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Carbon stock in forest restoration intercropped with *Eucalyptus* in the Atlantic Forest of Brazil

Estoque de carbono em restaurações florestais consorciadas com eucalipto na Mata Atlântica do Brasil

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ABSTRACT

This study aimed to evaluate above and below ground carbon stocks in three different forest restoration methods with native species, *Eucalyptus* and an area of semi-deciduous seasonal forest (as control) in the Atlantic Forest of Brazil. The restoration methods evaluated were active restoration without *Eucalyptus* (RAS), active restoration with *Eucalyptus* (RAE) and passive restoration (RPE) and compared to a secondary forest fragment (REF). The evaluation reservoirs were: trees, roots, herbaceous, litter and soil (layers of 0-5, 5-10 and 10-20 cm). The estimates show that the total carbon stock was higher in REF (64.68 Mg C ha^{-1}), followed by RAE (62.06 Mg C ha^{-1}), RPE (50.23 Mg C ha^{-1}) and RAS (33.84 Mg C ha^{-1}). The results show that a forest restoration with a greater diversity of native forest species does not imply having a greater carbon stock in the different compartments. The soil was the compartment with the largest carbon stock. The use of *Eucalyptus* plantations in forest restoration of native forest species can contribute to increase the capture and the storage of carbon in the different reservoirs of the ecosystem.

Keywords: *Climate change; Carbon kidnapping; Recovery of degraded areas; Environmental services.*

RESUMO

Este estudo teve como objetivo avaliar os estoques de carbono aéreo e subterrâneo em três diferentes métodos de restauração florestal com espécies nativas e eucaliptos e área de floresta estacional semidecídua (testemunha) na Mata Atlântica do Brasil. Os métodos de restauração avaliados foram restauração ativa sem eucalipto (RAS), restauração ativa com eucalipto (RAE) e restauração passiva (RPE) e comparada com fragmento florestal secundário (REF). Os reservatórios de avaliação foram: árvores, raízes, herbáceas, serapilheira e solo (camadas de 0-5, 5-10 e 10-20 cm). As estimativas mostram que o estoque total de carbono foi maior no REF (64,68 Mg C ha^{-1}), seguido pelo RAE (62,06 Mg C ha^{-1}), RPE (50,23 Mg C ha^{-1}) e RAS (33,84 Mg C ha^{-1}). Os resultados mostram que uma restauração florestal com maior diversidade de espécies florestais nativas não implica maior estoque de carbono nos diferentes compartimentos. O solo foi o compartimento com maior estoque de carbono. A utilização de plantações de eucalipto na restauração florestal de espécies florestais nativas pode contribuir para aumentar a captura e o armazenamento de carbono nos diferentes reservatórios do ecossistema.

Palavras-chave: *Mudanças climáticas; Sequestro de carbono; Recuperação de áreas degradadas; Serviços ambientais.*

1. INTRODUCTION

The restoration of tropical forests has entered the international political agenda as a means of fighting climate change, due to the ecosystem's service of capturing and storing carbon provided by forests (Shimamoto et al., 2014; Zanini et al., 2021), while increasing the number of species (Bustamante et al., 2019; Capellesso et al., 2020).

The services promoted by the restoration of forests, in addition providing an increase in biodiversity; additionally may generate economic, social and environmental benefits, collaborating with the fulfillment of the goals established by Brazil in the United Nation's Convention On Climate Change (UNFCCC), to restore 12 million hectares by the year 2030 according the Paris Treaty (Bustamante et al., 2019).

The Atlantic Forest is the Brazilian biome with the greatest biodiversity (Myers et al., 2000), but it is also the most degraded one, with only 12.4% of the original coverage remaining (Fundação SOS Mata Atlântica, 2020). The challenge for restoration is great because, in addition to the forest restoration being a very costly activity; about 70% of the Brazilian population lives in this biome (Brancalion et al., 2021). In Brazil, strategies which encourage the restoration of the Atlantic Forest have been discussed for a long time, guaranteeing the maintenance of the biodiversity (Brancalion et al., 2021), but it has not yet been able to reverse the situation of degradation. Despite technical, legal and political advances, the deforestation persists in the Atlantic Forest biome (Fundação SOS Mata Atlântica, 2020).

The restoration methods can include active, passive or assisted models (Zanini et al., 2021). In the active successional model, there is the introduction of native species and the desirable need for silvicultural treatments, while monitoring in order to guarantee a greater biodiversity (Rodrigues et al., 2009). On the other hand, in the passive model, the objective relies on the natural regeneration, requiring only in some cases isolation and forest protection, which transforms it into an assisted model (Zahawi et al., 2014).

