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ARTICLE

Study on Site Preparation and Restoration Techniques for Forest Restoration in Mining Tailings of Mariana, Brazil

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ABSTRACT

Ecological restoration in forest ecosystem is a priority in Mariana, Brazil. Thus, we evaluated the effects of passive and active restoration methods through different site preparation techniques by manipulating physical-chemical properties of substrates on tree community coverage in Mariana, Brazil. A total of 48 plots (12×12 m each) were established in two areas along the flood plains with accumulation of tailings. The following treatments were established: (1) planting of native tree seedlings with fertilization (PSf) and (2) without fertilization (PS); (3) direct seeding of native trees with fertilization (SDf) and (4) without fertilization (SD); (5) natural regeneration with fertilization (NRf) and (6) without fertilization (NR). Differences in substrate properties and tree community coverage were evaluated between treatments, the substrate properties and tree community coverage relationship, and main effects of substrate fertility and texture on tree community coverage. There were marked differences in substrate and plant coverage between treatments. On average, the highest plant coverage was found in treatment with fertilization, such as NRf (59,5%) and SDf (48%). However, the treatment with seedling planting (PSf and PS) and NR did not show differences (~37%), while the lowest values were observed in SD (23%). There is a strong relationship between substrate fertility and plant community coverage, with significant positive effects. We observed that the passive and active restoration methods can be complementary in the soil and plant community coverage recovery.

1. Introduction

The ecological restoration of degraded forest affected by the Fundão tailings dam in Marina, continues to have a high priority five years after ^[1]. The Fundão tailings dam collapse was one of the largest environmental disasters that deposit over 45 million m³ ore tailing into the environment, directly affected 863.7 ha of Permanent Preservation Areas (APP) associated to watercourses due to the flooding with the ore tailings, and ca 400 ha Atlantic forest were lost with the iron tailing ^[2,3]. Furthermore, some previous studies argue that these tailings layers deposited on soil can affect negatively the forest recovery ^[2,3]. Thus, differ-

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ent methods and site preparation techniques are critical to forest restoration. However, there is still an urgent need to know the effects of restoration methods and site preparation techniques on ecological indicators related ecosystem stabilization, such as plant community coverage.

The active related restoration techniques are based on management, i.e. seeding, planting seedlings, seed banks transposition from species-rich reference forest to degraded forests ^[4,5]. The natural second-growth forest that are regrowing after a disturbance (i.e. mining, agriculture) when protected from further disturbances it is considered passive restoration ^[4,6,7]. Both methods are complementary to a comprehensive restoration purpose ^[4,5,8]. However, it is still necessary to continue evaluating the response of different ecological indicators (i.e. soil fertility, plant coverage) to specific site preparation techniques along forest restoration.

Different site preparation techniques should be the way to improve the active and passive restoration strategies that are being applied and monitored ^[7,9]. Thus, restoration ecologists know that every environmental context requires specific site preparation techniques (i.e. fertilization) and restoration methods (i.e. seeding, seedling planting), and that site effects can be important driver of initial restoration trajectories ^[9-12]. The choice between different restoration methods and site preparation techniques depends on a compromise between goals and costs ^[7,13]. Restoration goal will depend on specific site preparation techniques and selecting key species ^[5,7,14]. However, plant species has different adaptation mechanisms to soil conditions (i.e. soil fertility and texture, organic matter, water retention content), thus initial conditions by site preparation techniques can be important [11,12].

Evaluating experimentally the responses of individual species to substrates, as well as the density-dependent relationships of species, which would require more experimental care, would require a lot of time, and would not allow generalization on a larger spatial scale ^[12,15]. For this reason, it is necessary to selected ecological restoration indicators with immediately direct effects on ecosystem stabilization and rapid assessment and monitoring; such as plant community coverage ^[16,17,18]. There are previous researches on the effects of site preparation techniques on seedling growth and survival, ^[14,19,20]; however, few have also compared the effects of restoration methods through different site preparation techniques on plant community coverage.

