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# The effect of ecological restoration methods on carbon stocks in the Brazilian Atlantic Forest

Anani Morilha Zanini [a,*], Rafaella Carvalho Mayrinck [b], Simone Aparecida Vieira [c], Plinio Barbosa de Camargo [d], Ricardo Ribeiro Rodrigues [a]

[a] ESALQ / USP - Escola Superior de Agricultura Luiz de Queiroz, Av Pádua Dias 11, São Dimas, Piracicaba, SP 13418 260, Brazil
[b] SENS/University of Saskatchewan, 116 - 110 Science Place, Saskatoon, SK S7N 5C9, Canada
[c] Instituto de Biologia, Universidade Estadual de Campinas, Unicamp, Cidade Universitária Zeferino Vaz, Rua Monteiro Lobato, 255, Campinas, SP CEP 13083-862, Brazil
[d] Centro de Energia Nuclear na Agricultura - USP, Piracicaba, São Paulo, Brazil. Piracicaba, SP 13418 260, Brazil

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ABSTRACT

Restoring degraded areas is an effective strategy to reestablish the environmental services provided by the forests including global warming mitigation by carbon sequestration. Restoring lands is especially important in the Atlantic Forest, a global hotspot in Brazil threatened by deforestation. To successfully restore degraded lands, it is necessary to apply the most suitable method for each situation. However, studies comparing restoration techniques are scarce. This lack of information hampers the Atlantic Forest restoration and, given the original complexity of its ecological dynamics, restoration success is even more challenging in this ecosystem. This study aimed to assess carbon stocks (above and belowground), carbon content (%), and carbon isotope at 5-year old sites implemented by different restoration methods in southeastern Brazil. The restoration methods tested were active restoration (AR), assisted restoration (AsR), and passive restoration in an abandoned pasture (AP), which were compared to a nearby pasture (P) and a remaining forest fragment (RF). The assessed pools were: tree, coarse roots, fine roots, herbaceous, litter, standing dead wood, fallen dead wood, and soil (0–1 cm, 5–10 cm, 10–20 cm layers). Total carbon stock was higher on RF (152.304 Mg C ha$^{-1}$), followed by the P (84.378 Mg C ha$^{-1}$), AR (66.414 Mg C ha$^{-1}$), AsR (65.73 Mg C ha$^{-1}$) and AP (65.581 Mg C ha$^{-1}$). The restoration areas sites are still too young to show significant differences in total carbon stock as a result of different restoration methods. However, carbon stock and carbon content (%) differed among the pools according to the method and, in all cases, the largest carbon pool was soil, which shows the importance of sampling every pool for carbon stock and carbon content (%) estimation. Isotope analysis showed that carbon inputs in the soil had different sources, C3 or C4 plants, depending on the method. We concluded that these young secondary areas are already sequestering carbon, which helps mitigate global warming, and that monitoring every pool is important for a complete assessment, not only to restore secondary forests and understand growth but also for other land-uses such as pastures. Besides, the results obtained can be generalized to other tropical forest ecosystems with similar conditions (local and landscape), constituting a relevant contribution to forest restoration and carbon-sequestration related sciences.

## 1. Introduction

Recovering forests is the most simple and effective way to remediate global warming. Carbon sequestration potential of areas recovering from harvests and secondary growth on abandoned agricultural land on the planet is estimated to be 4.4 Pg C y$^{-1}$. Moreover, by stopping deforestation and recovering degraded areas, 120 Pg C could be sequestered between 2016 and 2100 (Houghton and Nassikas, 2017). Only in the Latin America, second-growth forests could sequester 8.48 Pg C over 40 years, which could offset all the carbon released in the region by fossil fuel and industrial activities from 1993 to 2014 (Chazdon et al., 2016). Thus, through proper land management and

* Corresponding author.
*E-mail addresses:* ananizanini@usp.br (A.M. Zanini), sivieira@unicamp.br (S.A. Vieira), pcamargo@cena.usp.br (P.B. de Camargo), rrresalq@usp.br (R.R. Rodrigues).

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implementation of policies, society can probably still mitigate climate change and its devastating consequences (Houghton and Nassikas, 2017; Chazdon et al., 2016). Based on that, during the 2014 United Nations Climate Summit, 30 countries have committed to restore a total of 350 million hectares by 2030 aiming to mitigate climate change and to preserve biodiversity and water supply (Holl, 2017). However, fulfilling this huge forest restoration demand while maximizing carbon sequestration and reestablishing other environmental services provided by forests, in a practical and cheap way is not an easy task (Holl, 2017). Forest dynamic is complex to rebuild and it affects and is affected by many factors, from implementation to establishment of a mature stage (Holl, 2017). In the tropics, restoring forests is even more complex due to its climate and greater biodiversity, so studying the most applicable restoration methods and assessment procedures in the several different situations is crucial to understand and control this complex environment (Durigan and Melo, 2006; Rodrigues et al., 2009).