In the case of studies with successional models of restoration in the Atlantic Forest, there is a consensus among researchers that passive models, despite being slower and generally accumulating less carbon in the above biomass, are more advantageous due the low costs of restoration (Brancalion et al., 2021; Zahawi et al., 2014), which should be taken into account in restoration strategies for carbon kidnapping and storage. The areas must have a minimal natural regeneration potential, hence changing the logic of using active models to passive ones in the Atlantic Forest. In the Amazon forest, this model is preferred because the forest regeneration is faster due the large forest remnants and the greater natural resilience of that biome (Brancalion et al., 2021). In the Atlantic Forest with its considerable fragmentation and with the predominance of forest fragments with an average of only 3 ha (Fundação SOS Mata Atlântica, 2020), the passive restoration renders this situation more complex.

In addition to cost reduction, the passive models can outperform the active ones when considering the total stocks, that is, not only above biomass, but also litter, herbaceous, roots and soil, which in the case of the managed areas is more prone for carbon accumulation. Succession models with intercropping potential timber species, native or exotic, such as *Eucalyptus*, can increase carbon stocks and provide wood provisioning services (Millenium Ecosystem Assessment, 2005). This study aimed to evaluate above and below ground carbon stocks in three different forest restoration methods with native species and *Eucalyptus* in the Atlantic Forest of Brazil.

2. MATERIALS AND METHODS

2.1. Study area

The study was carried out in two locations, at the Rio Fundo farm (11° 06' 35" S and 37° 20' 11" W) in the municipality of Itaporanga d'Ajuda and at the Rural Campus of the Federal University of Sergipe (10°55'14.0"S 37°05'42.2"W) in the municipality of São Cristóvão, both located in the state of Sergipe (SE state), Brazil (Figure 1).

The climate in the two locations of the study areas is type as, dry season in summer and rainy season in winter, temperatures oscillating between 23°C and 31°C and average rainfall of 1600 mm (White & Silva, 2018). All sites are in the Semideciduous Seasonal Forest, in the Atlantic Forest biome (White & Silva, 2018). The soil was classified as Haplic Plinthossol (Santos, 2022). The main land use in the region is agriculture and livestock.

From 2013 onwards, stretches of the farm were incorporated into a Project for the Recovery of Degraded Areas (PRAD), as required by the Brazilian Institute of Environment and Renewable Natural Resources

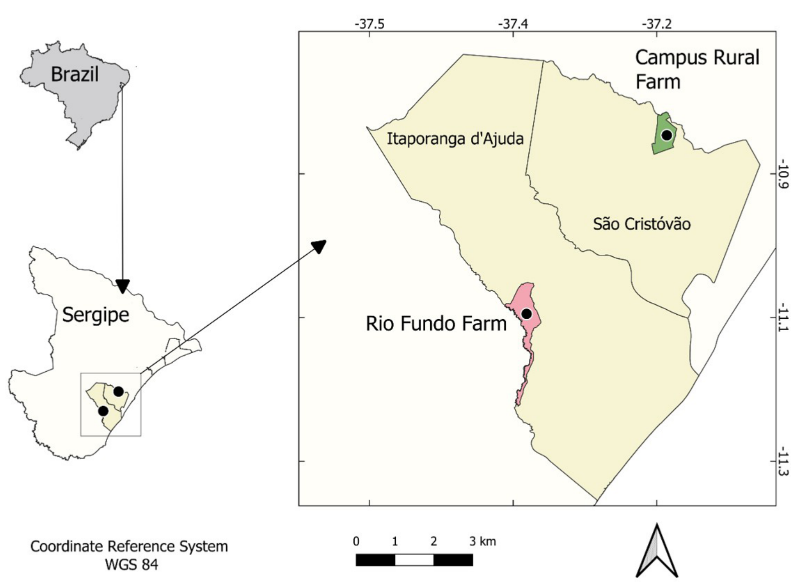

Figure 1. Study area in the state of Sergipe, Brazil.

- IBAMA, therefore, forest restoration projects were implemented with different methodologies.

2.2. Treatments

The forest restorations evaluated for carbon stocks were:

Active Restoration (RAS): reforestation with 30 native species planted with spacing of 4 x 4 meters in 2013, after clear cutting of individuals of *E. urophylla*. The seedling density was approximately 625 trees ha^{-1} and a total of 30 species were planted in an area of 4 ha.