Plant coverage in the proportional space occupied by each species within the community ^[21]. This ecological indicator predicts that species with a high coverage are more efficiently use resources ^[16,17,21]. Different studies have shown that plant coverage has been a proxy of ecological processes, and also a potential ecological indicator

of vegetation restoration ^[11,12,22]. Therefore, it has been observed that there is a positive relationship between plant coverage and plant species diversity ^[11,16,17] and ecosystem functioning, such as an increase in aboveground biomass ^[11,16,17,23]. An increase in plant coverage can generate favorable microclimatic conditions for the plant establishment and growth ^[24,25], as well as to maintain relative humidity and mineralization processes ^[18,26,27]. Furthermore, soil properties can strongly affect plant community coverage, and plants may also have strong feedback effects on soil properties ^[11,18,26].

In this context, we aimed to evaluate the effects of passive and active restoration methods through different site preparation techniques, mainly with the manipulation of substrates physical-chemical properties on plant community coverage in Mariana, Brazil. Therefore, we proposed the following specific objectives: (1) explain how different restoration methods and different site preparation techniques determine changes in plant community coverage; (2) analyze the substrate fertility and plan community coverage relationship. This study allows us to investigate the anticipation that site preparation techniques through the increase substrate fertility manipulation leading to increased plant community coverage at fine scale as an alternative for rapid ecosystem stabilization.

2. Material and Methods

2.1 Experimental Sites

Two close Atlantic forests areas were chosen on tailings dam in the district of Paracatu de Baixo (7754350 N, 686800 E), municipality of Mariana, Minas Gerais, Brazil (Fig 1). These areas correspond to Atlantic Forest which is a hotspot of plant diversity ^[27], and has a high carbon stock potential in the aboveground biomass ^[28]. However, Atlantic Forest one of the most threatened at global scale ^[29], where only around 10% remains of the original forests ^[30]. This region has a humid tropical climate (620 and 820 m elevation), with precipitation is 1340 mm year, relative humidity is 80%, and air temperature is 19°C ^[31].

The Fundão tailings dam occurred on 05 November 2015, where ca. 80% of the total volume (40 million m³) were release generating unsaturated sandy tailing along Gualaxo do Norte river (ca. 200 m) and forest ecosystems ^[32]. Mining tailings deposition affected different plant communities reducing their coverage, also generating tailings layers on the soil with contaminated particles that could limit the plant growth ^[33,34]. The tailings accumulated in our study area present different depths (ca 50-100 cm) on a flat and homogeneous topography along river, mainly affecting the Atlantic Forest.

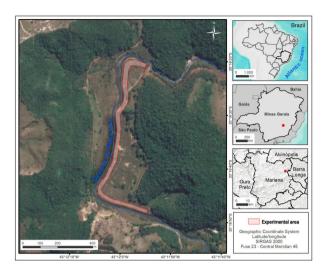


Figure 1. Study area along Gualaxo do Norte river, Mariana municipality, MG, Brazil

2.2 Design of Experiments

We established a randomized block design with six restoration treatments with eight replicates plots for each treatment on the tailing. Restoration treatments were as follows: planting of native tree seedlings with fertilization (PSf) and without fertilization (PS); seeding of native trees with fertilization (SDf) and without fertilization (SD); natural regeneration with fertilization (NRf) and without fertilization (NR), as a control treatment with no site preparation (Figure S1A - S1B). A total of 48 plots ($12 \times$ 12 m each) with a separation of five meters between plots were established in two areas along the tailings deposition (Figure S1A - S1B), in March of 2017, approximately 16 months after of the Fundão tailings collapse.

2.3 Site Preparation Techniques and Plant Material

Calcined dolomitic limestone (100 kgha⁻¹), agricultural gypsum (350 kgha⁻¹), and ammonium sulfate (100 kgha⁻¹) was applied in plots with fertilization and pH correction treatments. 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 (Table S1-S3. Appendix Material, SM). Thus, in each plot where correction and fertilization were predicted, Super Simples Phosphate (150 kg ha⁻¹) was used. Subsoiling was carried out with a depth of 60 centimeters of the planting lines of the cover species (see species list in table S1).