The Atlantic Forest is one of the tropical forest ecosystems in Brazil, the first threatened one since the Europeans settled in the country, and after that, deforestation and fragmentation have jeopardized the biome through its entire extension. The Atlantic Forest has only 28% of its original cover (Rezende et al., 2018), and, it is the fourth biodiversity hotspot in the world (Myers et al., 2000). It was estimated that 40% of its species are endemic, which means 8,567 species (Myers et al., 2000). Besides, fragmentation affects about 91% of the remaining Atlantic Forest fragments (Pütz et al., 2014) and about 46% of what is left is within 100 m or less from fragment edges; therefore, under strong edge effect (Ribeiro et al., 2009). Due to this fragmentation and consequent edge effect, the Atlantic Forest lost around 69 Tg C (±14 Tg C) between 2005 and 2014, equivalent to 9–24% of the total annual carbon loss associated with global tropical deforestation (Pütz et al., 2014). In addition to these issues, the Atlantic Forest's ability to recover from a disturbance may be drastically reduced after years of excessive exploitation (Tambosi et al., 2014; Poorter et al., 2016; MMA, 2017). Tambosi et al. (2014) found that 85% of the Atlantic Forest has low resilience capacity and that only 5% can be considered a good biodiversity source for the colonization of other areas. Poorter et al. (2016) mapped resilience in the tropical forests of South America and found that the least resilient areas are within the Atlantic Forest. This evidences that these lands need a more effective conservation policy, including the adaptive management of degraded natural forests and the restoration of deforested areas.

Resilience is a key factor to be considered in the selection of the most suitable restoration method (Tambosi et al., 2014; Ferreira et al., 2015; Poorter et al., 2016; Sansevero et al., 2017; César et al., 2018). Resilience can be described using the local and landscape components (Tambosi et al., 2014; Crouzeilles et al., 2016). Local resilience means that the site has good environmental conditions including soil (structure and nutrients) and climate (precipitation and temperature) to foster natural regeneration. Landscape resilience is about the potential the lands surrounding the restoration sites have to serve as source of propagules promoting genetic flux and colonization of adjacent areas (Johnstone et al., 2016). If local and landscape resilience are harmed beyond a certain point, it may be very difficult to re-establish forests naturally, leading to a new stable state different from the previous one (Campoe et al., 2014; Poorter et al., 2016). In the case the area is still resilient, it is possible to restore it through the process called passive restoration, in which the only action necessary is to fence the area to isolate it from disturbing factors (Wadt, 2003; César et al., 2018). To restore areas that need human interventions, other techniques are employed, such as the assisted restoration and active restoration (Ferreira et al., 2015; César et al., 2018; Osuri et al., 2019). The assisted restoration is recommended when the area is still resilient, but some interventions are needed. In these cases, techniques are used to benefit the desired species while hampering the development of others, such as weeds, that may compete with tree species (*Brachiaria*, for example, an invasive exotic grass common in pastures in the region). As part of this method, natural regeneration from the soil seed bank is stimulated to germinate and grow, and a few seedlings are planted to promote fast occupation of the area, avoid weed competition and increment local biodiversity (Rodrigues et al., 2009; Brancalion et al., 2015). Active restoration is used when the area has limited or no chance to recover by itself, so native species are planted in a strategic way to maximize restoration over time and mimic ecological succession (Kageyama and Gandara, 2004; Nave and Rodrigues, 2007; Rodrigues et al., 2009).

In real life, resilience is a gradient, not a categorical measure, which makes selecting the best restoration method a complex task. For this reason, studies comparing restoration methods are much needed. Gardon et al. (2020) pointed out that only 8% of the Brazilian forest-restoration related studies compared passive and active restoration in similar conditions and at the same time. In these studies (Ferreira et al., 2015; César et al., 2018), only aboveground biomass was assessed. This is not surprising, given the fact that it was only in 2003 that biomass started to be studied in restoration projects in the Atlantic Forest. This consists of important knowledge in forest restoration ecology in Brazil since biomass is one of the most important drivers of forest succession (Lohbeck et al., 2015; Toledo et al., 2018; Gardon et al., 2020). Thus, the lack of both adequate biomass sampling and paired comparisons of active and passive methods were pointed out as the main knowledge gaps in the forest restoration science (Gardon et al., 2020). Based on that, the goal of this study was to evaluate different methods (active, passive and assisted) for the restoration of areas in the Atlantic forest to understand their potential to reestablish above and belowground carbon stocks five years after implementation.

## 2. Material and methods

### 2.1. Study site

The study was conducted in three sites (*Capoava* (23° 12′ S 47° 10′ W), *Ingazinho* (23° 13′ S 47° 11′ W), and *Jequitiba Farms* (23° 13′ S 47° 10′ W)) in the municipality of Itu, SP, Brazil (Fig. 1). The climate on all sites is Cwa - dry winter and hot summer; annual average precipitation is 1,299.6 mm and the annual mean temperature is 21.3 °C (Alvares et al., 2013). All sites are in a Semideciduous Seasonal Forest, part of the Atlantic Forest (Veloso et al., 1991). The main land-use on the farms was pasture. In 2012, to comply with environmental legislation, forest restoration projects were implemented using different methods.