Active restoration with remaining eucalyptus (RAE): reforestation with 15 natives species planted with spacing of 4 x 4 meters, between the stumps of individuals of *E. urophylla*, which were thinned with an intensity of 50% in 2013. The density of seedlings was approximately 625 trees ha^{-1}. This area had a density of 312 to 313 adult *E. urophylla* individuals per hectare in an area of 5 ha.

Passive restoration (RPE): without planting native seedlings, abandonment the area after thinning 100% of the *E. urophylla* individuals; the area has been undergoing natural regeneration process since 2013, including regeneration of *Eucalyptus* on 4 ha.

Secondary forest (REF): Fragment of semi-deciduous seasonal forest, used as a control, age 20 years, approximately 100 ha, in the initial secondary succession stage.

2.3. Sampling

For each treatment, four plots (25 × 25 m – 625 m^2) were randomly allocated, making a sample area of 0.25 ha in total for each treatment. To avoid the border effects, the plots were allocated at least 50 m from the limits of the areas submitted to restoration treatments.

2.3.1. Aboveground carbon

2.3.1.1. Tree

All living trees within the plots that had at least a stem ≥ 5 cm diameter at breast height (DBH, 1.3 m) were measured for diameter (DBH) and for height (Ht) with a telescopic pole and determined to the most detailed botanical level. In the case of trees with more than one stem meeting the inclusion criteria, all stems were measured.

The botanical material collected was identified through bibliographic consultation and what was not was identified in the Herbarium of the Federal University of Sergipe. The classification system of APG IV (The Angiosperm Phylogeny Group, 2016) was adopted. All inventoried forest species are described in Almeida et al. (2025).

The wood density (p) was obtained from Chave et al. (2005) which provides wood density data for 2,456 tropical species. The biomass was determined using the equation developed for humid tropical forests by Chave et al. (2014) (Equation 1). The aboveground carbon content was assumed to be 47% (Eggelston et al., 2006). Based on the average biomass of the plot, it was possible to extrapolate the biomass (Mg ha^{-1}) and the carbon stock (Mg C ha^{-1}) for the whole area.

$$\text{Tree biomass} = AGB = 0.0673*\left(\text{p*DAP2*Ht}\right)*0.976 \qquad \text{(Eq. 1)}$$

2.3.1.2. Burlap and herbaceous

In the 625 m^2 plot, litter and herbaceous were collected within a square frame (1×1 m) at four random points. The samples were dried in an oven (60°C) until the weight stabilized. The dry biomass (g) was measured on a scale with precision of two decimal places. The value was extrapolated to the plot area and the plot mean in each treatment was calculated. Then, the samples were ground in a mill. A 100-gram subsample was sent to the Soil Laboratory of the Federal University of Sergipe (DA/UFS) to determine the carbon content (%) by oxidation of organic matter in the presence of sulfuric acid and potassium dichromate, according to EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária, 2009).

2.3.2. Carbon belowground

2.3.2.1. Roots

The roots were estimated using the indirect method so as to not disturb the restoration process by opening forest clearings. The equation developed by Cairns et al. (1997) (Equation 2), considers estimates of root biomass (BLG) from tree biomass (AGB), whose model takes into account an extensive literature review that defined the variable. The most important factor affecting the roots are aboveground biomass density, age, and latitude (Cairns et al., 1997).

The carbon content for soil roots was assumed to be 47% (Eggelston et al., 2006). Based on the average biomass of the plot, it was possible to extrapolate the biomass (Mg ha^{-1}) and the carbon stock (Mg C ha^{-1}) for the entire area.

$$\text{Root biomass} = -\exp\left(-1.085 + 0.926 * \ln\left(AGB\right)\right) \qquad \text{(Eq. 2)}$$

2.3.2.2. Ground

For the soil carbon, four random points were sampled in each 625 m^2 plot using an straight shovel and three subsamples were taken from layers 0–5, 5–10, and 10–20 cm deep (Empresa Brasileira de Pesquisa Agropecuária, 2009). The subsamples were mixed to compose the sample for each plot. The samples were air-dried and ground. Then, a 100 gram sample was used to determine the carbon content (Empresa Brasileira de Pesquisa Agropecuária, 2009). The soil density was determined in each plot by the volumetric cylinder method (Empresa Brasileira de Pesquisa Agropecuária, 2009). The soil carbon stock (Mg C ha^{-1}) was obtained from the Veldkamp (1994).

2.4. Data analysis

The statistical analysis of the biomass and carbon stock of the different pools for the treatments was performed in R (R Core Team, 2017). Levene and Shapiro-Wilk tests were applied to validate the requirements of analysis of variance (ANOVA), homogeneity of variance and normality of errors. Once all requirements were met, ANOVA was performed adopting a significance level of 5% and, when differences were detected, Tukey's test was applied with 95% confidence.