Six hundred sixty seedlings of each potential species were acquired. The seedlings were stored in a temporary

wood structure of approximately 40 m², covered with 50% shade. Initially, the seedlings were irrigated daily for approximately two weeks before planting. Two months after the experiment was implemented, an initial evaluation was made in May 2017 of the vegetation cover and soil physical and chemical parameters. The seedlings may undergo stress soon after planting if root growth is not sufficient to couple the seedlings to the water available in the soil. This stress can be minimized by preparing favorable planting sites with proper preparation. The roots of planted seedlings will have access to nutrient rich layers, which promotes seedling establishment and root growth ^[35].

2.4 Substrate Properties Sampled

The tailing top-substrate properties within each plot were sampled (at 0-10 cm depth), and after were measured following standard protocols ^[31]. Thus, we assessed available P, K, Ca, Mg, pH (H₂O), remaining phosphorus (Prem); exchangeable acidity (H + Al), effective cation exchange (t); potential effective cation exchange (T); organic matter (OM); bases saturation (%V). Furthermore, we assessed the soil texture, such as coarse sand (Sandc), fine sand (Sandt), and clay and silt contents (Figure S.1, from ESM). We present the average from three replicates of chemical properties of mining tailings after the Fundão tailings dam failure in Mariana (Table S4).

2.5 Plant Coverage Measurement

Finally, we estimated the plant relative coverage in each plot using a point-intercept method based on equally-spaced quadrat (100 intersections) ^[12]. Thus, we estimated the coverage proportion for each species in the quadrat and overall coverage ^[21].

2.6 Data Analyses

We carried out the data analyses using the R program 3.6.0 ^[36]. First, we tested we tested data distribution using Shapiro-Wilk and Q-Q plot ^[37]. We compare the tailing substrate properties and plant coverage between restoration treatments using the Kruskal-Wallis's and Dunn's test based on the 'dunn.test' package ^[38].

A principal components analysis (PCA) on the Pearson correlation matrix was used to reduce the number of redundant soil properties ^[39] using the 'FactoMineR' package ^[40]. Thus, we calculated correlations among tailing substrate properties and the ordination axes. We tested linear generalized models (GLMs with Poisson error distribution) to explain the main effects of soil predictor on plant coverage using the package 'lme4' ^[41] in the platform R 3.6.0 ^[36]. Then, we used the first PCA fertility (PCA1f) and texture (PCA1t) variables as predictors, based on 15 analyzed parameters (Figure S1-S2, from ESM). Thus, the first axis was considered as a proxy for soil fertility and soil texture across all tested models ^[39]. Was used also individual substrate properties and plant coverage as predictor, after checking the Q-Q graph ^[37]. When over-dispersion was observed in GLM, an empirical scaling of quasi-Poisson type was performed ^[37]. When two variables were strongly correlated ($r \ge 0.6$), was we included in separate models (Figure S3 from ESM). All models were calculated. The figures in this research were created using 'ggplot2' package ^[42].

3. Results

3.1 Substrate Fertility and Plant Coverage Pattern

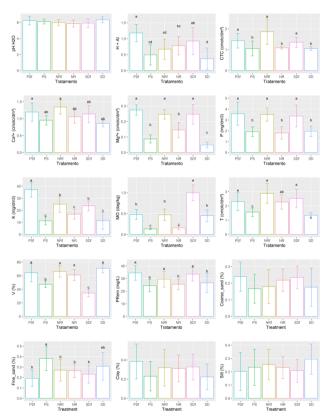


Figure 2. Differences of substrate properties between treatments. The following properties are included: P, K, Ca, Mg, pH (H₂O), organic matter (OM); exchangeable acidity (H + Al); effective cation exchange (t), potential cation exchange (M, P, P)

(T), P-Rem, bases saturation (%V), coarse sand (Sand_ c), fine sand (Sand_t), clay and silt. Treatments: seedlings with fertilization (PSf); seedlings without fertilization (PS); seeding with fertilization (SDf); seeding without fertilization (SD), regeneration with fertilization (RNf); regeneration without fertilization (RN). Different letters indicate that there is a difference (post-hoc p <0.05)</p> Significant differences in substrate properties between treatments were observed (Figure 2). Differences in substrate properties were observed between macronutrients, micronutrients, organic matter, and cation exchange capacity between treatments with and without fertilization. These properties present a similar pattern with no difference between fertilization treatments (PSf, NRf, SDf), and without differences between treatments without fertilization (PS, NR, SD). Conversely, it was observed as a general standard that substrate texture parameters are not markedly contrasting between treatments (Figure 2).

