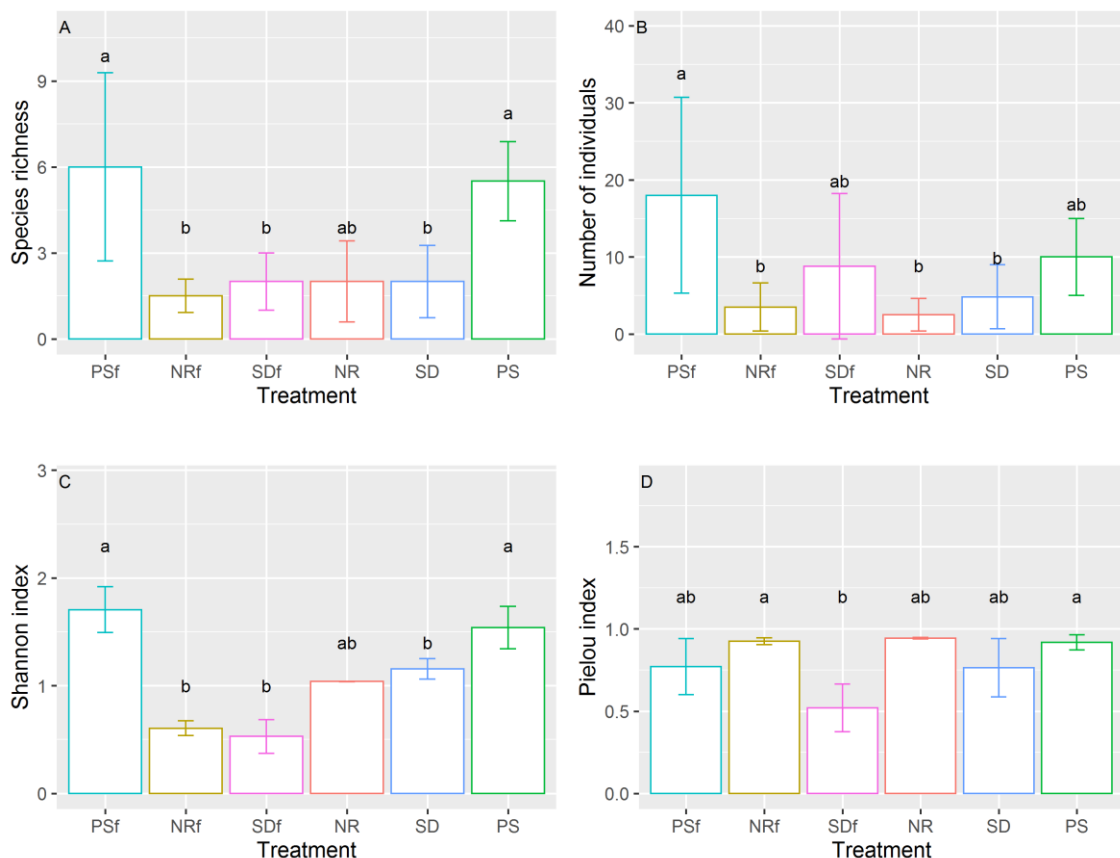
**Figure 3.** Ternary plots showing the relationships between clay, silt and sand proportions in different treatments: planting of native tree seedlings with fertilization and pH correction (PSf); planting of native tree seedlings without fertilization and pH correction (PS); seeding of native trees 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 (Alvarez et al. 1999) 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<sup>-1</sup>). K content was classified as low and P as very low, not reaching values above 2.35 mg dm<sup>-3</sup>.

Furthermore, all the other nutrients analyzed followed the same classification. Thus, the substrate demonstrates a low fertility. Likewise, we present the average from three replicates of chemical characteristics of the soil in areas contaminated with mining tailings after the Fundão tailings dam disruption in Mariana (Table SIII from SM).

#### 2.4.2 Differences in species diversity, abundance and aboveground biomass

Species diversity and abundance showed differences between treatments (Fig. 4). Species richness ( $\chi^2 = 16.08$ ,  $df = 5$ ,  $p < 0.01$ ), abundance ( $\chi^2 = 11.31$ ,  $df = 5$ ,  $p < 0.01$ ), Shannon index ( $\chi^2 = 14.80$ ,  $df = 5$ ,  $p < 0.01$ ), and Pielou index ( $\chi^2 = 9.72$ ,  $df = 5$ ,  $p < 0.01$ ) showed differences between treatments. However, there are no differences in aboveground biomass ( $p = 0.11$ ).

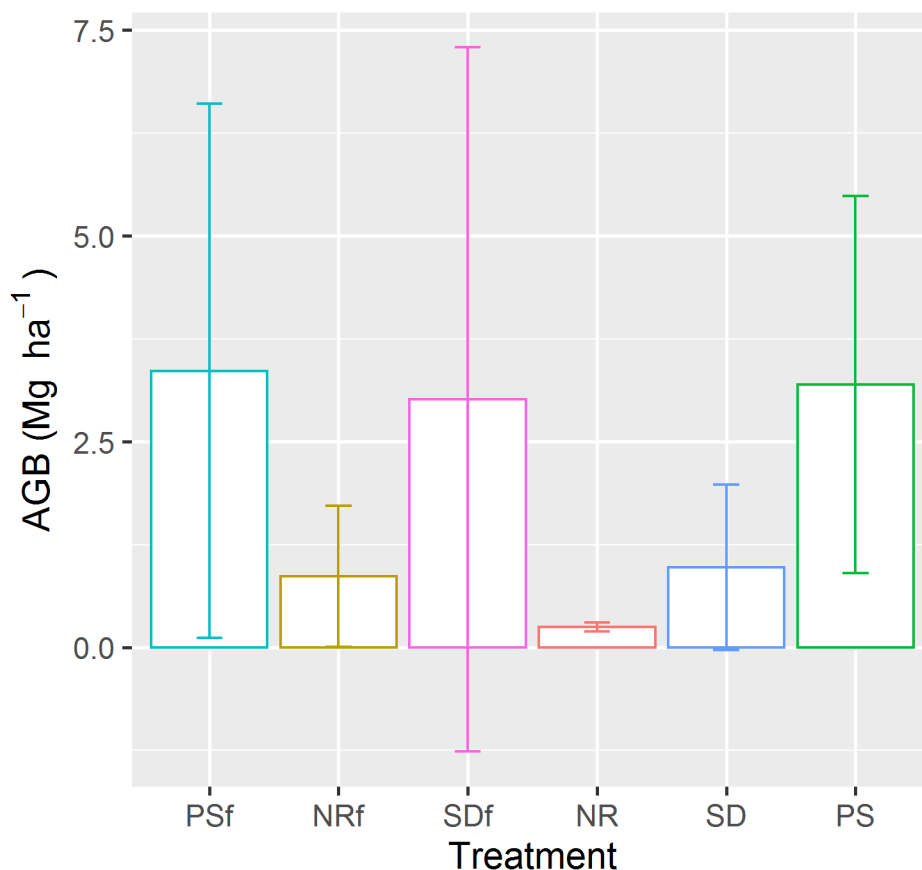


**Figure 4.** Differences in tree species richness, number of individuals, and Shannon-Wiener's diversity and Pielou's evenness index. A randomized block design with six restoration treatments was used, consisting of six replicates for each treatment:



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).

The AGB stored in the plots ranged from 0.06 Mg ha<sup>-1</sup> (NR) to 10.49 Mg ha<sup>-1</sup> (PSf). On average, the highest AGB storage was found for PSf (3.36 Mg ha<sup>-1</sup>), PS (3.19 Mg ha<sup>-1</sup>), SDf (3.01 Mg ha<sup>-1</sup>) and SD (0.97 Mg ha<sup>-1</sup>). 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<sup>-1</sup>) and NR (0.25 Mg ha<sup>-1</sup>) (Fig. 5).



**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).

## 2.5 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 (Lehmann and Schroth 2003; Fink et al. 2016) since the establishment of the experiment. 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.

This 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 (i.e. Gioria et al. 2012; Gioria and Pyšek 2015). Pilocelli (2020), also studying 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. Accordingly, the 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 fertilization/ pH correction were applied compared with the same methods, but without fertilization and pH correction (Fig. 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. Wheeler et al. (2016) found similar results, where in the first ten years after planting of seedlings the AGB accumulation was  $0.95 \text{ Mg ha}^{-1} \text{ y}^{-1}$  and the natural regeneration was hindered by elephant grass (*Pennisetum purpureum*). In the study site, weed control seems to be particularly important, as the native tree species suffer strong competition from invasive grasses (Gioria et al. 2012; Gioria and Pyšek 2015). Ferez et al. (2015) 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 6 years of the restoration interventions ( $18.2$  and  $5.2 \text{ Mg C ha}^{-1}$ , respectively).

Several studies have shown that soil properties have important effects on plant communities in early forest succession (e.g. Powers and Marín-Spiotta 2017; Stuble et al. 2017), mainly, soil nutrients content which can favor the biomass accumulation and shape plant diversity in secondary tropical forests (Estrada-Villegas et al. 2019; van Breugel et al. 2019). For instance, fertilization with N and P increased seedling richness, reduced mortality (Ceccon et al. 2003), accelerated plant growth (Davidson et al. 2004) and also induce changes in plant species composition (Siddique et al. 2010). Freitas et al. (2019) have evaluated 72 direct-seeded sites between one and 10 years old, three seedling planting sites and six natural regeneration sites aged from 7 to 9 years old in Mato Grosso, Brazil. 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 (Donato et al. 2012). Moreover, a more diverse community is expected to use more efficiently

the available resources due to their complementary needs (Williams et al. 2017; Lu et al. 2018). The 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 (Martínez-Ramos et al. 2016; Stuble et al. 2017).

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 (Fig. 4). This result suggests evenness 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 (Lorenzi 2000; Rodrigues et al. 2010). Studies have shown similar Pielou index values in sites under restoration processes in Brazil, for example, 0.60 (Colmanetti et al. 2016), 0.73 (Miranda-Neto et al. 2014) and 0.811 (Lopes et al. 2018).

The Shannon index ranged from 0.528 (PS) to 1.705 (PSf) (Fig. 4). However, it's a low value compared to those found in areas under restoration processes in Minas Gerais, 3.258 (Balestrin et al. 2019) and 3.103 (Lopes et al. 2018). Also, in the state of Minas Gerais, Silva et al. (2018) found 2.35 for the natural regeneration after 18 months of restoration. Likewise, Elfili and Resende (2003) stated that the Shannon index normally vary from 1.3 to 3.5, but can reach values above 4.70 for tropical forests; nevertheless, it is worth mentioning again that the data was collected at an early stage, after less than 2 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.

## 2.6 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. 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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### 3 ARTICLE 3 - FUNCTIONAL COMPOSITION ENHANCES PLANT ABOVEGROUND BIOMASS STORAGE UNDERGOING ACTIVE RESTORATION ON MINING TAILINGS IN MARIANA, BRAZIL

#### 3.1 ABSTRACT

Ecological restoration activities on the areas with dam tailing in Mariana region, Brazil, combining different methods of active and passive restoration are being applied. However, there is still a major need to know the efficiency of these methods. We hypothesized that aboveground biomass (AGB) increment is promoted by the simultaneous effects of restoration treatments and technosol quality through functional diversity. These are then also related to the community-weighted mean of the community of values of functional traits (CWM) related to the mass ratio hypothesis (MRH) in different restoration treatments. We evaluated different Linear mixed-effect models to determine direct effects of abiotic, taxonomic, stand structural and functional attributes undergoing different restoration methods (planting of nursery-grown seedlings, seeding and natural regeneration) and the effect of soil fertilization on AGB. Stems and biomass distribution were categorized into the following functional groups: successional strategy (pioneer, early secondary and late secondary), dispersal syndrome (autochoric, anemochoric and zoochoric), nitrogen fixation (fixer and non-fixer), wood density (softwood and hardwood) and also if the species were either planted or naturally regenerated. As expected, at this early stage of the restoration process, the more representative successional group was the pioneer species and the anemochoric syndrome mainly represented by naturally regenerated stems. The autochoric dispersal and the nitrogen-fixing trees showed to be biomass dominant groups, driven mostly by *Senna alata*, because of its high abundance and wood density (hardwood). We found that high CWM of wood density and active restoration methods determine higher levels of AGB on mining tailings where natural regeneration is limited. Our results support the MRH, and explain how functional composition as the main predictor determines AGB during restoration. We conclude that functional dominance overrides the effects of species richness and abundance on AGB, and hence, it is important to test the mutual effects of functional composition when testing the effects of restoration methods on forest functioning.