### 2.2. Treatments

The treatments tested were:

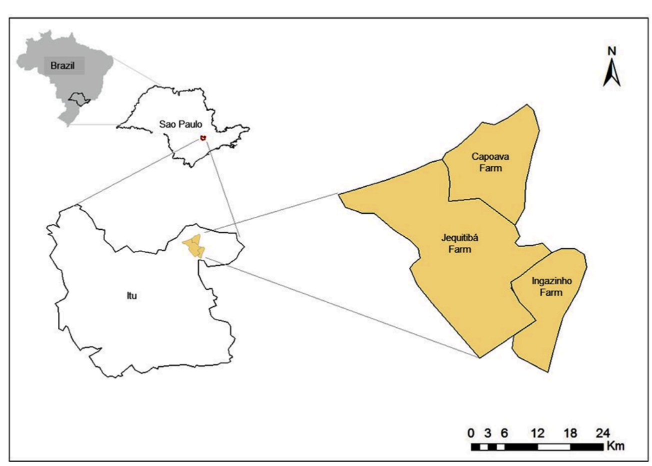


**Fig. 1.** Location of our experimental sites on the farms *Jequitibá (*23° 13′ S 47° 10′ W), *Capoava* (23° 12′ S 47° 10′ W)*,* and *Ingazinho* (23° 13′ S 47° 11′ W) in Itu, São Paulo, Brazil.

*Active restoration (AR):* seedlings of native species were planted in a staggered manner, as in Rodrigues et al. (2009), in two phases, with a 1.5-year gap. In the first phase, short-lived fast-growing species were planted to quickly shade the soil and reduce the need for weed control, creating environmental conditions for other species to establish. Nitrogen-fixing (N-fixing) plants were also planted in this phase, intercropped with native species in alternated rows, to supply nutrients to the plants and help with weed control. In the second phase, a more diverse and functional group of species was introduced, aiming to increasing biodiversity and restoring forest dynamics. Seedling density was approximately 1,666 trees ha$^{-1}$ and a total of 80 species were planted (Fig. 2A).

*Assisted restoration (AsR):* management was made to improve the environmental conditions for forest regeneration, such as weed control and fencing of the area. Fast-growing species were added to occupy the area not colonized yet by natural regeneration. One year later, other native species were added to increase biodiversity. A total of 72 species were added. Density was around 1,111 trees ha$^{-1}$, including the naturally occurring plants and the planted seedlings (Fig. 2B)

*Passive restoration on an abandoned pasture (AP):* Passive restoration was applied, so no human intervention was made, except by fencing the area to prevent cattle access and by fire monitoring. The area was proper for agriculture and used to be a pasture, but it was abandoned. It has low resilience capacity and low biodiversity (Fig. 2C).

*Remaining forest fragment (RF):* this nearby Seasonal Semi-Deciduous forest was used as control. It is a small preserved area, of approximately 30 ha, in an advanced stage of succession and occurrence of key native species and lianas (Fig. 2D).

*Pasture (P):* well-managed pasture formed by African grasses used for extensive livestock production (Fig. 2E).

### 2.3. Sampling

For each treatment, there were five plots (30 × 30 m – 900 m$^2$) randomly located, making a sample area of 0.45 ha total. To avoid edge effects, plots were placed at least 30 m from the limits of the areas subjected to the restoration treatments.

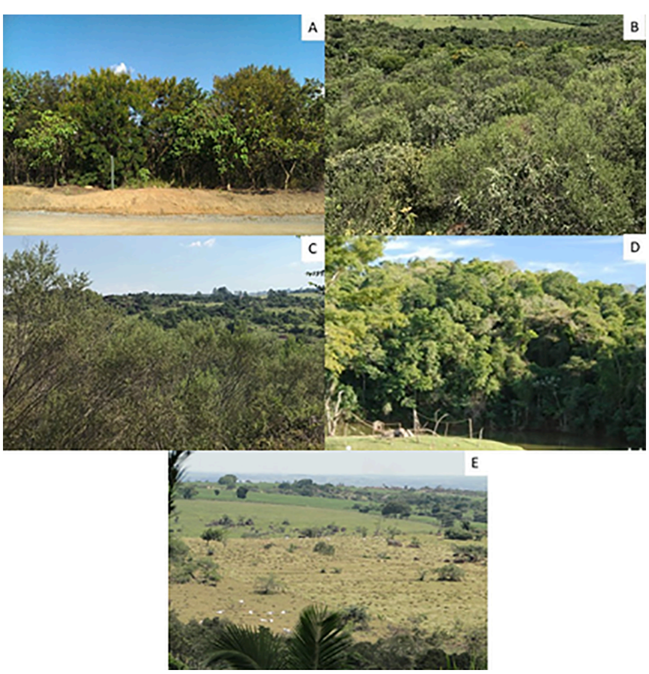


**Fig. 2.** Treatments applied in the area in Itu, Sao Paulo, Brazil. A: AR (Active restoration), B: AsR (Assisted restoration), C: AP (Abandoned pasture) five years after planting, D: RF (Remaining forest fragment), and E: P (Pasture).