Significant differences in plant coverage between treatments were observed (Figure 3). On average, the highest plant coverage was found in treatment with fertilization, such as NRf (59,5%) and SDf (48%). However, the treatment with seedling planting (PSf and PS) did not show differences (~37%), while the lowest values were observed in SD (23%).

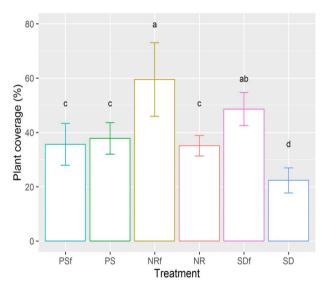


Figure 3. Differences in plant coverage between treatments: planting seedlings with fertilization (PSf); seedlings without fertilization (PS); seeding with fertilization (SDf); seeding without fertilization (SD), regeneration with fertilization (RNf); regeneration without fertilization (RN). Different letters indicate that there is a statistical differences (post-hoc p <0.05)

3.2 Descriptors of Substrate Fertility

The PCA analysis indicated that the first two axes explained ~ 47% of the variability in substrate data (Figure 4). The PCA1 explained 32.1% in substrate data and correlated positively with fertility indicators, mainly nutrients, such as Mg (R = 0.82, p <0.05), potassium

(R = 0.79, p <0.05), phosphorous (R = 0.60, p <0.05) and cation exchange capacity (R = 0.50, p <0.05), and negatively with pH (R = -0.26, p <0.05), silt (R = -0.43, p <0.05) and fine sand (R = -0.68, p <0.05). The PCA₂ explained 16.7% of the variation in substrate data and correlated negatively with coarse sand (R = -0.54, p <0.05), and clay (R = 0.53, p <0.05), but positively with a silt (R = 0.72, p <005). The phosphorous presented a high correlation with PCA1 and PCA2, showing a marked difference between treatments (Fig 3, Figure S.3 from ESM).

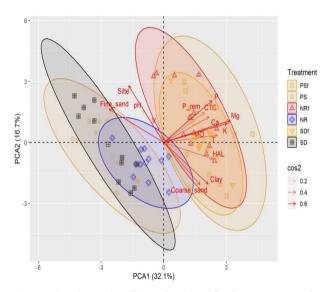


Figure 4. Dimensionality-reduction of substrate properties based on Principal Component Analysis (PCA) across different restoration treatments. The following properties are included: P, K, Ca, Mg, pH (H₂O), organic matter (OM); exchangeable acidity (H + Al); effective cation exchange (t), potential cation exchange (T), P-Rem, bases saturation (%V), coarse sand (Sand_c), fine sand (Sand_t), clay and silt. Treatments: seedlings with fertilization (PSf); seedlings without fertilization (PS); seeding with fertilization

(SDf); seeding without fertilization (SD), regeneration with fertilization (RNf); regeneration without fertilization (RN)

3.3 Substrate Properties and Plant Coverage Relationships

Our main univariate tested model explained the strong effects of substrate fertility (PCA1f) on plant coverage (GLM: t = 2.59, p <0.001, Figure 5). Thus, it is assumed that the initial site condition through fertilization promotes higher plant coverage as ecological indicator.