**Keywords:** biodiversity-ecosystem function, functional identity, functional traits, community-weighted mean, hyperdominant species

### 3.2 INTRODUCTION

The Fundão dam rupture accident in Mariana, Minas Gerais, released almost 40 million cubic meters of tailings in the Gualaxo do Norte, Carmo and Doce rivers. The deposition of tailings on the banks of these rivers and their tributaries was restricted to a 113 km stretch that runs from the Fundão dam to the Risoleta Neves hydroelectric plant, called Environmental Area 1 (Fundação Renova 2019; Martins et al. 2020). The ecological restoration activities on the affected areas combining different methods and techniques of active and passive restoration are being applied (Martins et al. 2020). These methods are typically used in contrasting situations, while passive restoration is usually successful where the vegetation spontaneously recovers (Holl and Aide 2011; Holl 2017), and active restoration is recommended in areas where natural regeneration is hindered or limited (Holl 2012; Martins 2018). However, there is still a major need to know the efficiency of these methods on the areas affected and how they are responding. For example, at early restoration stages are expected higher diversity and aboveground biomass accumulation for planted seedlings compared with non-planted species (Wheeler et al. 2016). Thus, monitoring ecological indicators of rapid assessment and direct positive effects on ecosystem stabilization contributes to a better understanding of the efficacy of restoration practices and guides further interventions that may be needed.

In tropical forest, the stand age-dependent forest attributes (functional, taxonomic and structural attributes) concomitantly shape ecosystem functioning; such as aboveground biomass stock (Yuan et al. 2016; Ali et al. 2017; Poorter et al. 2017). Thus, understanding the relationships between functional traits composition, species richness and composition, stand structural (i.e. wood density and height) and, aboveground biomass stock is important for global carbon accounting (Lohbeck et al. 2015; Ali et al. 2016; Poorter et al. 2016; Ouyang et al. 2019), given that carbon stock within aboveground biomass in restoring forests is one of the most important processes for mitigating climate change (Villa et al. 2020a). These changes during restoration can be evaluated using functional attributes associated with regeneration (e.g. Poorter et al. 2017; 2019), such as seed dispersal syndrome and regeneration strategies (Tabarelli and Peres 2002; Westoby 2002; Santo-Silva et al. 2012, 2016; Tabarelli 2012; Rozendaal and Chazdon 2015). In this sense, there is strong evidence that during the early stages of tropical forest succession, light-demanding

pioneer species dominate (Villa et al. 2018, 2019) along with non-zoochoric pioneer species (Tabarelli et al. 2010, 2012). However, little is known about the ecosystem functioning distribution (i.e. biomass stock) of dominant species and functional groups during active restoration. Furthermore, further research is still needed to explore the direct effects of forest attributes on aboveground biomass in tropical forest undergoing active restoration. For example, abiotic attributes (i.e. soil nutrients), taxonomic attributes (i.e. species richness), structural attributes (i.e. stem density and tree size), and functional trait composition (i.e. wood density).

There is sufficient evidence to demonstrate a positive relationship between biodiversity and ecosystem functioning (BEF) in tropical forests (Chisholm et al. 2013; Poorter et al. 2015, 2017). The BEF relationship is very important for the understanding of how species diversity affects the dynamics of production and storage of aboveground biomass (AGB) (e.g., Poorter et al. 2015; Villa et al. 2020b), including in areas undergoing secondary succession in human-modified forest landscapes (Lohbeck et al, 2015; Villa et al. 2020b). Previous studies show that in tropical forests the dominant species (i.e. higher basal area or wood density) are the most representative species in ecosystem processes (e.g. Bastin et al. 2015; Fauset et al. 2015; Rodrigues et al. 2019). This phenomenon of a disproportionate influence of a small number of species on ecosystem functioning is also called “hyperdominance” (e.g. Bastin et al. 2015; Rodrigues et al. 2019) and has been applied to describe the relative contribution to ecosystem services, such as biomass and carbon storage at regional and fine scales (Fauset et al. 2015; Rodrigues et al. 2019).

Thus, the mass ratio hypothesis (MRH) postulates that aboveground biomass is driven by functional traits of the most abundant species in communities (Grime 1998). There is substantial evidence to support this hypothesis wherein aboveground biomass should be closely related to the community-weighted trait mean (CWM) in natural forests (Prado-Junior et al. 2016; Yuan et al. 2016; Ali et al. 2017). Under the predictions of MRH, many studies have reported that the CWM of trait values determines aboveground biomass due to the dominance of few productive and high functioning species in natural second-growth tropical forests (Lohbeck et al. 2015; Lohbeck et al. 2016; Ali et al. 2017; Poorter et al. 2017). Functional composition are not only predictors of aboveground biomass in natural forests, but also there is

another explanatory variable, such as abiotic (e.g., soil and climate) and structural attributes (e.g., stem count) that explain variation in aboveground biomass (Chiang et al. 2016; Ali et al. 2016, 2017; Poorter et al. 2017; Fotis et al. 2018). For example, soil nutrients determine resource availability (Paoli et al. 2005), and their availability triggers faster plant growth (Russo et al. 2005; Quesada et al. 2012).

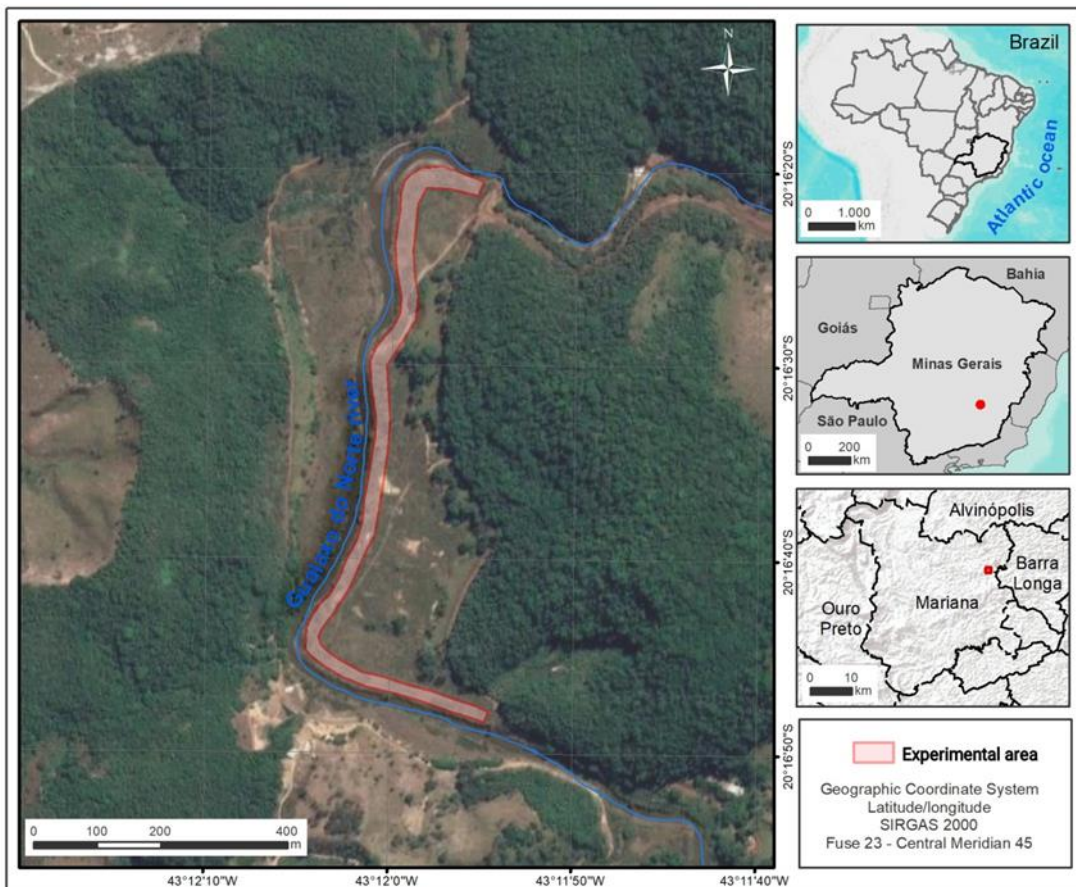
Here, we aim to evaluate the effects of abiotic (i.e. technosol nutrients), taxonomic (i.e. tree species richness), stand structural (i.e. stem count), functional attributes (functional composition of wood density and height) undergoing different restoration methods (i.e. planting of nursery-grown seedlings, direct seeding and natural regeneration) on AGB, as well as to evaluate the AGB distribution pattern among functional groups by restoration treatment in areas affected by the Fundão tailings dam collapse in Mariana, Minas Gerais state, southeastern Brazil. We estimate the biomass distribution by functional groups, such as successional strategy, dispersal syndrome, nitrogen fixation, wood density categories and restoration strategy. Thus, we respond to the following research questions: 1) How are the tree species richness and composition pattern by functional groups and restoration treatments? 2) What is the relative importance of tree functional groups among the stem dominant and biomass dominant species by restoration treatments? 3) How do abiotic (Technosol nutrients) and stand tree attributes (taxonomic, structural, and functional) determine aboveground biomass? 3) What is the relative effect of abiotic (Technosol nutrients), stand tree attributes (taxonomic, structural, and functional) and restoration methods on aboveground biomass? We hypothesize that the effects of restoration methods and functional trait composition overrule the effects of taxonomic and stand structural attributes on aboveground biomass.

### **3.3 MATERIALS AND METHODS**

#### *3.3.1 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 (Fig. 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 (EMBRAPA 1997). The tailings accumulated in the study area presented 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 (Carmo et al. 2017). 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 by the collapse of the Fundão dam.



**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.



### 3.3.2 *Experimental design*

Approximately 16 months after of the Fundão dam collapse, in March of 2017, 36 plots varying from the size of 300 to 500 m<sup>2</sup> 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/correction (SDf) and without fertilization and pH correction (SD); natural regeneration with fertilization/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 SM). Two years and 4 months post restoration interventions were demarcated a regular area of 144 m<sup>2</sup> (12 × 12 m) in the center of each plot, where the data was collected, so that a possible edge effect would be eliminated.

### 3.3.3 *Site preparation techniques and fertilization*

Calcined dolomitic limestone (100 kg ha<sup>-1</sup>), agricultural gypsum (350 kg ha<sup>-1</sup>), ammonium sulfate (100 kg ha<sup>-1</sup>) and super simple phosphate (150 kg ha<sup>-1</sup>) were applied to improve the substrate fertility in plots where correction and fertilization were foreseen. Subsoiling was carried out 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.

### 3.3.4 *Technosol sampling and analyses of chemical and physical properties in the laboratory*

The technosol 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 technosol properties, composite

samples of 5 sub-samples were taken at the edges and center of each plot. Technosol 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 (EMBRAPA 1997). All 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<sup>-1</sup> 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 (Walkley and Black 1934). 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:  $SB = Ca^{2+} + Mg^{2+} + K^{+} + Na^{+}$ ;  $CEC\ pH7\ (T) = SB + (H+Al)$ ;  $t = Ca^{2+} + Mg^{2+} + K^{+} + Al^{3+}$ ;  $V = (SB/T) \times 100$ , respectively. Remaining phosphorus (Prem) was analyzed by colorimetry after reacting the samples with a solution of 60 mg L<sup>-1</sup> of P.

### 3.3.5 *Plant inventory and functional traits*

In each plot, all trees with diameters at breast height (DBH)  $\geq 2$  cm were identified to the species level and tagged for measurement. The *Angiosperm Phylogeny Group IV* (APG IV 2016) was used to classify species into families.

We used categorical and quantitative tree functional traits for different analyzes of this study. Categorical functional traits were divided into the following categories: successional strategy (pioneer, early secondary and late secondary species), dispersal syndrome (anemochoric, autochoric and zoochoric), nitrogen fixation (fixer and non-fixer species), and for wood density categories as softwood ( $wd < 0.6\ g.cm^{-3}$ ) or hardwood species ( $wd \geq 0.6\ g.cm^{-3}$ ). These criteria have previously been adopted in other studies (Tabarelli and Peres 2002; Santo-Silva et al. 2012; Rozendaal and Chazdon 2015; Piotto et al. 2020). We consider this trait categorical classification as functional groups in this study. The species-specific stem

wood density (WD) and height (quantitative tree functional traits) is a key trait for plant growth, survival and forest carbon storage during succession (Poorter et al. 2019). Wood density reflects a trade-off between volumetric growth and survival because of resistance against biophysical hazards (Westoby et al. 2002; Santo-Silva et al. 2016). We gathered species' wood density values from the Global Wood Density Database (Chave et al. 2009).