#### 2.3.1. Aboveground carbon

*2.3.1.1. Tree.* All living trees within the plots that had at least one stem and diameter at breast height (DBH, 1.3 m) ≥ 5 cm were tagged, measured (for height and diameter), and determined to the most detailed botanical level. DBH was measured using a tape and height (Ht) using a digital hypsometer. In the case of trees with more than one stem meeting the inclusion criteria, all stems were measured. Vegetative material from unknown species was collected for proper identification. Wood density (Wd) was retrieved from Chave et al. (2006), which provides wood density data for 2,456 tropical species. Biomass was determined using the equation from Zanini (2019) (1), who tested many equations for the same area in a previous study. The aboveground carbon content was assumed to be 45.3% (Zanini, 2019). Based on the average plot biomass, it was possible to extrapolate biomass (Mg ha$^{-1}$) and carbon stock (Mg C ha$^{-1}$) to the entire area.

$$Biomass = 29.126 - 4.519DBH + 0.054DBH^2 + 0.569DBH*Ht - 0.005DBH^2*Ht \quad (1)$$

*2.3.1.2. Litter and herbaceous.* In the 900 m$^2$ plot, litter and herbaceous were collected inside a square frame (25 × 25 cm) at five random points. Samples were dried in an oven (40 °C), until weight stabilization. Dry biomass (g) was measured on a scale with precision of two decimal places. The value was upscaled to the plot area and the plot average in each treatment was calculated. Then, samples were ground in a mill and sifted to 0.250 mm. A subsample of 100 mg was sent to the Isotopic Ecology Laboratory at the Center of Nuclear Energy in Agriculture at the University of Sao Paulo (CENA/USP) and an elementary analyzer Carlo Erba model 1110 was used for carbon content (%) determination. After computing carbon content (%) and biomass, these values were multiplied to determine carbon stock (Mg C ha$^{-1}$).

*2.3.1.3. Dead biomass.* Trees standing dead and fallen wood were sampled in the 900-m$^2$ plot. For dead standing trees, all individuals with DBH ≥ 4.8 cm were measured, as in Harmon and Sexton (1996). DBH was measured using a tape and height was measured with a digital hypsometer to all individuals with at least one stem of DBH ≥ 4.8 cm. For fallen dead wood, all individuals of DBH ≥ 2 cm were sampled using the line intersect method for 100 m (Van Wagner, 1968) as in Guzman (2014). To calculate dead biomass, the volume was multiplied by wood density, and for carbon stock determination, dead biomass was multiplied by carbon content (%), both considering its decomposition degree, as in Vieira et al. (2011).

#### 2.3.2. Belowground carbon

*2.3.2.1. Roots.* Fine roots (diameter less than 2 mm) and coarse roots (diameter greater than 2 mm) were sampled using two different methods. For fine roots, two points were randomly sampled in the 900 m$^2$ plot, as for litter, using an auger, at 0–10 cm, 10–20 cm, and 20–30 cm layers, totaling 14 cm$^3$ sampled in each point. Fine roots were handpicked, washed, and dried in an oven (40 °C), until weight stabilization. Dry biomass (g) was measured on a scale using a two-decimal places precision. Next, samples were ground in a mill and sifted to 0.250 mm. A subsample of 100 mg was sent to the Isotopic Ecology laboratory at the Center of Nuclear Energy in Agriculture at the University of Sao Paulo (CENA/USP) for carbon content (%) determination, using an elementary analyzer Carlo Erba model 1110. To calculate fine root biomass, the temporal prediction method (Metcalfe and Williams, 2007) was used, as in Silva (2015). Carbon stock (Mg C ha$^{-1}$) was determined by multiplying fine roots biomass by carbon content (%). For coarse root determination, it would be necessary to open pits through the forest, which would disturb the restoration process. Thus, a model developed by Cairns et al. (1997) to estimate root biomass was used. They made an extensive literature review looking for the most important variable affecting roots to compose their model, which were aboveground biomass density, age, and latitude.

*2.3.2.2. Soil.* For soil carbon, five random spots were sampled in each 900 $m^2$ plot using an auger and three subsamples were taken from the 0–5, 5–10, 10–20, and 20–30 cm depth layers. The subsamples were mixed to compose the sample for each plot. Samples were air-dried, ground, and sifted to 0.250 mm. Next, a 100 mg sample was sent to the Center of Nuclear Energy in Agriculture at the University of Sao Paulo (CENA/USP). Carbon content (%) and the $^{13}C/^{12}C$ isotope ratio were analyzed using an elementary analyzer Carlo Erba model 1110.

Soil density was measured in each plot using a volumetric ring (82.644 $cm^3$). Rings were dried in an oven (105 °C) for 72 h and then had their weight measured using a two-decimal-place scale. Density (g/$cm^3$) was the result of dry weight divided by ring volume (82,644 $cm^3$). Soil carbon stock (Mg C $ha^{-1}$) was obtained from the equation of Veldkamp (1994).

### 2.4. Data analysis

Statistical analysis of soil bulk density, biomass, and carbon stock of the different pools for the different treatments was made in R (R Development Core Team, 2018). Levene's and Shapiro – Wilk tests were applied to validate the requirements for the analysis of variance (ANOVA), homogeneity of variance, and normality of errors. With all requirements satisfied, ANOVA was performed adopting a 5% level of significance, and when differences were detected, the Tukey test was applied with a 95% confidence.

## 3. Results

### 3.1. Carbon stock and soil density

Table 1 shows carbon stock on each pool including soil layers (0–5, 5–10, 10–20 cm) as well as soil density for each treatment. Fig. 3 shows the percentage of total carbon stock (TCS) of each pool for each treatment. Belowground carbon stock was greater than aboveground carbon, and soil was the largest belowground carbon pool, for all restoration methods. In the layer 0–5 cm, carbon stock was greater in RF and P compared to the other treatments. The same trend was observed on the deepest layer (10–20 cm). On the middle layer (5–10 cm), RF, AR, AsR, and AP had the highest carbon stock, and P had the lowest. Coarse roots were a significant carbon stock pool on RF (7.62 Mg $Cha^{-1}$, 5% TCS) due to the presence of old trees. Despite the presence of trees in AR and AsR, they are only five years old, so they did not have time yet to grow trees with a large root system.