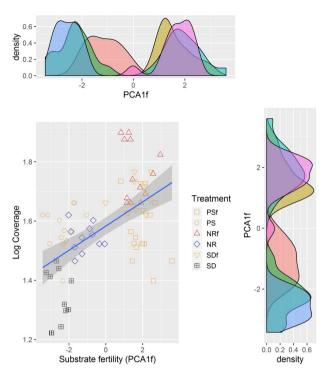


Figure 5. Plant coverage and the main predictor (substrate fertility). Color fill figures indicate treatments, and lines represent the prediction, and the shaded indicate the confidence interval (95 %). Treatments: seedlings with fertilization (PSf); seedlings without fertilization (PSf); seeding with out fertilization (SDf); regeneration with fertilization (RNf); regeneration without fertilization (RN)

4. Discussion

4.1 Changes in Plant Community Coverage

Our results indicated marked changes in substrate properties and plant coverage between treatments of site preparation techniques, and the main model explained significant effects of substrate fertility on plant community coverage. Plant community coverage as ecological indicator can be sensitive to initial site conditions and site effects treatments, which can shape the successional trajectories ^[9], and during early restoration or regeneration stages ^[11,19]. Thus, we presume that the initial site conditions through different fertilization techniques promote mineralization process, consequently increases substrate fertility and biomass production that determine higher plant community coverage.

Local-scale restoration in the Atlantic forest must also be of great priority (i.e. Mariana), due to the high environmental heterogeneity among sites that can be determinant for initial site conditions during restoration. However, in our study, the seeding of native trees with fertilization showed also high plant coverage. Thus, seeding can be an efficient method for rapid recovery of structure mainly in sites where natural regeneration is limited and planting seedlings can be expensive ^[19,20]. Different studies argued that seeding is a low-cost alternative for forest restoration ^[43,44,45], where can there be a reduction of up to 40% of the costs avoiding cost for planting ^[43], with a high density of seeds planted ^[19,44]. We propose that seeding and seedling planting with fertilization as initial site conditions, it is possible to select species with higher potential for biomass production and plant coverage, in this way accelerate the establishment of new natural seedlings during regeneration process.

The natural regeneration can depend on multiple environmental factors ^[46, 47]. For example, a recent study shows that Neotropical second-growth forests differ drastically in their ability to recover biomass (average of 66 years to recover 90% of aboveground biomass), mainly shape by the variation in soil fertility and water availability, due to that high rainfall and low water deficit allow extending the growing season and increasing productivity ^[48]. However, studies at site-scale are essential for supply initial conditions during forest restoration according to each environmental context and degradation level. For this reason, in our study approach we analyzed the importance of the relationship between plant community coverage and substrate properties in forest restoration. Likewise, we consider that due to the high substrate fertility in the initial site conditions and plant coverage in seeding of native trees with fertilization and natural regeneration with fertilization treatments; can also have important positive effects on soil recovery along forest sucession. Furthermore, our results coincide with some recent meta-analyzes for tropical forests demonstrated for the first time that the success of ecological restoration is higher for passive restoration (i.e. natural regeneration) in relation to active restoration for vegetation structure due to the influence of biotic and abiotic drivers ^[13,49]. This study provides premises to forest management based on ecological indicators of restoration, such as tailing substrate fertility and community plant coverage, which may shape the sucessional trajectories of Atlantic forest restoration in Mariana.

We presume the high substrate texture variability is due to possible materials exchanges with the tailing. Therefore, it is possible that the less coarse-texture substrate may have lower variability of site conditions or microhabitats; however can affect positively the seed germination and seedlings growth due their high moisture content ^[6,12]. These processes of natural establishment of plants in sites probably induce an increase in plant coverage. Furthermore, previous studies on soil stabilization showed that management of weed species during passive restoration is desirable to reduce invasive alien species and subsequently increase the diversity of native woody species. ^[50]. Therefore, a recent study demonstrated that limestone substrate had a positively relationship with abundance, being most beneficial for native plant species establishment and higher coverage ^[12]. However, after the Fundão tailings dam failure in Mariana, there are no previous studies for method comparisons because these are the first results with this approach.