3. RESULTS

3.1. Carbon stocks

Table 1 shows the carbon stocks in each reservoir, including soil layers (0–5, 5–10, 10–20 cm) for each treatment.

Belowground carbon stocks were higher than aboveground carbon, considering the soil as the largest carbon reservoir for all restoration methods under study (Table 1). The carbon stock in the roots presented a significantly higher value in REF (9.04 Mg C ha^{-1}) in relation to the other treatments. The largest aboveground carbon stock was in the tree compartment (26.86 Mg C ha^{-1}). The litter was

Table 1: Carbon stocks in above and below ground compartments in areas with different restoration methods.

	Reservoirs	REF (Mg C ha^{-1})	RAS (Mg C ha^{-1})	RAE (Mg C ha^{-1})	RPE (Mg C ha^{-1})
Above the ground	Trees	26.86a	8.92b	14.42ab	7.96b
	Herbaceous	0.27b	0.16b	0.46b	1.31a
	Burlap	2.72a	0.99b	1.74a	1.18b
Subtotal		29.85	10.07	16.62	10.45
Below the ground	Roots	9.04a	3.05b	4.90b	2.79b
	Soil (0-5cm)	7.66c	6.00d	10.13b	11.85a
	Soil (5-10 cm)	6.21b	4.90b	11.03a	10.56a
	Soil (10-20 cm)	11.92c	9.82c	19.38a	14.58b
Subtotal		34.83	23.77	45.44	39.78
	Total (Mg C ha $^{-1}$)	64.68a	33.84b	62.06a	50.23ab

RAS: Active restoration, RAE: Active restoration with remaining *Eucalyptus*, RPE: Passive restoration, REF: Secondary Forest. *Significant differences are indicated by different letters by the 5% Tukey test.

the second largest aerea biomass in the treatments, being statistically equal between the RAE (1.74 Mg C ha^{-1}) and the REF (2.72 Mg C ha^{-1}).

In the herbaceous reservoir, the RPE treatment (1.31 Mg C ha^{-1}) was significantly higher than the others. Total carbon stocks were higher in REF (64.68 Mg C ha^{-1}), RAE (62.06 Mg C ha^{-1}) and RPE (50.23 Mg C ha^{-1}), were statistically similar, but higher than the total carbon stock of RAS (33.84 Mg C ha^{-1}).

In the 0–5 cm layer, the carbon stock was significantly higher in RPE (11.85 Mg C ha^{-1}) compared to the other treatments. In the intermediate layer (5-10 cm), RPE (10.56 Mg C ha^{-1}) and RAE (11.03 Mg C ha^{-1}), both intercropped with *Eucalyptus*, were statistically equal and superior to the other treatments. In the deepest layer (10–20 cm), RAE had the highest carbon stock (19.38 Mg C ha $^{-1}$).

4. DISCUSSION

After 10 years of implementation of the different forest restoration models, the results show that the presence of *Eucalyptus* trees in RAE and RPE provided a total carbon stock similar to REF. Despite having only native species, RAS did not result in a total carbon stock similar to REF.

The presence of the *E. urophylla* in the RAE and RPE treatments favored a greater accumulation of carbon in the soil and roots compared to the RAS and REF treatments. The *Eucalyptus* trees present in these treatments increased the litter supply and had a more developed root system since these were not removed, which contributes to a higher carbon content in the below ground biomass. In this way, even REF presenting a greater diversity of species, it did not result in a greater stock of carbon in the soil and in the roots, highlighting the role of *E. urophylla* in RAE and RPE, which was to be able to promote an increase of carbon stock in the soil and roots even with only 10 years of implementation. The REF area is a secondary forest with no history of forest degradation or deforestation for over 40 years. The REF has around 30 native forest species, including late secondary and climax species, such as *Cupania vernalis*, *Byrsonima sericea*, *Duguetia lanceolata* and *Pterodon emarginatus* (Almeida et al., 2025).

Thus, the total belowground carbon stocks was in RAE (45.44 Mg C ha^{-1}) and RPE (39.78 Mg C ha^{-1}), REF (34.83 Mg C ha^{-1}), while RAS was the one which had the lowest stock (23.77 Mg C ha $^{-1}$). In other words, the presence of *E. urophylla* is a determining factor in a greater total below ground carbon. Some studies show that the carbon stock below ground is slowly reestablished, taking many years to show significant change (Cunningham et al., 2015; Jones et al., 2019). Macedo et al. (2008) and Nogueira et al. (2011) also did not find significant differences in below ground carbon between the different methods applied to restore an area of Atlantic Forest, 10 and 13 years after implementation, respectively. In this way, despite the short time since the implementation of RAE and RPE (10 years), the presence of *Eucalyptus* was essential to promote higher values of below ground carbon stocks.