### 3.3.6 Quantification of functional composition

Functional composition index was computed to discriminate the main generic properties of functional diversity in a community, i.e., the dominance of functional traits (most abundant species) (Conti and Díaz 2013; Ali et al. 2017). For the quantification of functional composition, the community weight mean (CWM) values of each functional trait (wood density and height) were calculated as the mean value of the trait in the community, weighting by species' relative abundance (Eq. 1) (Garnier et al. 2004). We used the relative abundance of species rather than basal area to avoid circular redundancy derived from the use of DBH to calculate basal area and aboveground biomass (Conti and Díaz 2013; Ali et al. 2017).

$$CWM_{\chi} = \sum_{i=1}^s (p_i * t_i) \quad \text{Eq. 1}$$

where  $CWM_{\chi}$  is the CWM for trait  $\chi$  in each plot,  $s$  is the number of species in the plot,  $p_i$  is the relative abundance of the  $i$ th species in the plot and  $t_i$  is the trait value for the  $i$ th species. The CWM was calculated for each plot from the species abundance and functional traits using the FD package (Villéger et al. 2008; Laliberté and Legendre 2010).

### 3.3.7 Aboveground biomass estimation

The aboveground biomass of individual stems was calculated using the general allometric equation (Eq. 2) proposed by Chave et al. (2014), based on tree DBH (cm), and species' wood density ( $\rho$ , g cm<sup>-3</sup>). We used Neotropical data from the Global Wood Density Database (Chave et al. 2009) to obtain the wood density for each species, using genus or family averages whenever species-level information was not available.

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

The total aboveground biomass (AGB) per plot was the sum of the AGB of all trees with DBH  $\geq$  2 cm, which was then converted to megagrams per hectare (Mg ha<sup>-1</sup>) (Ali et al. 2017).

### 3.3.8 Stem and biomass distribution between functional groups

We estimated the stem and biomass distribution divided into the following functional groups: successional strategy (pioneer, early secondary and late secondary), dispersal syndrome (autochoric, anemochoric and zoochoric), nitrogen fixation (fixer and non-fixer), wood density (softwood and hardwood) and also if the species were either planted or naturally regenerated. We accounted the number of species required to accumulate 50% of biomass stock and stems, which allow us to assess, respectively, the number of biomass dominant and stem dominant species in each treatment. Dominant or hyperdominant species are the species that together accumulate at least 50% of the total biomass/stems when ranked by a decreasing order of contribution to the total biomass or number of stems (Bastin et al. 2015; Fauset et al. 2015).

### 3.3.9 Statistical analyses

All analyses were run in the software R 3.6.0 (R Core Team 2019), and for graphs illustration in this study, we used the 'ggplot2' package (Hadley 2015). We calculated rarefied species richness for all restoration treatments plots using "iNext" package (Hsieh et al. 2016). To compare the variation of community composition between restoration treatments a Non-metric multidimensional scaling (NMDS) analysis was performed using 'metaMDS' function based on Jaccard similarity (Clarke 1993; Oksanen et al. 2018). After checking the stress generated by the NMDS, we corroborate the non-metric fit based on stress using linear regression (Fig. S1 from Supplementary Material, SM). Furthermore, we used the 'MDSrotate' function, which rotates an external environmental variable (AGB) to be parallel to the first multidimensional scaling dimension (Oksanen et al. 2018; Villa et al. 2018, 2020b). All different functions of NMDS are available within the "vegan" package

(Oksanen et al. 2018). Thus, we studied species richness and community composition based on two categorical functional attributes associated with forest regeneration (successional strategy, dispersal syndrome, nitrogen fixation, and wood density categories).

Technosol nutrients properties were summarized by principal component analysis (PCA) on the correlation matrix separately using the 'FactoMineR' package (Husson et al. 2018). For that, all variables were centered and standardized, and correlations between pairs of individual variables and PCA scores were presented. To reduce any strong correlations between pairs of nutrients properties, we used the first axis of PCA for variability of chemical properties-related technosol fertility (PCA1f) as variables in the following analyses (i.e. Schmitz et al. 2020).

We used linear mixed-effects models (LMMs, with random and fixed effects) to test the main effects of multiple drivers (taxonomic, abiotic, structural and functional forest attributes) and restoration treatments on aboveground biomass. Predictors with fixed effects (continuous explanatory variables) were grouped into four categories of forest attributes, such as taxonomic attributes (i.e. species richness), abiotic attributes, i.e. variability of chemical properties-related technosol fertility (PCA1f), functional attributes (CWM WD and height), and structural attributes (stem count). The plots were considered as a random effect (1 | plots) in all models. The Gaussian error distribution were tested (Fig. S2 from SM), after that the distributions of residuals were visually checked and the most suitable distribution and link function (i.e. normality was confirmed by the Q-Q graph and Shapiro–Wilk test) was evaluated (Zuur et al. 2009; Crawley 2012). We assessed collinearity between selected predictor variables using Spearman correlation analysis; when two variables were strongly correlated ( $r \geq 0.7$ ), were included in univariate models (Fig. S3 from SM). We have used a chemical-based PCA to extract the scores (PCA1f) of variability of chemical properties-related technosol fertility (Fig. S4 from SM). All models were calculated using the package 'lme4' (Bates et al. 2019) in the platform R (R Core Team 2019).

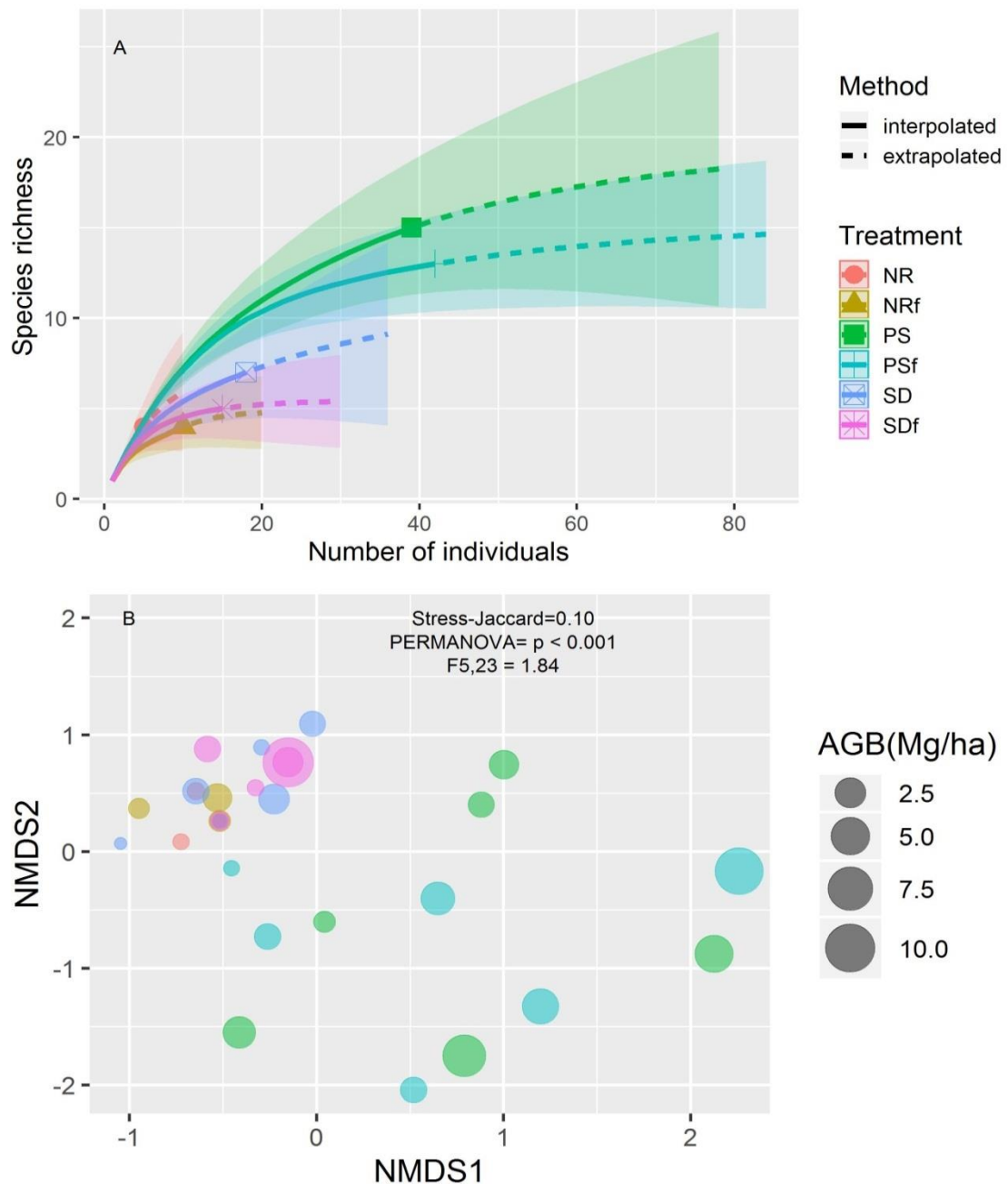
Finally, we compared the most parsimonious model using a multi-model inference approach with the *Dredge* function of the "MuMIn" package (Barton 2017), based on the Akaike Information Criterion (AIC), considering all models with  $AIC < 2.0$  as equally plausible (Burnham and Anderson 2002; Burnham et al. 2011). We

also used the estimates of the predictors' coefficients to interpret parameter estimates on a comparable scale using the “jtools” package (Long 2020).

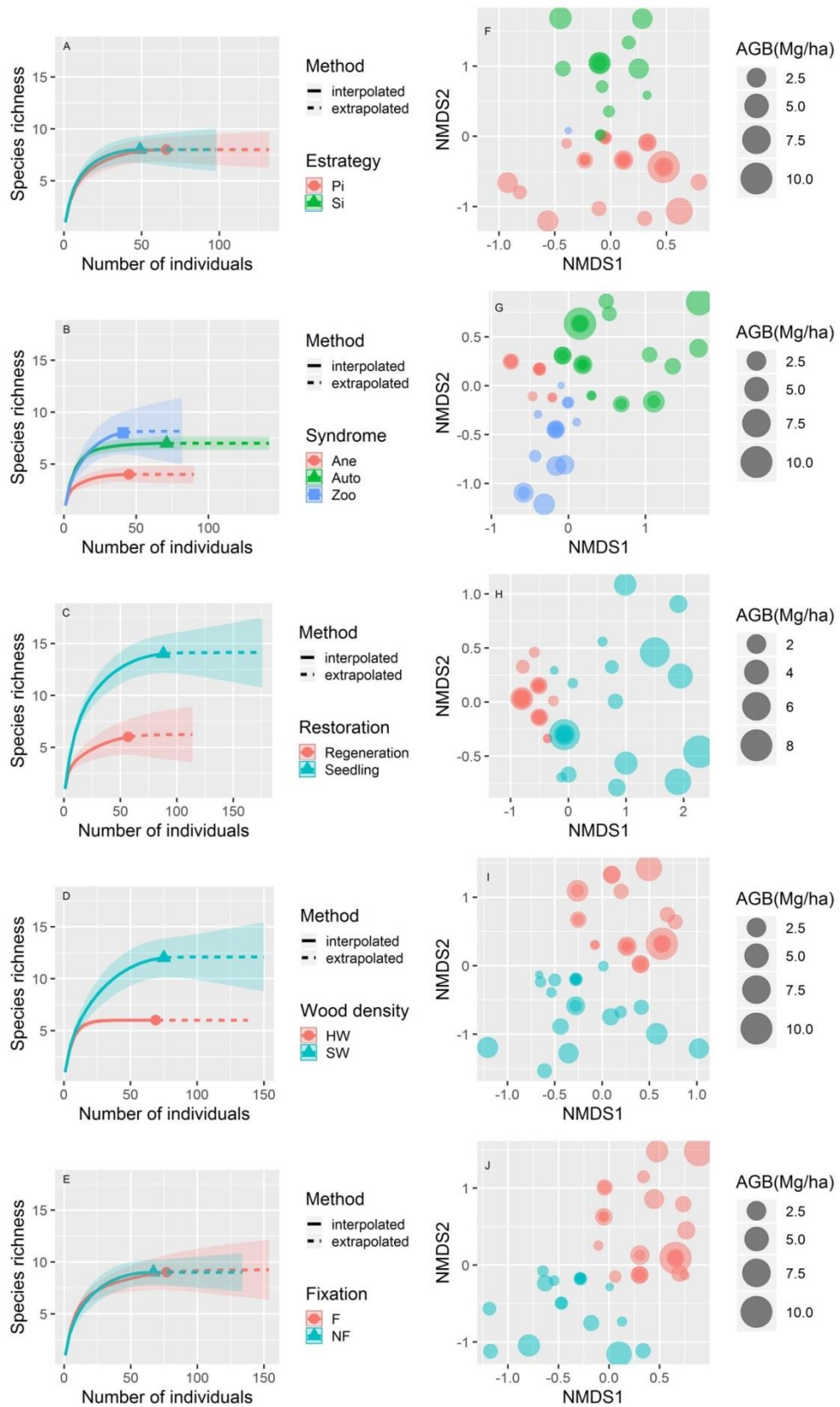
### **3.4 RESULTS**

#### *3.4.1 Species richness and community composition*

Species richness differed significantly between planting seedling treatments in relation to different seeding and natural regeneration treatments (Fig. 2A). However, there are not differences between planting seedling treatments (with and without fertilization), which presented the highest richness observed. Species richness for most functional groups have significant differences, except between nitrogen fixers and non-fixers, and between pioneers and secondary species (Fig. 3A-J).