4.2 Effects of Substrate Properties on Plant Coverage

Our results showed that substrate fertility affect positively plant coverage. These results corroborate this relationship (fertility and coverage) that has been observed in different types of plant communities and ecosystems ^[11,16,17]. Despite not having quantified the direct effects of plant coverage quality on substrate properties, we presumed that an increase in plant coverage (as proxy of aboveground biomass) promotes a higher organic matter accumulation^[11]. This result reflected the higher substrate fertility in PSf, SDf, and NRf where present the highest values of phosphorous, effective cation exchange capacity, percentage of bases saturation, and micronutrients. Thus, it has been demonstrate that mineral content is absorbed by the organic matter and clay minerals in the soil, which are responsible for elevated micronutrients concentrations [51]. Furthermore, probably there is a closed nutrients cycling in our experiment where treatments with higher fertility (as main predictor), allows a higher plant coverage and consequently higher aboveground biomass production, which promotes the nutrient turnover through litterfall and decomposition. The organic matter turnover to the soil allows maintaining high nutrients levels and substrate fertility. Conversely, a decline in soil organic matter levels induces a decrease in soil aggregate stability and soil moisture ^[51,52].

Although the results indicate that both restoration methods (active and passive) with fertilization promotes higher plant community coverage, it is possible enhance diversity and dominance of native species in degraded forest, compared to passive restoration. Our study also demonstrated that the different effect site is important for ecological restoration in Mariana, beyond passive restoration ^[5,50]. Thus, active restoration with dominant-pioneer species (rapid growth and coverage) can reduce soil loss during initial restoration ^[50,53].

5. Conclusions

This study demonstrates how the effects of restoration

methods (active and passive) and site initial conditions, mainly through the substrate fertilization, determines an increase in plant community coverage. Specifically, the results showed a positive relationship between mining tailings substrate fertility and plant community coverage, mainly due to the effects of fertilization treatments. However, the natural regeneration with fertilization was the treatment that induced higher plant community coverage in the study area. Although the active and passive restoration may be complementary and are being applied efficiently in the areas affected by mining tailings in Mariana. We assume that passive restoration in second-growth forest that are regrowing after anthropogenic disturbances still represents an important driver for the plant coverage recovery in Mariana, Minas Gerais, Brazil.

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Appendixes: Supplementary data

Table S1. Species of native seedlings for the cover phase

Family	Common name	Specie
Euphorbiaceae	Capixingui	Croton floribundus
Euphorbiaceae	Sangra d'água	Croton urucurana
Fabaceae	Ingá-banana Inga vera	
Fabaceae	Sena-aleluia/piteira	Senna pendula
Fabaceae	Pau-cigarra	Senna multijuga
Fabaceae	Pata-de-vaca	Bauhinia forficata
Malvaceae	Mutamba	Guazuma ulmifolia
Malvaceae	Algodoeiro	Heliocarpus popayanen- sis
Solanaceae	Fumo-bravo	Solanum granulosole- prosum
Anacardiaceae	Aroeira-pimenteira	Schinus terebinthifolius

Table S2. Native tree species sown. Seed quantity (g ha⁻¹)

Family	Common name	Specie	g ha ⁻¹
Cannabaceae	Crindiúva	Trema micrantha	4
Euphorbia- ceae	Capixingui	Croton floribundus	20
Euphorbia- ceae	Sangra d'água	Croton urucurana	6

Fabaceae	Orelha de negro	Enterolobium contortisi- liquum	78
Fabaceae	Mutamba	Guazuma ulmifolia	4
Fabaceae	Monjoleiro	Senegalia polyphylla	30
Fabaceae	Fedegosão	Senna macranthera	16
Fabaceae	Pau-cigarra	Senna multijuga	8
Malvaceae	Algodoeiro	Heliocarpus popayanen- sis	4
Solanaceae	Fumo-bravo	Solanum granulosole- prosum	6

 Table S3. Green manure for planting lines between each seedling

Family	Common name	Specie	g ha ⁻¹
Fabaceae	Crotalária	Crotalaria ochro- leuca	3.5
Fabaceae	Guandu-arbóreo	Cajanus cajan	26.1
Fabaceae	Crotalária-juncea	Crotalaria juncea	14.0
Fabaceae	Estilosantes	Stylosanthes capi- tata	1.2
Fabaceae	Fedegoso gigante	Senna alata	1.3
Pedaliaceae	Gergelim	Sesamum indicum	1.6
			47.7