The major aboveground carbon stock pool in RF was trees (27.77 Mg $Cha^{-1}$, 17.9% TCS). Litter was the greatest aboveground pool for the other treatments: 5.8% TCS (3.805 Mg $Cha^{-1}$) in AP, 4.9% TCS in AR (3.236 Mg $Cha^{-1}$), 4.3% TCS in AsR (2.719 Mg Cha-1), 3.4% TCS (2.829 Mg $Cha^{-}1$) in P and 9.1% TCS (13.87 Mg Cha-1) in RF. Herbaceous was a significant aboveground pool for AP (3.198 Mg $Cha^{-1}$, 4.9% TCS) and P (2.088 Mg C $ha^{-1}$, 2.5% TCS).

Total carbon stock was greatest on RF (152.304 Mg $Cha^{-1}$), followed by P (84.378 Mg $Cha^{-1}$), AR (66.414 Mg $Cha^{-1}$), AsR (63.030 Mg $Cha^{-1}$) and AP (65.581 Mg $Cha^{-1}$), in this order. Soil density was 1.4 g $cm^{-3}$ in P, AR, AsR and AP, and 0.9 g $cm^{-3}$ in RF.

### 3.2. Carbon content (%)

Carbon content was assessed in all pools and treatments (Table 2). Carbon content in trees was assumed 45.3%, as determined in a previous research by Zanini (2019) studying the same tree community. Carbon content in deadwood was considered 46.05% as in Vieira et al. (2011), who studied an Atlantic Forest fragment in the same state as our study site. The carbon content average in herbaceous was 41.86%, and there was no significant difference between the treatments. Litter carbon content average was 33.65%, greater in RF (38.63%) and AP (38.08%) compared to the other treatments. Fine roots' carbon content average was 32.2%, higher in RF (40.51%) and P (37.38%) compared to the other treatments. Average carbon content decreased with soil depth in all treatments: 3.37%, 2.48%, and 1.93% to the 0–5, 5–10, and 10–20 cm depth layers, respectively, 2.56% on average. Soil carbon content was highest in RF and was higher in P compared to the other treatments, in all soil layers, except the layer 10–20 cm (2.54% in RF × 2.91% in P).

### 3.3. Carbon isotope

Carbon isotope analysis was done down to 20 cm below ground for all treatments. The amount of $\delta^{13}C$ is related to C3-C4 plant proportion in the area and is used to indicate short-term changes (Mosquera et al., 2012a). The higher the $\delta^{13}C$ the greater C4-related carbon in the area (Hobbie and Werner, 2004). As one can see in Fig. 4, $\delta^{13}C$ was lowest on RF (−25.8%) followed by AsR (−19.4%), AR (−17.1%), AP (−15.6%) and P (−14.4%).

The percentage of carbon from C3 and C4 plants in total carbon stocked in the soil in each treatment is shown in Fig. 5. Carbon from C3 plants was 92%, 51%, and 38% of the C stocks of RF, AsR, and AR, respectively. P and AP were the treatments with the greatest amount of herbaceous, so they had the highest proportion of carbon from C4 plants: 83%, and 81%, respectively.

**Table 1**
Carbon stock (Mg C $ha^{-1}$) and soil density (g $cm^{-3}$) for each treatment in each pool ± standard deviation. AR: Active restoration, AsR: Assisted restoration, AP: Abandoned pasture, RF: Remaining forest fragment, P: Pasture. Letters compare values in the row, according to the Tukey test with 95% of confidence.

| | Pools | RF (Mg C $ha^{-1}$) | | AR (Mg $Cha^{-1}$) | | AsR ($MgCha^{1}$) | | AP ($MgCha^{-1}$) | | P ($MgCha^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Aboveground | Tree | 27.277 ± 16.05 | a | 2.656 ± 3.97 | b | 2.331 ± 4.35 | b | 0.279 ± 0.97 | c | 0 ± 0 | c |
| | Herbaceous | 0.111 ± 0.08 | c | 0.433 ± 0.31 | b | 0.621 ± 0.11 | b | 3.198 ± 2.03 | a | 2.088 ± 0.59 | a |
| | Litter | 13.867 ± 7.23 | a | 3.236 ± 1.72 | b | 2.719 ± 1.62 | b | 3.805 ± 2.02 | b | 2.829 ± 0.81 | c |
| | Dead wood | 11.224 ± 2.34 | a | 0.03 ± 0.23 | b | 0.04 ± 0.23 | b | 0 ± 0 | b | 0 ± 0 | b |
| | Fallen dead wood | 2.351 ± 1.83 | a | 0.251 ± 0.41 | b | 0.313 + 0.61 | b | 0 ± 0 | b | 0 ± 0 | b |
| Subtotal | | 54.719 | | 5.892 | | 5.050 | | 7.003 | | 4.917 | |
| Belowground | Fine roots | 4.237 ± 1.35 | a | 0.944 ± 0.553 | b | 0.719 ± 0.67 | b | 2.879 ± 2.06 | a | 3.543 ± 2.43 | a |
| | Coarse roots | 7.624 ± 11.27 | a | 0.780 ± 1.05 | b | 0.679 ± 1.14 | b | 0.091 ± 0.28 | c | 0 ± 0 | c |
| | 0–5 cm soil | 37.081 ± 12.01 | a | 19.005 ± 3.59 | b | 16.215 ± 2.847 | b | 18.903 ± 4.78 | b | 21.762 ± 11.39 | a |
| | 5–10 cm soil | 25.771 ± 6.84 | a | 25.062 ± 5.89 | a | 24.743 ± 4.84 | a | 23.418 ± 5.44 | a | 20.484 ± 9.05 | b |
| | 10–20 cm soil | 22.761 ± 7.55 | a | 14.017 ± 2.79 | b | 14.65 ± 2.36 | b | 13.008 ± 3.45 | b | 33.672 ± 7.81 | a |
| Subtotal | | 97.474 | | 58.084 | | 40.958 | | 58.208 | | 79.461 | |
| | Total ($MgCha^{-1}$) | **152.304** | **a** | **66.414** | **b** | **65.730** | **b** | **65.581** | **b** | **84.378** | **b** |
| | Soil density ($gcm^{-3}$) | 1.01 ± 0.11 | b | 1.44 ± 0.12 | a | 1.40 ± 0.11 | a | 1.39 ± 0.25 | a | 1.39 ± 0.04 | a |