**Figure 2.** Individual-based rarefaction curves (A) and Non metric multidimensional scaling (NMDS) based on species composition (B) from different treatments along an aboveground biomass (AGB) gradient in Mariana. Treatments: PSf = planting of native tree seedlings with fertilization and pH correction; PS = planting of native tree seedlings without fertilization and pH correction; SDf = seeding of native trees with fertilization/correction; SD = seeding of native trees without fertilization and pH correction; NRf = natural regeneration with fertilization/correction; NR = natural regeneration without fertilization and pH correction.



**Figure 3.** Individual-based rarefaction curves and Non metric multidimensional scaling (NMDS) based on species composition from different treatments and



functional groups along an aboveground biomass (AGB) gradient in Mariana. Functional groups: Successional group (Pi = pioneer; ES = early secondary; LS = late secondary); Dispersal syndrome (Zoo = zoochoric; Ane = anemochoric; Auto = autochoric); Wood density (HW = hardwood; SW = softwood); Nitrogen Fixation (F = fixer; NF = non-fixer). Treatments: PSf = planting of native tree seedlings with fertilization and pH correction; PS = planting of native tree seedlings without fertilization and pH correction; SDf = seeding of native trees with fertilization/correction; SD = seeding of native trees without fertilization and pH correction; NRf = natural regeneration with fertilization/correction; NR = natural regeneration without fertilization and pH correction.

Plant community composition showed significantly differences between some restoration treatments on two NMDS axis (Figure 2B). Thus, planting seedling (with and without fertilization) presented significant differences with different seeding and natural regeneration treatments (with and without fertilization).

#### 3.4.2 Biomass and abundance (stem count) distribution

The total AGB per treatment ranged from 0.50 Mg ha<sup>-1</sup> (NRf) to 20.16 Mg ha<sup>-1</sup> (PSf) (Table 1). All the treatments have either one or two AGB dominant species, cumulating more than 50% of total community AGB. The species per treatments are *Inga vera* and *Croton floribundus* (PS), *Mimosa pigra* (NR), *Senna alata* (SD), *Senna alata* and *Inga vera* (PSf), *Mimosa pigra* (NRf) and *Senna alata* (SDf). Thus, only these four species were classified as AGB dominant in the community. *Senna alata* demonstrated the highest number of stems in three treatments (SD, PSf and SDf), *Vernonanthura phosphorica* in two (NR and NRf) although it was not classified as AGB dominant and *Inga vera* in one (PS).

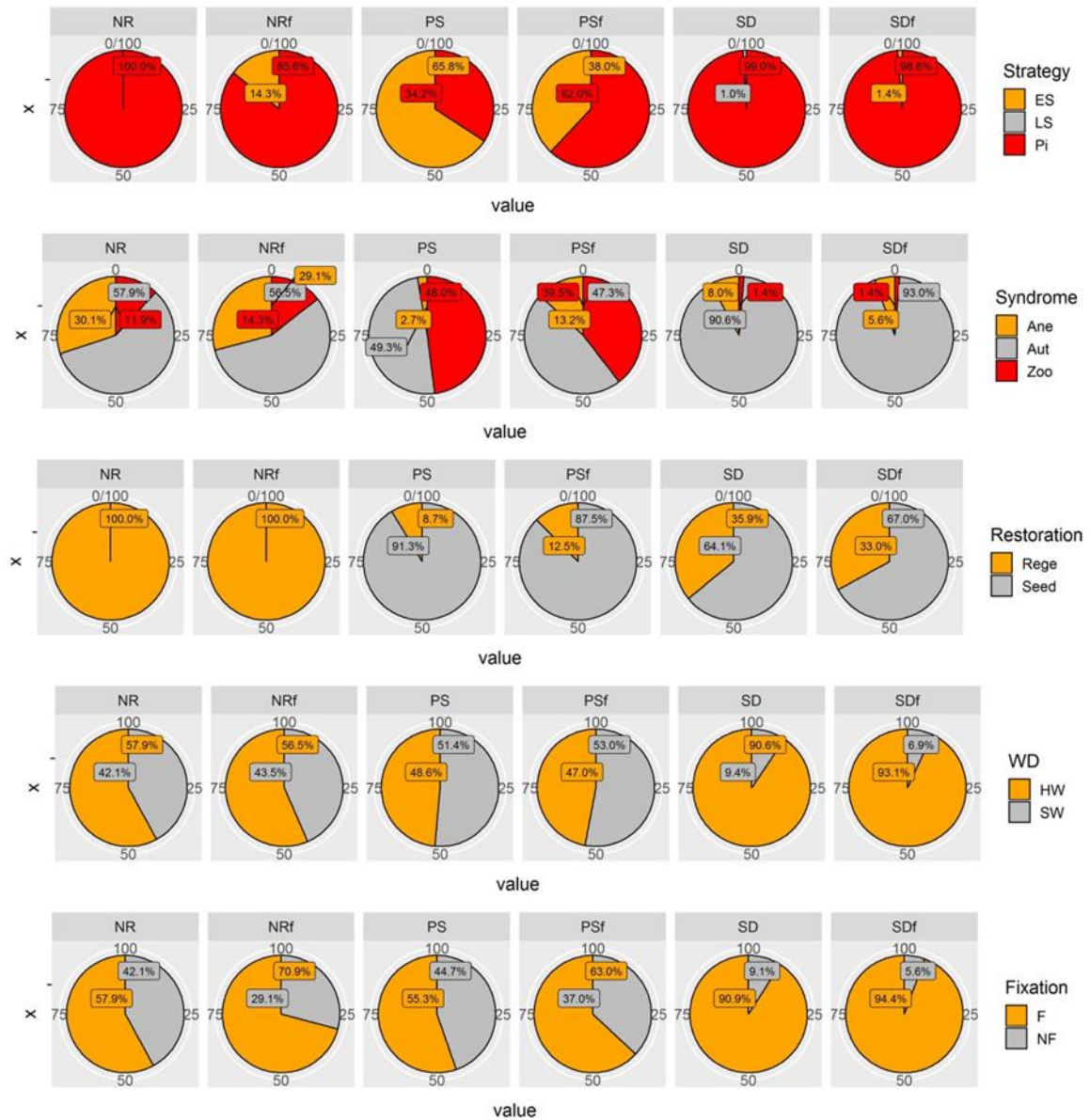
**Table 1:** Biomass species distribution per treatment. Biomass dominant species are indicated in bold and stem dominant with an \*(cumulating more than 50% of total community AGB or stems).

Treat	Species	Stem	DS	NF	RS	SG	WD	AGB (Mg ha <sup>-1</sup> )	AGB Acum (Mg ha <sup>-1</sup> )	Pr AGB (%)	Pr Acum AGB (%)
PS	<i>Inga vera</i> *	14	Zoo	fix	pla	ES	SW	6.21	6.21	32.42	32.42
PS	<i>Croton floribundus</i> *	9	Aut	nonf	pla	ES	HW	5.05	11.27	26.39	58.81
PS	<i>Croton urucurana</i>	3	Zoo	nonf	pla	P	SW	2.73	14	14.26	73.08
PS	<i>Mimosa pigra</i>	2	Aut	fix	reg	P	HW	1.32	15.31	6.87	79.94
PS	<i>Senna alata</i> *	12	Aut	fix	pla	P	HW	1.23	16.54	6.4	86.35
PS	<i>Senna pendula</i>	6	Aut	fix	pla	P	HW	0.87	17.41	4.56	90.91
PS	<i>Bauhinia forficata</i>	4	Aut	fix	pla	ES	HW	0.84	18.25	4.36	95.27
PS	<i>Guazuma ulmifolia</i>	2	Ane	nonf	pla	ES	SW	0.23	18.48	1.18	96.45
PS	<i>Solanum lycocarpum</i>	2	Zoo	nonf	reg	P	SW	0.2	18.68	1.03	97.49
PS	<i>Vernonanthura phosphorica</i>	1	Ane	nonf	reg	P	SW	0.15	18.83	0.8	98.29
PS	<i>Heliocarpus popayanensis</i>	1	Ane	nonf	pla	ES	SW	0.14	18.97	0.73	99.01
PS	<i>Senna multijuga</i>	2	Aut	fix	pla	ES	SW	0.13	19.1	0.68	99.69
PS	<i>Solanum granulosoleprosum</i>	1	Zoo	nonf	pla	P	SW	0.05	19.15	0.27	99.96
PS	ni	1			pla			0.01	19.16	0.04	100
NR	<i>Mimosa pigra</i>	1	Aut	fix	reg	P	HW	0.29	0.29	57.94	57.94
NR	<i>Vernonanthura phosphorica</i> *	2	Ane	nonf	reg	P	SW	0.15	0.44	30.14	88.08
NR	<i>Solanum granulosoleprosum</i>	1	Zoo	nonf	pla	P	SW	0.06	0.5	11.92	100
SD	<i>Senna alata</i> *	16	Aut	fix	pla	P	HW	3.6	3.6	61.79	61.79
SD	<i>Mimosa pigra</i>	3	Aut	fix	reg	P	HW	1.63	5.23	27.89	89.68
SD	<i>Vernonanthura phosphorica</i>	7	Ane	nonf	reg	P	SW	0.47	5.7	7.98	97.66
SD	<i>Trema micrantha</i>	1	Zoo	nonf	pla	P	SW	0.06	5.75	1.02	98.67
SD	Unidentified	1			pla			0.06	5.81	0.97	99.65
SD	<i>Enterolobium contortisiliquum</i>	1	Zoo	fix	pla	LS	SW	0.02	5.83	0.35	100
PSf	<i>Senna alata</i> *	27	Aut	fix	pla	P	HW	5.84	5.84	28.99	28.99
PSf	<i>Inga vera</i> *	13	Zoo	fix	pla	ES	SW	4.86	10.71	24.11	53.11
PSf	<i>Croton urucurana</i>	5	Zoo	nonf	pla	P	SW	2.89	13.6	14.35	67.45
PSf	<i>Vernonanthura phosphorica</i> *	22	Ane	nonf	reg	P	SW	1.81	15.41	9	76.45
PSf	<i>Croton floribundus</i>	8	Aut	nonf	pla	ES	HW	1.69	17.1	8.36	84.81
PSf	<i>Senna pendula</i>	11	Aut	fix	pla	P	HW	1.03	18.12	5.09	89.9
PSf	<i>Heliocarpus popayanensis</i>	9	Ane	nonf	pla	ES	SW	0.84	18.96	4.17	94.07
PSf	<i>Mimosa pigra</i>	1	Aut	fix	reg	P	HW	0.7	19.67	3.48	97.55
PSf	<i>Bauhinia forficata</i>	4	Aut	fix	pla	ES	HW	0.22	19.89	1.09	98.64
PSf	<i>Solanum granulosoleprosum</i>	5	Zoo	nonf	pla	P	SW	0.2	20.08	0.98	99.63
PSf	<i>Senna multijuga</i>	2	Aut	fix	pla	ES	SW	0.06	20.14	0.28	99.91
PSf	<i>Trema micrantha</i>	1	Zoo	Nonf	pla	P	SW	0.02	20.16	0.09	100