 Table S4. Average from three replicates of chemical characteristics of the soil in areas contaminated with mining tailings

Parameter	Average
pH (H2O)	7.0
P (mg dm-3)	8.3
K (mg dm-3)	12.6
Ca2+ (cmolc dm-3)	0.7
Mg2+ (cmolc dm-3)	0.0
Al3+ (cmolc dm-3)	0.0
H + Al (cmolc dm-3)	0.1
SB (cmolc dm-3)	0.2
CEC(t) (cmolc dm-3)	0.2
CEC(T) (cmolc dm-3)	0.3
V %	70
OM (dag kg-1)	0.5

Notes: 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.



Figure S1A. Experimental design in study area (bottom image area I), district of Paracatu de Baixo (7754350 N, 686800 E), municipality of Mariana, Minas Gerais, Brazil



Figure S1B. Experimental design in study area (bottom image area II), district of Paracatu de Baixo (7754350 N, 686800 E), municipality of Mariana, Minas Gerais, Brazil

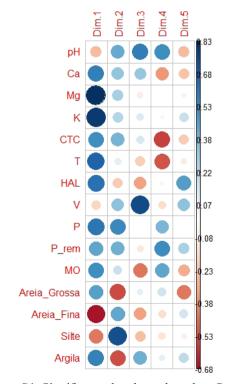


Figure S1. Significance levels are based on Spearman correlation coefficients between soil parameters and

principal components of soil PCA from 78 plots of different treatments. For analysis: P, K, Ca, and Mg available, exchangeable acidity (H + Al); pH (H_2O) ; organic matter (OM); effective cation exchange capacity (t); potential

cation exchange capacity (T); remaining phosphorus (P-Rem); percentage of bases saturation (V); and the soil texture as coarse sand (Sand_c); fine sand (Sand_t); clay and silt contents were included

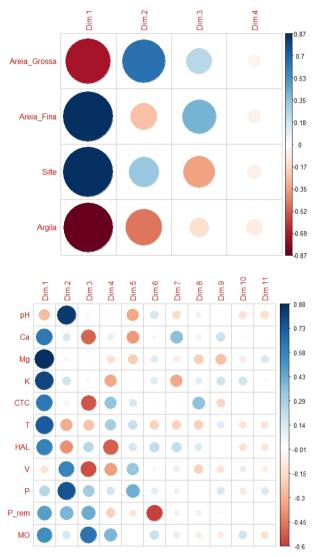


Figure S2. Significance levels are based on Spearman correlation coefficients between soil parameters and principal components of both texture PCA and fertility PCA from 78 plots of different treatments. For analysis: P, K, Ca, and Mg available, exchangeable acidity (H +

Al); pH (H₂O); organic matter (OM); effective cation exchange capacity (t); potential cation exchange capacity (T); remaining phosphorus (P-Rem); percentage of bases saturation (V); and the soil texture as coarse sand (Sand_c); fine sand (Sand_t); clay and silt contents were included

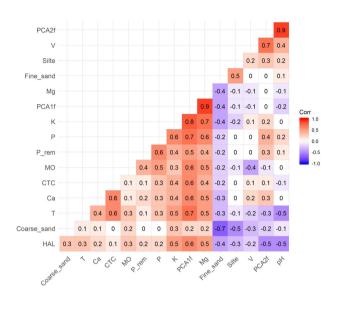


Figure S3. Spearman correlation among all individual variables measured in different treatments. For analysis: PCA axes, P, K, Ca, and Mg available, exchangeable acidity (H + Al), pH (H₂O), organic matter (OM); effective cation exchange capacity (t), potential cation exchange capacity (T), P-Rem, percentage of bases saturation (V), and the soil texture as coarse sand (Sand c), fine sand (Sand t),

clay and silt contents were included

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