Furthermore, it was observed that in all results (except for the 5–10 cm depth in the RAS), the amount of carbon increased with increasing soil depth. Similar results were also reported by Zaninovich & Gatti (2020) for open-canopy forests in the Atlantic Forest. This is possibly due to more favorable conditions for root deposition and increased biological activity at this depth (Fernandes et al., 2024)

The 0–5 cm layer, RPE (11.85 Mg C ha^{-1}) followed by RAE (10.13 Mg C ha $^{-1}$) showed the highest carbon stocks. Despite a lower diversity of species, these two treatments have a higher percentage of *Eucalyptus* trees and a greater amount of herbaceous material, which consists of grasses, which contributes to a greater accumulation of organic matter in the more superficial layers. The same trend was observed at depths of 5-10 cm and 10-20 cm in RPE and RAE, being superior to RAS and REF. In Ethiopia, the reforestation of degraded farmlands and pastures with *Eucalyptus* raised soil C stocks in the 0-20 cm layer to almost 70% of the level found in a 30-year dry subtropical highland natural forest (Assefa et al., 2017) .

In RAE and RPE, the carbon stock composed of herbaceous (0.46 and 1.31 Mg C ha^{-1}, respectively) is higher than the REF and RAS areas (0.27 and 0.16 Mg C ha $^{-1}$, respectively). On the other hand, RAS and REF showed relatively small carbon stocks of herbaceous plants, but most of it was in the arboreal reservoir, which shows that the restoration area (RAS) is having a behavior more similar to the secondary forest (REF).

In relation to the litter, REF and RAE had a greater contribution of litter, which could potentially represent a greater stock of carbon in the soil due to the nutrient cycling process. Among the restoration methods, the one with the highest amount of litter was RAE (1.74 Mg C ha^{-1}), which may increase carbon stocks in other compartments. Litter stock is affected by environmental factors such as tree presence, tree density, species richness and age (Carpanezzi et al., 1997), precipitation, temperature, soil moisture, soil organisms, degree of disturbance and level of area degradation (Martins & Rodrigues, 1999), decomposition rate and growth rate (Machado et al., 2008).

The aboveground carbon stock was highest in REF (29.85 Mg C ha^{-1}), followed by RAE (16.62 Mg C ha^{-1}), RPE (10.45 C ha^{-1}) and RAS (10.07 Mg C ha^{-1}). It is observed that restorations with *Eucalyptus* (RAE and RPE) had a significantly higher aboveground carbon stock compared to RAS. This is due to the rapid growth and consequent efficiency of *Eucalyptus* in capturing atmospheric carbon (Viera & Rodríguez-Soalleiro, 2019).

When restoring a degraded area, you want it to approximate a reference model area (Gann et al., 2019), which, in this study, was the secondary forest area (REF). In this sense, RAE was the forest restoration that the aboveground biomass stock was closest to the secondary forest area (REF). RAE had the highest tree density and eucalyptus individuals, factors that were determining for a higher carbon stock in the above biomass. Despite RAS having greater native species diversity, this did not result in a greater total carbon stock.

The total carbon stock was significantly higher in the REF (64.68 Mg C ha^{-1}) and RAE (62.06 Mg C ha^{-1}) compared to the other treatments (RPE: 50.23 Mg C ha^{-1} and RAS: 33.84 Mg C ha^{-1}). Studies show that restoration areas in the Atlantic Forest need time for growth changes to be detected (Zanini et al., 2021). Gardon et al. (2020) found that most Brazilian forest restoration areas began to show significant differences between passive and active methods after the 5th to 10th year after implementation.

5. CONCLUSION

The results show that a forest restoration with a greater diversity of native forest species does not imply a greater carbon stock in the different compartments. The soil was the compartment with the largest carbon stock. The use of *Eucalyptus* plantations in forest restoration of native forest species can contribute to an increase in the capture and the storage of carbon in the different compartments of the ecosystem.

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AUTHOR CONTRIBUTIONS

MMF: conceptualization, funding acquisition, supervision, writing; MBJ: conceptualization, funding acquisition, supervision, writing; MVAV: data curation, formal analysis; BCGCM: data curation, formal analysis; ASR: conceptualization, funding acquisition, supervision, writing; JRG: conceptualization, funding acquisition, supervision, writing; RNAF: conceptualization, funding acquisition, supervision, writing; MRMF: conceptualization, writing.