Treat	Species	Stem	DS	NF	RS	SG	WD	AGB (Mg ha <sup>-1</sup> )	AGB Acum (Mg ha <sup>-1</sup> )	Pr AGB (%)	Pr Acum AGB (%)
NRf	<i>Mimosa pigra</i>	5	Aut	fix	reg	P	HW	1.95	1.95	56.52	56.52
NRf	<i>Vernonanthura phosphorica</i> *	8	Ane	nonf	reg	P	SW	1.01	2.96	29.13	85.66
NRf	<i>Senegalia polyphylla</i>	1	Zoo	fix	reg	ES	SW	0.5	3.45	14.34	100
Sdf	<i>Senna alata</i> *	44	Aut	fix	pla	P	HW	10.1	10.1	67.02	67.02
Sdf	<i>Mimosa pigra</i>	3	Aut	fix	reg	P	HW	3.92	14.02	26.04	93.06
Sdf	<i>Vernonanthura phosphorica</i>	6	Ane	nonf	reg	P	SW	0.84	14.86	5.58	98.64
Sdf	<i>Senegalia polyphylla</i>	1	Zoo	fix	reg	ES	SW	0.2	15.07	1.36	100

**Treat** = treatment; **DS** = dispersal syndrome; **RS** = restoration strategy; **WD** = wood density categorie; **SG** = successional group; **NF** = nitrogen fixation; **AGB Acum** = aboveground biomass accumulated; **Pr AGB** = proportion of aboveground biomass; **Pr Acum AGB** = proportion accumulated of aboveground biomass; **PSf** = planting of native tree seedlings with fertilization and pH correction; **PS** = planting of native tree seedlings without fertilization and pH correction; **Sdf** = seeding of native trees with fertilization/correction; **SD** = seeding of native trees without fertilization and pH correction; **NRf** = natural regeneration with fertilization/correction; **NR** = natural regeneration without fertilization and pH correction; **fix** = fixer; **nonf** = non-fixer; **reg** = regeneration; **pla** = planted (seeding/seedling); **P** = pioneer; **ES** = early secondary; **LS** = late secondary; **Zoo** = zoochoric; **Ane** = anemochoric; **Aut** = autochoric; **HW** = hardwood; **SW** = softwood.

The natural regeneration contributed with 12.5% and 8.7% of the total biomass accumulated in the PSf and PS treatments, respectively. On the other hand, it represented 33% (Sdf) and 35.9% (SD) of the biomass where direct seeding was applied as the restoration method (Fig. 4). Except for PS, all the treatments had the pioneers' group as AGB dominant, while the less representative was the late secondary. The autochoric and the nitrogen fixers were the species which accumulated more biomass. However, when considering the wood density, the soft and hardwood species had similar AGB distribution, except for the plots where direct seeding was used which more than 90% of the biomass was stocked by hardwood species.



**Figure 4.** Proportional aboveground biomass distribution of functional groups per treatment. Functional groups: Restoration strategy (rege = regeneration; seed = seeding/seedling); Successional group (Pi = pioneer; ES = early secondary; LS = late secondary); Dispersal syndrome (Zoo = zoochoric; Ane = anemocoric; Aut = autocoric); Wood density (HW = hardwood; SW = softwood); Nitrogen Fixation (F = fixer; NF = non-fixer). Treatments: PSf = planting of native tree seedlings with fertilization and pH correction; PS = planting of native tree seedlings without fertilization and pH correction; SDf = seeding of native trees with fertilization/correction; SD = seeding of native trees without fertilization and pH correction; NRf = natural regeneration with fertilization/correction; NR = natural regeneration without fertilization and pH correction.

### 3.4.3 Linear mixed-effect models

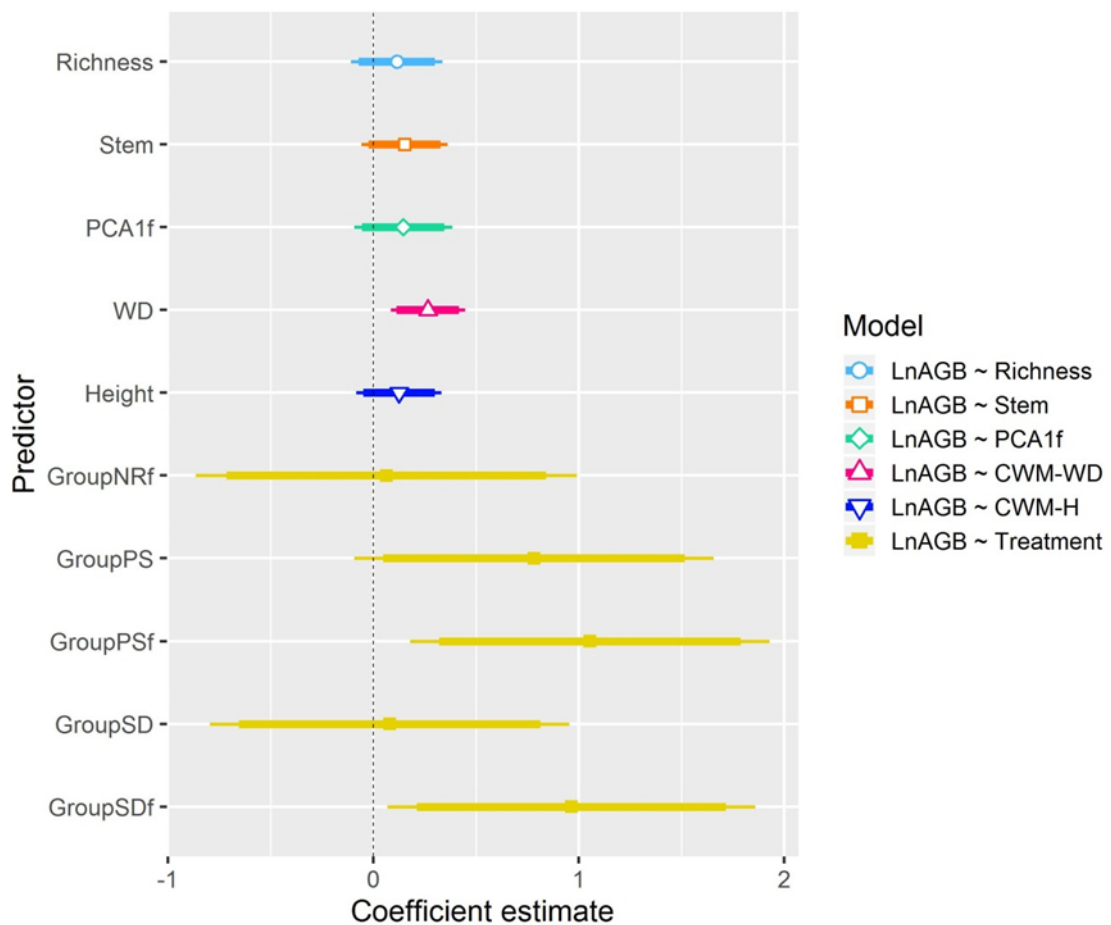
The linear mixed-effect models showed that variation in aboveground biomass was explained mainly by functional composition of wood density and based-treatments in active restoration. We found that these two predictors explained 98% of the variation in AGB (Table 2). Thus, according to the best models ( $\Delta\text{AIC} < 2$ ), CWM-WD had the strongest positive effect on AGB (Fig. 5, Est. = 2.49,  $t = 2.27$ ,  $p < 0.001$ ) with a significant correlation (Fig. 5), and explained 86% of their variation (Table 2). Moreover, there was also a significant positive relationship between treatments and AGB (Table 2), mainly planting seedling (Fig. 5, Est. = 0.89,  $t = 1.91$ ,  $p < 0.001$ ) and seeding with fertilization (Est. = 0.92,  $t = 1.86$ ,  $p < 0.001$ ). Conversely, other predictors had no effect on AGB (Fig. 5 and table 2).

**Table 2:** Candidate mixed effect models predicting the aboveground biomass (AGB) with Gaussian error distribution (linear mixed effects model - lme). Predictors are taxonomic attributes (species richness), abiotic attributes (variability of chemical properties-related soil fertility as PCA1f), functional attributes using community weight mean (CWM) of wood density (WD), structural attributes (stem count), and restoration treatments. Result of information-theoretic-based model selection is indicated (Akaike criterion corrected for small samples). AICc = Akaike information criterion for small samples;  $\Delta\text{AICc}$  = Difference between the AICc of a given model and that of the best model ( $\Delta\text{AIC} < 2$ ); and AICcWt = Akaike weights (based on AIC corrected for small sample sizes). Models with significant effects (\*).

Response variable	Model	R <sup>2</sup>	Estimate	t	p	df	LL	AICc	$\Delta\text{AIC}$	AICcWt
AGB	~CWM-WD	0.76	2.49	2.27	0.001*	4	-23.12	55.9	0.0	0.86
	~Treatment	0.67	0.21	5.12	0.01*	7	-24.26	57.23	1.33	0.12
	PSf		0.89	1.91	0.01*					
	SDf		0.92	1.86	0.01*					
	NRf		0.29	0.59	0.56					
	PS		1.0	2.13	0.23					
	SD		0.25	0.53	0.59					

	NR	0.08	0.16	0.34					
~CWM-Height	0.24	0.37	1.49	0.14	4	-27.51	64.7	8.78	0.011
~PCA1f	0.16	0.024	0.41	0.68	4	-29.02	67.7	11.79	0.002
~Richness	0.22	0.024	0.56	0.60	4	-29.46	68.6	12.68	0.002
~Stem count	0.28	0.02	1.70	0.10	4	-30.21	70.1	14.18	0.001

**PSf** = planting of native tree seedlings with fertilization and pH correction; **PS** = planting of native tree seedlings without fertilization and pH correction; **SDf** = seeding of native trees with fertilization/correction; **SD** = seeding of native trees without fertilization and pH correction; **NRf** = natural regeneration with fertilization/correction; **NR** = natural regeneration without fertilization and pH correction



**Figure 5.** Standardized regression coefficients of different linear mixed-effects

models (LMMs. with random and fixed effects) to test the main effects of predictors on aboveground biomass (AGB). The following predictors are included according to the main models: taxonomic attributes, such as species richness, abiotic attributes using variability of chemical properties related to technosol fertility as PCA1, functional attributes using community weight mean (CWM) of wood density (WD) and height, structural attributes (stem count) and treatments. The associated 95% confidence intervals and the relative importance of each factor expressed as the percentage of explained variance are indicated. Treatments: 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/correction (SDf) and without fertilization and pH correction (SD); natural regeneration with fertilization/correction (NRf) and without fertilization and pH correction (NR).

### **3.5 DISCUSSION**

These results demonstrate the relationships among forest attributes, such as taxonomic (species richness), abiotic (technosol fertility), structural (stem count) and functional attributes (CWM of WD and height values) with aboveground biomass undergoing different restoration methods in the Atlantic forests. The functional composition of WD and active restoration methods had a key role to predict aboveground biomass. More specifically, aboveground biomass increased directly with CWM-WD and, SDf and PSf methods based in the best models tested. Therefore, the high aboveground biomass in restored forests could be better explained by the MRH (based on wood density) than by the species richness and abundance. Our findings are essential to understand the functional diversity of the Atlantic forest undergoing active restorations that play important roles in ecosystem functioning recovery.

### 3.5.1 Aboveground biomass distribution between species and functional groups

The variation of AGB is influenced by several factors including abiotic factors, such as soil properties (Hanif et al. 2019; Villa et al. 2020a). Soil properties, mainly nutrients availability, are well known for their influence and fast response in plant growth rate and biomass accumulation (Russo et al. 2005; Quesada et al. 2012; Eisenhauer et al. 2018). However, our result showed that the fertilization and pH correction on the mining tailings have not influenced the biomass accumulation significantly, but all treatments that were fertilized had higher AGB means. In tropical forests, soil nutrients may also contribute to AGB acquisition indirectly through plant functional traits and taxonomic diversity (Díaz et al. 2007; Ali and Yan 2018; Bu et al. 2018). Different plant functional traits improve acquisition and efficient use of soil nutrients and available resources due to the plant complementary use of those resources (lower niche overlap among species) which have a positive effect on the carbon input (Díaz et al. 2011; Zhang et al. 2012; Bu et al. 2018; Zuo et al. 2018).