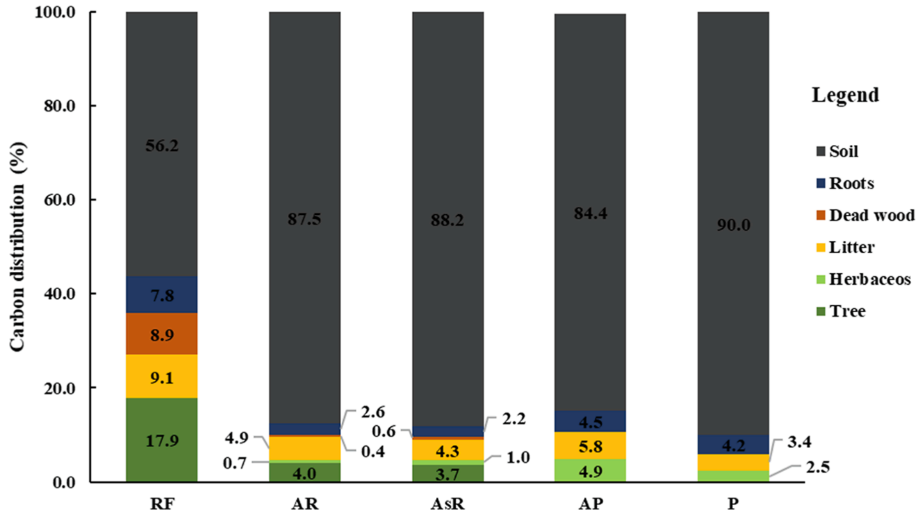


**Fig. 3.** Proportion of each pool in the total carbon stock of the different treatments. AR: Active restoration, AsR: Assisted restoration, AP: Abandoned pasture, RF: Remaining forest fragment, CP: Pasture in Itu, São Paulo, Brazil.

**Table 2**
Carbon content (%) ± standard deviation of different pools in each treatment. AR: Active restoration; AsR: Assisted restoration; AP: Abandoned pasture, RF: Remaining forest fragment, P: Pasture, 0–5 cm, 5–10 cm, 10–20 cm depth soil layers. Letters compare values in the row, according to the Tukey test at 95% confidence.

| Pools | RF | | AR | | AsR | | AP | | P | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Herbaceous | 41.76 ± 0.1 | a | 42.11 ± 0.63 | a | 42.22 ± 0.76 | a | 42.00 ± 0.63 | a | 41.01 ± 1.28 | a | **41.86 ± 0.84** |
| Litter | 38.63 ± 2.93 | a | 29.65 ± 9.88 | b | 31.12 ± 10.5 | b | 38.08 ± 5.76 | a | 32.47 ± 4.71 | b | **33.65 ± 8.71** |
| Fine roots | 40.51 ± 4.05 | a | 33.41 ± 10.2 | b | 27.18 ± 7.96 | b | 29.92 ± 7.44 | b | 37.38 ± 5.14 | a | **32.22 ± 8.95** |
| 0–5 cm | 7.31 ± 2.42 | a | 2.51 ± 0.51 | b | 2.32 ± 0.43 | b | 3.01 ± 0.52 | b | 5.22 ± 1.71 | a | **3.37 ± 1.84** |
| 5–10 cm | 4.48 ± 1.44 | a | 1.94 ± 0.37 | b | 2.10 ± 0.38 | b | 2.15 ± 0.34 | b | 3.83 ± 1.18 | a | **2.48 ± 1.07** |
| 10–20 cm | 2.54 ± 0.70 | a | 1.74 ± 0.39 | b | 1.78 ± 0.41 | b | 1.68 ± 0.27 | b | 2.91 ± 0.69 | a | **1.93 ± 0.59** |