Dominant species are the disproportionate contribution of a small number of species accounting for at least half of the stem abundance or biomass accumulation (Bastin et al. 2015; Fauset et al. 2015; Rodrigues et al. 2019). Consequently, these few dominant species are the most representative of some essential ecosystem services even in diverse tropical forests (Lohbeck et al. 2016; Staggemeier et al. 2017). For example, a study conducted on a forest fragment, in Minas Gerais, less than 4% of the species were classified as biomass hyperdominant and less than 8% as stems hyperdominants (Rodrigues et al. 2019). On the other hand, in the Amazonian basin, only 1.4% and 0.91% of the tree species were accounted as stems and biomass hyperdominants, respectively (ter Steege et al. 2013; Fauset et al. 2015). We found two biomass dominant and three stems dominant species where planting of nursery-grown seedlings were applied (PS and PSf). While, for the other treatments (SD, SDf, NR, NRf), only one biomass and stem dominant specie was found (Tab. 1).

Considering all the treatments separately, four species were stem hyperdominant (*Inga vera*, *Senna alata*, *Croton floribundus* and *Vernonanthura phosphorica*) and four were biomass hyperdominant (*Inga vera*, *Senna alata*, *Croton floribundus* and *Mimosa pigra*). Such difference is due to the fact that *Vernonanthura*



*phosphorica* has a softwood and smaller diameter and total height compared with the other dominant species. Together, these traits explain why *Vernonanthura phosphorica*, despite having so many stems, contributed relatively little to the biomass stock. In contrast, *Mimosa pigra*, despite having fewer stems, has a hardwood and usually many branches from a single tree which contributed to the biomass accumulation. Furthermore, our results are consistent with the ones obtained by Rodrigues et al. (2019) and Fauset et al. (2015) where approximately one third of the biomass hyperdominant species were not framed as stem hyperdominant. All the regenerating trees were pioneers, except for *Senegalia polyphylla* (early secondary); moreover, the dominance of these successional groups was expected due to the early stage of the restoration interventions (28 months). In terms of biomass, the only treatment that the pioneers' group was not the dominant was PS, mainly because of the species *Inga vera* and *Croton floribundus*, both early secondary and biomass hyperdominant. The late secondary group was the least representative with only one species (*Enterolobium contortisiliquum*) found in a SD plot. Thus, the more favorable conditions created by pioneer species in degraded sites shows to be crucial because it allows the establishment of trees that previously would not have been able to colonize that habitat (Elgar et al. 2014; Wheeler et al. 2016).

Planted and seeded trees accumulated much more biomass than naturally regenerated species. However, at early restoration stages are expected higher AGB for active restoration compared with passive restoration (e.g. Wheeler et al. 2016). Since invasive grasses often hinder natural regeneration by acting as barriers to native species recruitment (Gioria et al. 2012; Gioria and Pyšek 2015), we believe that the main filter for the regeneration in the study area was the strong competition with invasive grasses, such as *Urochloa* sp and *Cynodon dactylon*. Additionally, Pilocelli (2020) found fast natural regeneration and high diversity also studying an area affected by the mining tailings in the municipality of Marina, but where invasive species was not a biotic filter. Therefore, the control of invasive grasses is fundamental for the restoration (Holl 2012), notably in the areas affected by the Fundão dam collapse.

Overall, soft and hardwood species had similar AGB distribution, except for the treatments where direct seeding was used. More than 90% of the biomass was

stocked by hardwood species in these treatments because of *Senna alata* and *Mimosa pigra*, biomass dominant species. In this sense, AGB is related to the growth rate, the capability to use available resources and how much carbon is stored; consequently, hardwood species are able to store more carbon at the same space available (Violle et al. 2007; Fauset et al. 2015; Poorter et al. 2015). Moreover, functional attributes are directly linked with the carbon stock in the AGB (Rawat et al. 2019). Although the autochoric syndrome was a biomass dominant group for all the treatments (mainly driven by *Senna alata* and *Mimosa pigra*), the anemochoric syndrome was an important dispersal mechanism when considering the number of stems of naturally regenerated species. Once the vegetation cover and the seed bank are lost, naturally the first propagules to arrive and colonize the impacted area are dispersed by the wind (Holl 2012). The nitrogen-fixing trees accumulated between 55.3 % (PS) and 94.4 (SDf) of the AGB, which are essential for the restoration process of severely degraded lands due to their recognized role for the improvement of the soil conditions (Wang et al. 2010; Chaer et al. 2011).

### 3.5.2 *Functional traits composition shape aboveground biomass*

These results showed that beyond the species richness and abundance, the functional composition through wood density is the main predictor that explains the increase in biomass. Recently, a study on second-growth Amazon forests re-growing after shifting cultivation, showed that functional composition as the main predictor determines aboveground biomass testing the mass ratio hypotheses (MRH), and conclude that functional dominance overrides the effects of abiotic factors and taxonomic attributes (Villa et al. 2020b). Thus, fast-growing trees, especially during active restoration, can reach rapid aboveground biomass accumulation; but the most diverse plantations, including those with large trees and high wood densities, are likely to also have the ability to recover and maintain AGB storage over the long time (Conti and Diaz 2013; Hulvey et al. 2013). Different ecosystem services are provided when a higher number of tree species are employed (Lamb 2018). Nevertheless, relatively moderate tree diversity is also able to have positive effects on ecosystem service; although different species may be more effective than others depending on their traits and on the ecosystem functioning (Lamb 2018).

Studies have shown that tree abundance and biomass, increase in a predictable way during early succession (Lasky et al. 2014; Derroire et al. 2016), but the trajectories can vary, reflecting that different mechanisms might interfere in this dynamic (Estrada-Villegas et al. 2019). After disturbances, early pioneer species colonize the site leading to a fast biomass accumulation before stabilizing and slightly declining with stand age (He et al. 2012; Lasky et al. 2014; Quinn and Thomas 2015; Sheil and Bongers 2020). However, where has occurred intense degradation and loss of plant diversity due to anthropogenic disturbances, such as in the study area, active restoration techniques often show better outcomes, faster plant coverage and higher biomass recovery when the species and methods are chosen carefully (Crouzeilles et al. 2017). Also, a meta-analysis carried out by Martin et al. (2013) estimated that AGB recovers more quickly than tree diversity would reach old-growth forest levels in tropical secondary forests, suggesting that key species selection enhancement follows forest structure and biomass recovery paths.

We compared natural forest regeneration, direct seeding and planting of seedlings under different site treatments and, as predicted, overall, active restoration manifested higher tree species richness and abundance leading to higher biomass recovery, although biomass was not significantly different between treatments. Furthermore, previous studies have found that passive and active restoration actions did not differ significantly in the levels of biodiversity or ecosystem services in agroecosystems (Barral et al. 2015). Furthermore, in a meta-analyse carried by Meli et al. (2017) was concluded that planting trees in agricultural land does not result in consistently faster or more complete recovery than passively restored sites. Nevertheless, Crouzeilles et al. (2017) have found that ecological restoration success is higher for natural regeneration than for active restoration in tropical forests. However, we have to consider that mined sites are almost on all occasions restored adopting an association of planting, soil amendments and recontouring topography because of the highly degraded conditions (Meli et al. 2017). Moreover, we have to consider the uniqueness of the study area due to its long history of land use based on pasture for livestock and the depositions of tons of mining tailings after the Fundão Dam Collapse. Therefore, it is still unclear the predictions to future outcomes on forest recovery; for instance, some forests recover similar biomass values within few decades while others take more than 80 years (Martin et al. 2013; Poorter et al.

2016). On the other hand, a recent study shows that during second-growth forest, it was necessary about 20 years for degraded forests to return to 80% of the species richness of the old-growth forest; however, only about 30% will return in terms of species composition (Rozendaal et al. 2019). Taking this into consideration, what we can state about this study is that active restoration is showing better responses so far and more monitoring studies are needed to evaluate the success of ecological restoration along time.

### *3.5.3 Implications for forest management and restoration*

Active restoration is frequently used where natural regeneration faces limitations, sites with long disturbance background take longer to naturally regenerate or even not recover due to aggressive grasses dominance, long distance from sources of propagules, soil limitations or grazing (Bertacchi et al. 2016; Gilman et al. 2016). In order to avoid ecosystem collapse the restoration projects should aim plant diversity, ecosystem function and structural complexity (Lindenmayer et al. 2016). Thus, with active restoration approaches it's possible to manage the number of species, individuals and the functional groups used for a specific goal. Direct seeding and seedling planting are the most common active methods employed, despite being more expensive compared to passive restoration (Grossnickle and Ivetić 2017; Martins 2018). These methods are showing better responses for the study area, where the invasive grasses are a problem and the original soil structure was totally modified because of the mine waste deposited during the Fundão Dam Collapse. As a consequence, an initial control of the invasive grasses is essential where the projects aim the restoration via natural regeneration.

However, few studies have addressed the synergistic effect of multiple drivers that threaten the relationship between biodiversity and ecosystem functioning in Atlantic forests undergoing restoration. A broader perspective on biodiversity and ecosystem functioning relationships is crucial for sustainable ecosystem management in light of species loss and intensified anthropogenic disturbance under future climate change conditions (Schuldt et al. 2018). Thus, we propose that exploring these relationships between ecological restoration methods can provide

fundamental insights into recovery and sustainable management of ecosystem services in the study area.

### 3.6 CONCLUSION

This study contributes to the further understanding of the biodiversity-ecosystem functioning relationships during Atlantic forest restoration. We found that high CWM of wood density and active restoration methods determine higher levels of aboveground biomass on mining tailings where natural regeneration is limited. These results support the mass ratio hypotheses, and explain how functional composition as the main predictor determines aboveground biomass during tropical forest restoration. We conclude that functional dominance overrides the effects of species richness and abundance on aboveground biomass, and hence, it is important to test the mutual effects of functional composition when testing the effects of restoration methods on forest functioning. As expected, at this early stage of the restoration process, the more representative successional group was the pioneer species and the anemochoric dispersal syndrome mainly represented by naturally regenerated stems. Lastly, the autochoric dispersal and the nitrogen-fixing trees showed to be biomass dominant groups, driven mostly by *Senna alata*, because of its high abundance and wood density (hardwood).

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## FINAL CONSIDERATIONS

Based on the results obtained in this study, it is possible to state that the tailings deposition does not impede the restoration of the affected environments. In general terms, the study area showed good vegetation cover by tree and herbaceous species, covering practically all the technosol.

Analyzing the data jointly (AGB, richness, abundance, functional groups), active restoration resulted in better responses in the study area. The main filter for natural regeneration was the presence of invasive grasses, especially those of the genus *Urochloa* and *Cynodon dactylon*. Because in other studies, where invasive grasses were controlled or were not effectively present, performed in areas that had the same impact, natural regeneration showed high diversity and rapid colonization. In practical terms, the control of invasive species is essential for the success of the restoration projects in areas affected by the tailings from the Fundão dam

The dynamics of the tailings was similar to a common soil in relation to the resistance to penetration: during the rainy period it presented less compaction and in periods of drought occurred an increase of this value. Therefore, the most propitious time for the vegetation management, planting or sowing, would also be during the rainy season, in order to avoid possible impediments to root growth and water restrictions.

The results of the chemical analysis showed that the tailings have a low natural fertility, however, the soils of the region, due to the high degree of weathering, have equivalent fertility. The only element analyzed that obtained responses above those considered normal was Fe, which was already expected due to the origin of the tailings.

Finally, these results allow more precise decisions to be made regarding the recovery of environments impacted by the Fundão dam. However, it is worth mentioning that the monitoring of the study area is of fundamental importance to elucidate the direction that the plant community will follow and the self-sustainability of these ecosystems.