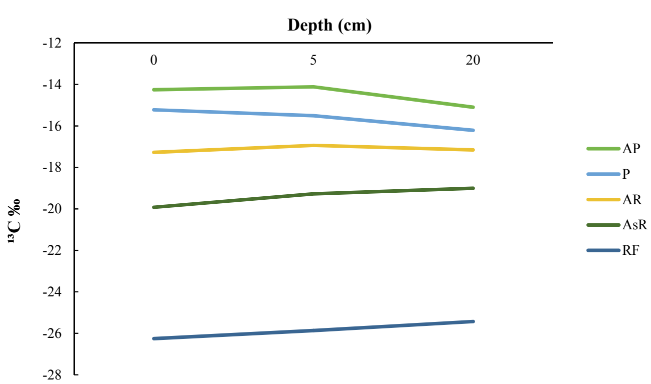


**Fig. 4.** Average $\delta^{13}C$ in the soil amongst the treatments at different soil depths (0–20 cm). AR: Active restoration, AsR: Assisted restoration, AP: Abandoned pasture, RF: Remaining forest fragment, P: Pasture.

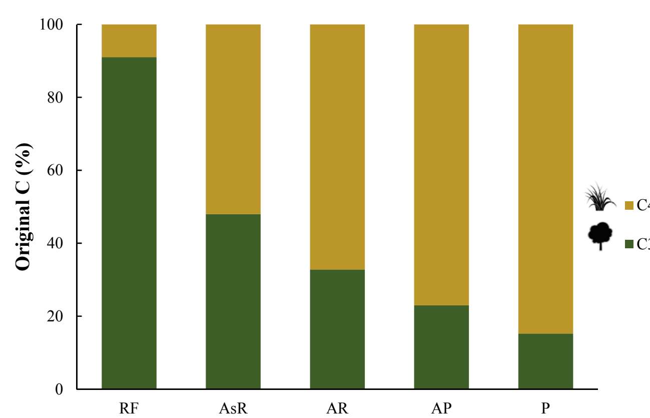


**Fig. 5.** Proportion of carbon from C3 and C4 plants in soil (0–20 cm) under each treatment. AR: Active restoration, AsR: Assisted restoration, AP: Abandoned pasture, RF: Remaining forest fragment, CP: Pasture.

## 4. Discussion

### *4.1. The reference site - RF*

Reference forest must be evaluated since it is nearby forest fragments working as a potential source of propagules for degraded areas, reducing isolation effect and also indicating potential carbon stock for the restoration sites (Ferez et al., 2015; Magnago et al., 2015; Robinson, 2015; Azevedo et al., 2018; Matos et al., 2020; Rosenfield and Müller, 2019; Gardon et al., 2020). Thus, a reference forest was used to evaluate the effective recovery of the carbon stock by implementing the restoration methods. Other research also used neighbor forest fragments to assess the progress of the restoration areas. In general, even though these forest fragments are inserted in the same biome, their carbon stocks diverge.

Carbon stock on RF was 152.304 $MgCha^{-1}$. RF is a small fragment (30 ha), containing a lot of lianas, which can cause mortality of the host trees (Ingwell et al., 2010), or at least reduce growth rate and slow regeneration within canopy gaps (Schnitzer, 2002), indicating degradation (Nogueira et al., 2011; Viani et al., 2015; D'Albertas et al., 2018). Thus, we can infer that the fragment did not reach maturity yet, and if it was larger, the edge effect would be less pronounced, so carbon stock would be greater, as evidenced by other studies (Paula et al., 2011; Pütz et al., 2014; Magnago et al., 2015; Scarano, 2015; Toledo et al., 2018; D'albertas et al., 2018; Matos et al., 2020). For example, Ferez et al., (2015) found 181.5 Mg C $ha^{-1}$ in a 1,400 ha forest fragment and Matos et al. (2020) found in average 369.25 $MgCha^{-1}$ in Atlantic Forest fragments of sizes up to 23,480 ha. Size is not the only factor affecting carbon stock, but it is an important one since it determines to which extent the edge effect affects the fragment (Paula et al., 2011; Pütz et al., 2014; Scarano, 2015; Matos et al., 2020). Besides size, building corridors linking the fragments promotes the increase of carbon stock, richness, abundance and reduces extinction risk for many species (Paula et al., 2011; Magnago et al., 2015; Rezende et al., 2018; Matos et al., 2020; Safar et al., 2020).

### 4.2. Belowground carbon stock

Soil, fine, and coarse root pools composed belowground carbon stock. Belowground carbon stock was larger than aboveground carbon stock in all treatments, which is in agreement with several studies done in tropical forests (Vieira et al., 2011; Ferez et al., 2015; Jones et al., 2019). For example, Vieira et al. (2011) also found higher belowground carbon stock than aboveground carbon stock, studying the Atlantic Forest along a 1000-m altitude gradient, for all elevations. Ferez et al. (2015) and Jones et al. (2019), studying an Atlantic Forest fragment in Sao Paulo, Brazil, and a tropical forest restoration area in Panama, respectively, also found larger belowground carbon stock than aboveground carbon stock. It raises attention to the fact that in most studies, the belowground pool is not considered (Jones et al., 2019), for two main reasons. First, because sampling is time- and resource-demanding, and second because its significance for total carbon stock is mistakenly underestimated. However, precise carbon sequestration estimates considering all pools are important to better understand secondary forests' growth, carbon sequestration rates, and the total ecosystem carbon stock (Jones et al., 2019; Osuri et al., 2019) so carbon stock information can be more reliable for global warming mitigation plans.