## APPENDICES

### APPENDIX A – ARTICLE 1

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

Family	Species
Anacardiaceae	<i>Schinus terebinthifolius</i>
Euphorbiaceae	<i>Croton floribundus</i>
Euphorbiaceae	<i>Croton urucurana</i>
Fabaceae	<i>Senna alata</i>
Fabaceae	<i>Inga vera</i>
Fabaceae	<i>Senna pendula</i>
Fabaceae	<i>Senna multijuga</i>
Fabaceae	<i>Bauhinia forficata</i>
Malvaceae	<i>Guazuma ulmifolia</i>
Malvaceae	<i>Heliocarpus popayanensis</i>
Solanaceae	<i>Solanum granulosoleprosum</i>

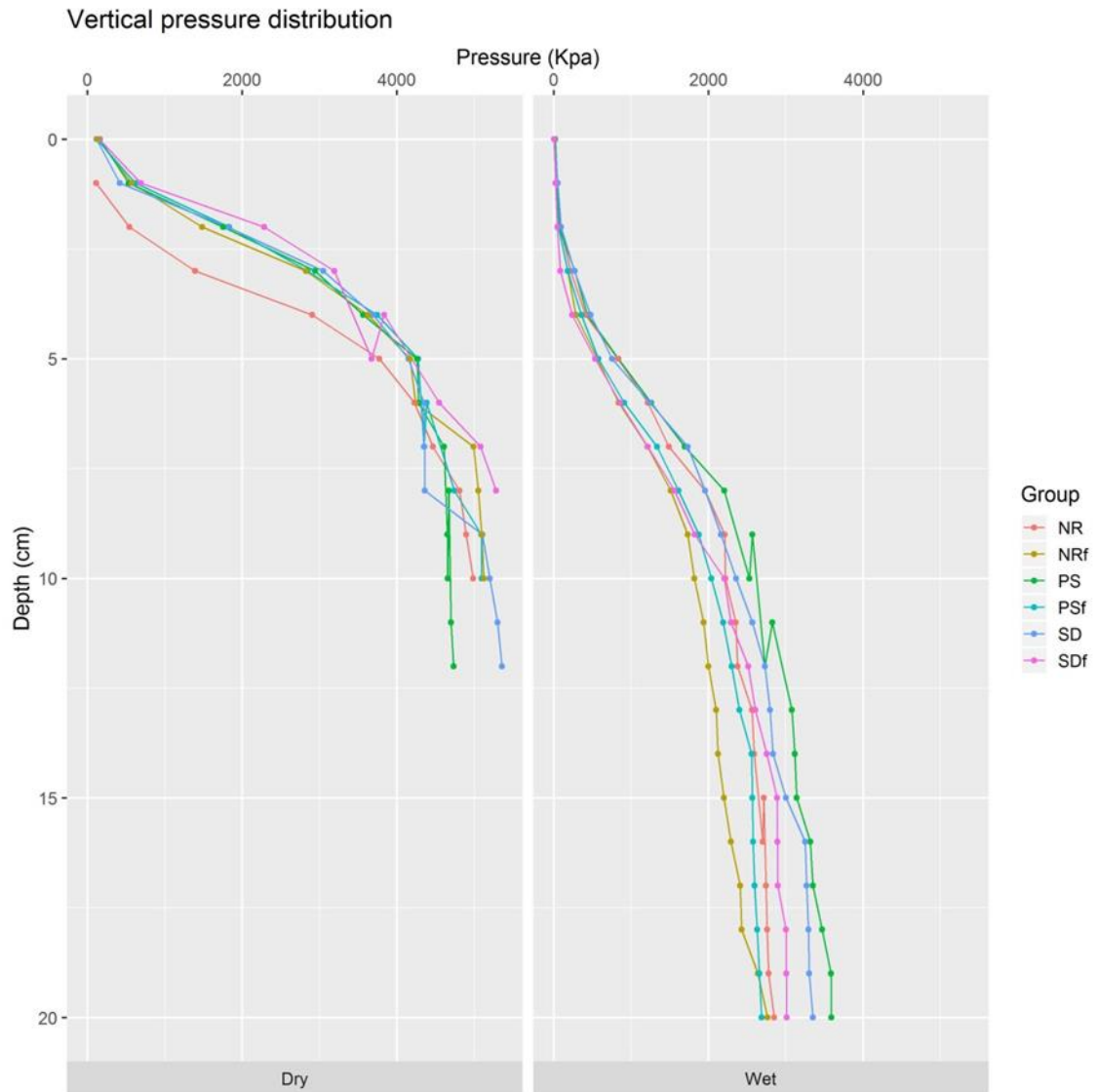
**Table SII.** Native tree species seeded, seed quantity (g ha<sup>-1</sup>)

Family	Species	g ha <sup>-1</sup>
Cannabaceae	<i>Trema micrantha</i>	4
Euphorbiaceae	<i>Croton floribundus</i>	20
Euphorbiaceae	<i>Croton urucurana</i>	6
Fabaceae	<i>Enterolobium contortisiliquum</i>	78
Fabaceae	<i>Guazuma ulmifolia</i>	4
Fabaceae	<i>Senna alata</i>	10
Fabaceae	<i>Senegalia polyphylla</i>	30
Fabaceae	<i>Senna macranthera</i>	16
Fabaceae	<i>Senna multijuga</i>	8
Malvaceae	<i>Heliocarpus popayanensis</i>	4
Solanaceae	<i>Solanum granulosoleprosum</i>	6

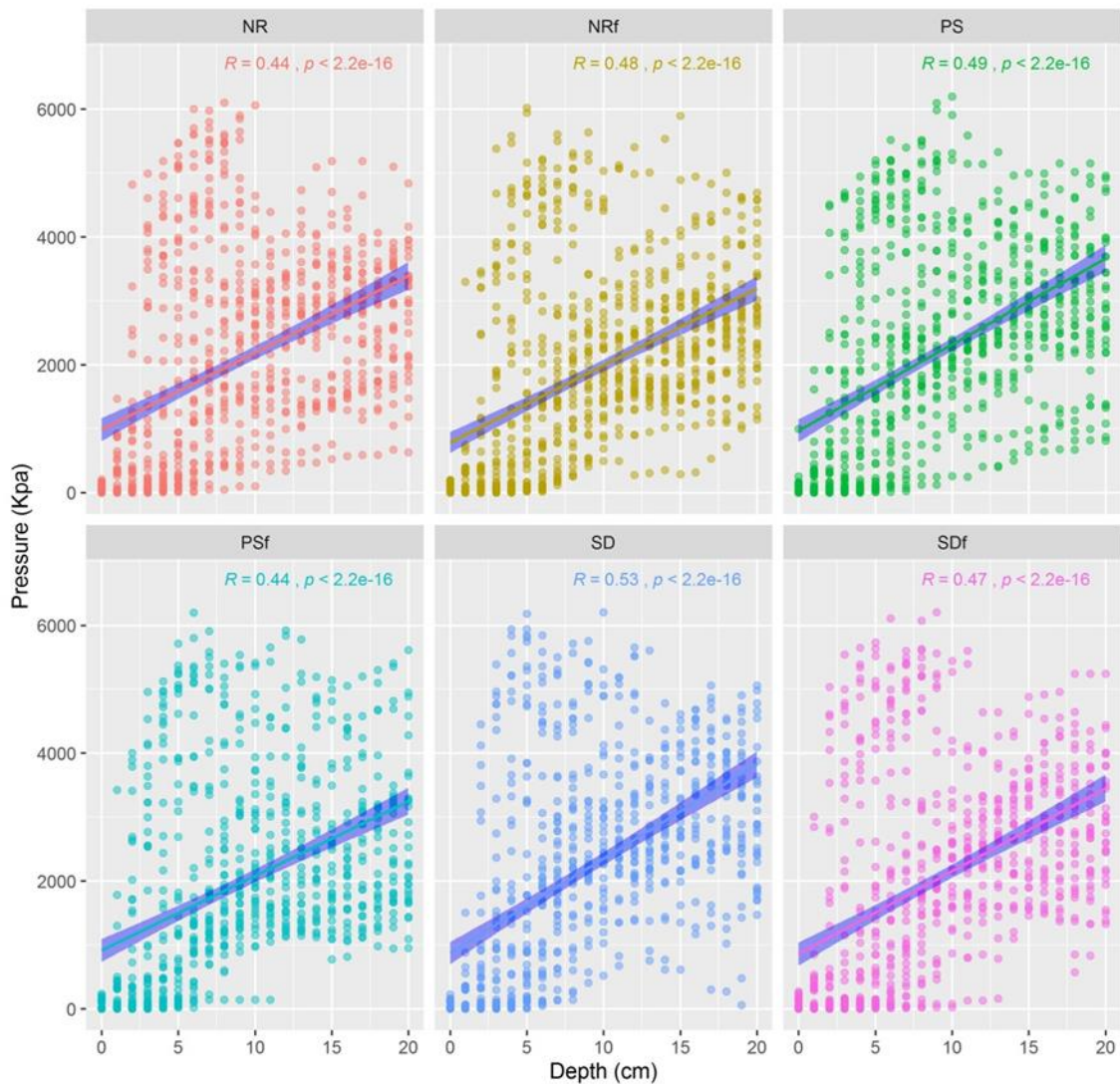




**Figure S1.** General view of the study area after the Fundão tailings dam collapse (a) and actual view with vegetation cover (b). Gualaxo river, district of Paracatu de Baixo (7754350 N, 686800 E), municipality of Mariana, Minas Gerais, Brazil. Photo: RENOVA, November 2015 (a) and December 2019 (b).



**Figure S2.** Vertical substrate penetration resistance distribution. Treatments: Seedlings with fertilization/pH correction (PSf); Seedlings without fertilization/pH correction (PS); Natural Regeneration with fertilization/pH correction (NRf); Natural Regeneration without fertilization/pH correction (NR); Seeding with fertilization/pH correction (SDf); Seeding without fertilization/ pH correction (SD).



**Figure S3.** Penetration resistance and the main predictors' relationship according with LMM approach. The effect of depth on pressure between treatments. Color fill circles indicate data per treatments. Solid lines represent the fitted values (prediction) of the models, and the shaded area the 95 % confidence interval of the predicted values of each model. Treatments: Seedlings with fertilization/pH correction (PSf); Seedlings without fertilization/pH correction (PS); Natural Regeneration with fertilization/pH correction (NRf); Natural Regeneration without fertilization/pH correction (NR); Seeding with fertilization/pH correction (SDf); Seeding without fertilization/ pH correction (SD).

## APPENDIX B – ARTICLE 2

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

<b>Family</b>	<b>Species</b>
Anacardiaceae	<i>Schinus terebinthifolius</i>
Euphorbiaceae	<i>Croton floribundus</i>
Euphorbiaceae	<i>Croton urucurana</i>
Fabaceae	<i>Senna alata</i>
Fabaceae	<i>Inga vera</i>
Fabaceae	<i>Senna pendula</i>
Fabaceae	<i>Senna multijuga</i>
Fabaceae	<i>Bauhinia forficata</i>
Malvaceae	<i>Guazuma ulmifolia</i>
Malvaceae	<i>Heliocarpus popayanensis</i>
Solanaceae	<i>Solanum granuloseprosum</i>

**Table SII.** Native tree species seeded, seed quantity (g ha<sup>-1</sup>)

<b>Family</b>	<b>Species</b>	<b>g ha<sup>-1</sup></b>
Cannabaceae	<i>Trema micrantha</i>	4
Euphorbiaceae	<i>Croton floribundus</i>	20
Euphorbiaceae	<i>Croton urucurana</i>	6
Fabaceae	<i>Enterolobium contortisiliquum</i>	78
Fabaceae	<i>Guazuma ulmifolia</i>	4
Fabaceae	<i>Senna alata</i>	10
Fabaceae	<i>Senegalia polyphylla</i>	30
Fabaceae	<i>Senna macranthera</i>	16
Fabaceae	<i>Senna multijuga</i>	8
Malvaceae	<i>Heliocarpus popayanensis</i>	4
Solanaceae	<i>Solanum granuloseprosum</i>	6

**Table SIII.** Average from three replicates of chemical characteristics of the soil in areas contaminated with mining tailings

Parameter	Average
pH (H <sub>2</sub> O)	7.0
P (mg dm <sup>-3</sup> )	8.3
K (mg dm <sup>-3</sup> )	12.6
Ca <sup>2+</sup> (cmolc dm <sup>-3</sup> )	0.7
Mg <sup>2+</sup> (cmolc dm <sup>-3</sup> )	0.0
Al <sup>3+</sup> (cmolc dm <sup>-3</sup> )	0.0
H + Al (cmolc dm <sup>-3</sup> )	0.1
SB (cmolc dm <sup>-3</sup> )	0.2
CEC(t) (cmolc dm <sup>-3</sup> )	0.2
CEC(T) (cmolc dm <sup>-3</sup> )	0.3
V %	70
OM (dag kg <sup>-1</sup> )	0.5

SB = Sum of exchangeable bases; CEC (t) = Effective cation exchange capacity; CEC (T) = Cation exchange capacity at pH 7.0; V = base saturation index; OM = organic matter. Fonte: Silva-Junior et al. (2018)

Silva-Junior CA, Coutinho AD, Oliveira-Júnior JF, Teodoro PE, Lima M, Shakir M, Gois G, Johann JA (2018) Analysis of the impact on vegetation caused by abrupt deforestation via orbital sensor in the environmental disaster of Mariana, Brazil. Land Use Policy 76: 10–20. <https://doi.org/10.1016/j.landusepol.2018.04.019>

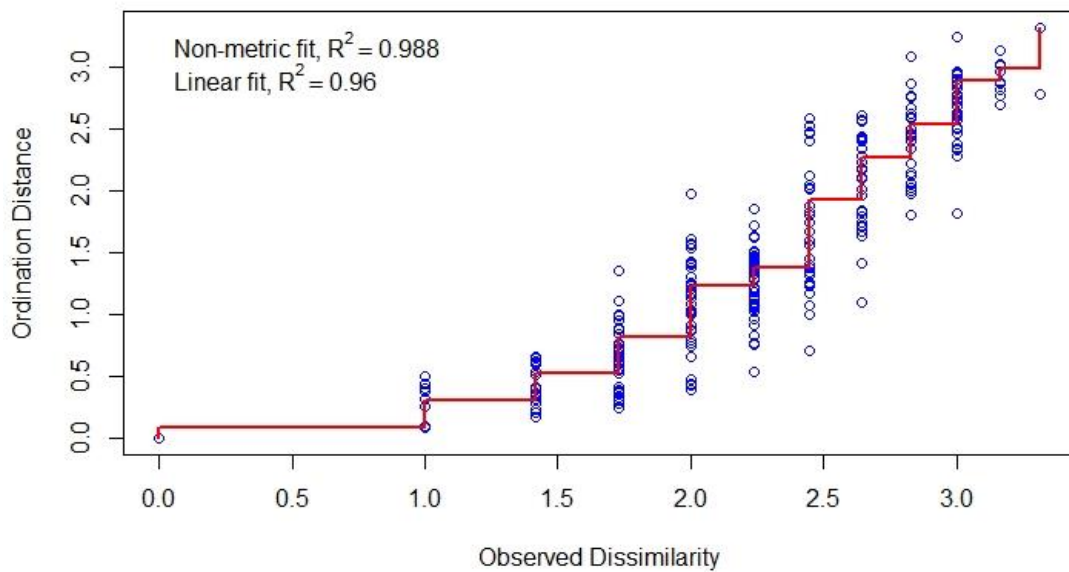
## APPENDIX C – ARTICLE 3

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

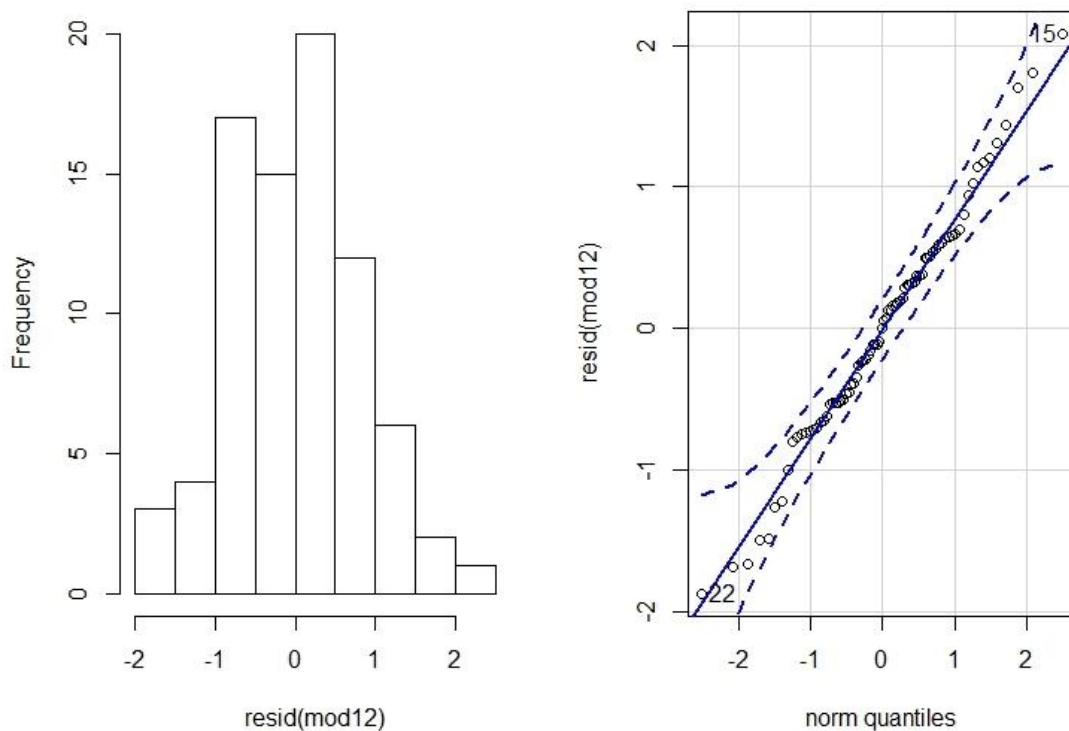
Family	Species
Anacardiaceae	<i>Schinus terebinthifolius</i>
Euphorbiaceae	<i>Croton floribundus</i>
Euphorbiaceae	<i>Croton urucurana</i>
Fabaceae	<i>Senna alata</i>
Fabaceae	<i>Inga vera</i>
Fabaceae	<i>Senna pendula</i>
Fabaceae	<i>Senna multijuga</i>
Fabaceae	<i>Bauhinia forficata</i>
Malvaceae	<i>Guazuma ulmifolia</i>
Malvaceae	<i>Heliocarpus popayanensis</i>
Solanaceae	<i>Solanum granuloseprosum</i>

**Table SII.** Native tree species seeded, seed quantity (g ha<sup>-1</sup>)

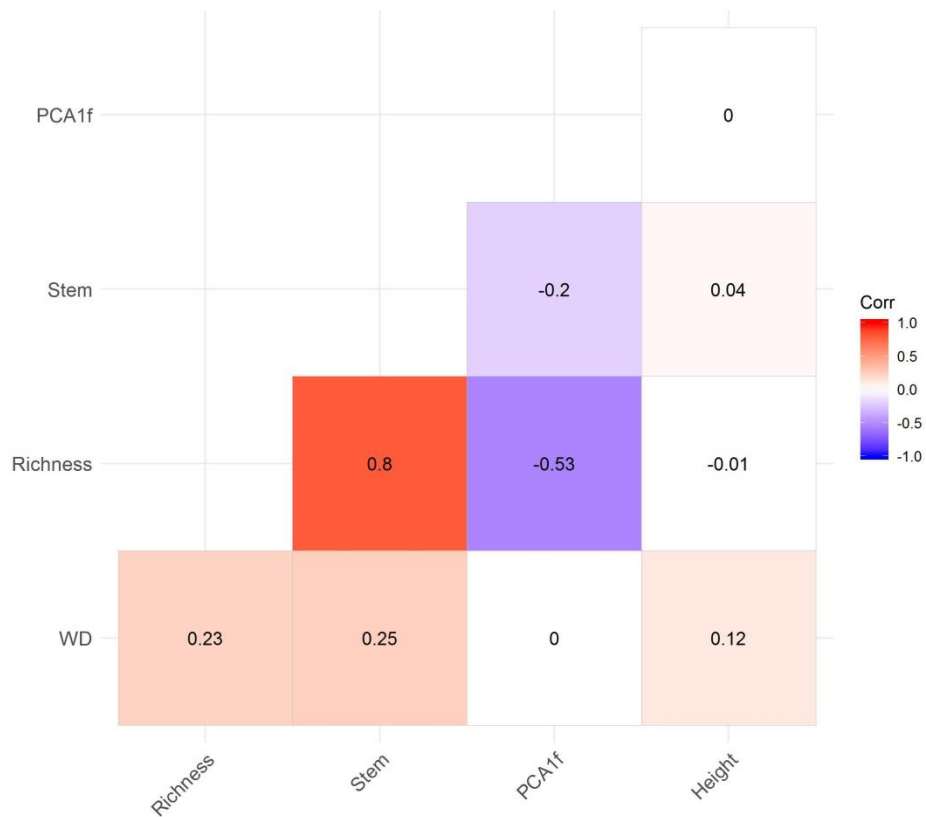
Family	Species	g ha <sup>-1</sup>
Cannabaceae	<i>Trema micrantha</i>	4
Euphorbiaceae	<i>Croton floribundus</i>	20
Euphorbiaceae	<i>Croton urucurana</i>	6
Fabaceae	<i>Enterolobium contortisiliquum</i>	78
Fabaceae	<i>Guazuma ulmifolia</i>	4
Fabaceae	<i>Senna alata</i>	10
Fabaceae	<i>Senegalia polyphylla</i>	30
Fabaceae	<i>Senna macranthera</i>	16
Fabaceae	<i>Senna multijuga</i>	8
Malvaceae	<i>Heliocarpus popayanensis</i>	4
Solanaceae	<i>Solanum granuloseprosum</i>	6



**Figure S1.** Shepard plot showing the nonmetric fit based on stress using linear regression between Euclidean distance in reduced space and the Jaccard distance (original dissimilarities).

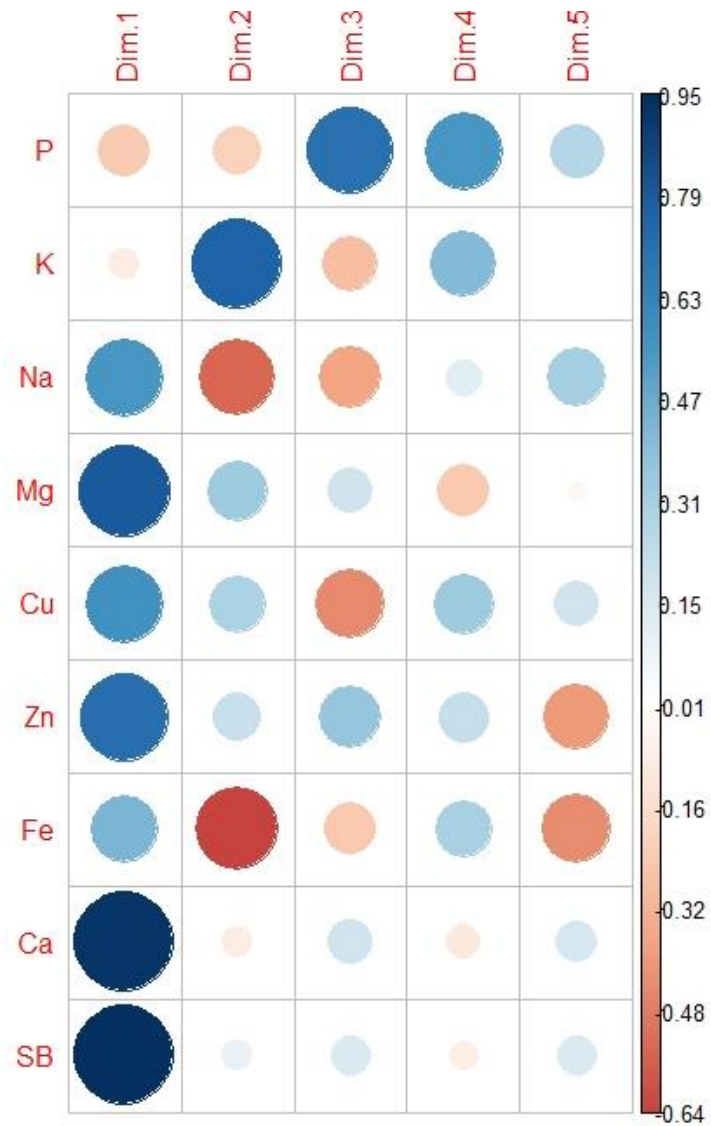


**Figure S2.** Example to test the most suitable distribution and link function using histogram and Q-Q considering the best models with  $AIC < 2.0$ .



**Figure S3.** Spearman correlation among all individual variables measured. For analysis biotic attributes (species richness), abiotic attributes (chemical properties-related technosol fertility (PCA1f), and functional attributes using community weight mean (CWM) of wood density (WD) and height, structural attributes (stem count) were included.





**Figure S4.** Significance levels are based on Spearman correlation coefficients between chemical properties-related substrate fertility and principal components of PCA from plots of different treatments.