RF had the largest belowground carbon pool (97.474 Mg C $ha^{-1}$), showing the importance of forest cover to increase the soil carbon sink. P had the next largest belowground carbon pool (79.461 Mg C $ha^{-1}$). Indeed, pastures can sequester significant amounts of carbon in the soil (Brown and Lugo, 1990; Segnini, 2005). For example, Szakács (2011), found 54.4 Mg C $ha^{-1}$ in a pasture in SP, Brazil in the 0–50 cm layer. Assad et al., (2013) studied more than 100 pastureland soils in Brazil, and found soil carbon stocks between 20 and 100 Mg C $ha^{-1}$. Normally, soils lose carbon when forests are converted to pastures (Fearnside and Barbosa, 1998; Guo and Gifford, 2002; Stefano and Jacobson, 2017) and after that, they can behave either as a C sink or a source, depending on management (Fearnside and Barbosa, 1998; Desjardins et al., 2004; Mosquera et al, 2012a). In the Amazon, for example, the 0–20 cm soil layer lost 4.9 Mg C $ha^{-1}$ in the 15 years following deforestation (Fearnside and Barbosa, 1998). Even though P had a large carbon stock, restoring a pasture to a forest through passive restoration may be problematic, mainly because it hinders seedlings establishment due to competition with grasses, as shown by some studies (Steininger, 2000; Sansevero et al., 2017; César et al., 2018), which makes P potential carbon sequestration low. Matos et al (2018) compared carbon stocks of pasture and forest under passive restoration in the Atlantic Forest and found that 30 years after implementation, the restoration site had recovered around 20% of the original carbon stock, while the pasture had recovered only 3%.

Total belowground carbon did not differ among AR (58.084 Mg C $ha^{-1}$), AsR (40.958 Mg C $ha^{-1}$), and AP (58.208 Mg C $ha^{-1}$), which can be explained by the young age of the project. Several studies have shown that belowground carbon stock is reestablished slowly, taking many years to show significant change (Macedo et al., 2008; Nogueira et al., 2011; Cunningham et al., 2015; Jones et al., 2019). For example, Jones et al. (2016), studying a restoration site in a tropical forest in Panama, found that belowground carbon stock took 40 years to be reestablished. Macedo et al., (2008) and Nogueira et al. (2011) also did not find significant differences in belowground carbon amongst the different methods applied to restore an Atlantic Forest area, 13 and 10 years after implementation, respectively. Even in a Free-Air Carbon dioxide Enrichment (FACE) experiment, which increased the surrounding atmospheric carbon concentration by 25%, carbon increase in the soil was detectable only 6 to 10 years after the beginning of the experiment (Smith, 2004).

On the 0–5 cm layer, RF (37.081 Mg C $ha^{-1}$) and P (21.762 Mg C $ha^{-1}$) had the largest carbon stocks, followed by AR (19.005 Mg C $ha^{-1}$), AP (18.903 Mg C $ha^{-1}$) and AsR (16.215 Mg C $ha^{-1}$). Superficial soils are more affected by vegetation cover (Don, 2011). They had more roots from trees and grasses and probably had less carbon mineralization due to lower temperature as a result of vegetation cover (Bernoux et al., 2002; Marín-Spiotta and Sharma, 2013; Luo et al., 2020). In the next layer, 5–10 cm depth, RF (25.771 Mg C $ha^{-1}$), AR (25.062 Mg C $ha^{-1}$), AsR (24.743 Mg C $ha^{-1}$), and AP (23.418 Mg C $ha^{-1}$) showed the highest values, followed by P (20.484 Mg C $ha^{-1}$). In the deepest layer, 10–20 cm, P (33,672 Mg C $ha^{-1}$) had the highest value, followed by RF (22.761 Mg C $ha^{-1}$), then AR (14.017 Mg C $ha^{-1}$), AsR (14.65 Mg C $ha^{-1}$) and AP (13.008 Mg C $ha^{-1}$).

Besides soil, carbon present in coarse and fine roots is a significant share of the belowground carbon stock. Carbon stock of coarse roots was higher in RF (10.62 Mg C $ha^{-1}$), followed by AR (3.63 Mg C $ha^{-1}$) and AsR (2.7 Mg C $ha^{-1}$), followed by AP (0.12 Mg C $ha^{-1}$) and P (0 Mg C $ha^{-1}$), in this order. It shows that planting trees in the area was effective to increase carbon sequestration in the coarse roots pool. Another fact to add is the presence of N-fixing plants in AR, which probably affected positively the roots pool (Nadelhoffer, 2000; Zhu et al., 2013). Carbon stock in fine roots was greater in RF (3.03 Mg C $ha^{-1}$), AP (2.88 Mg C $ha^{-1}$), and P (4.32 Mg C $ha^{-1}$), followed by AR (1.01 Mg C $ha^{-1}$) and AsR (0.8 Mg C $ha^{-1}$). It can be explained by the fact that RF presented fine roots from trees; P and AP did not have trees but were dominated by grasses, so their fine roots compensated for the absence of fine roots from trees. Fine roots can be an ally for soil carbon stock, since they can easily grow deep in the soil, and increase carbon stock in deeper layers, which enforce carbon sequestration by hampering carbon release to the atmosphere (Mosquera et al., 2012b). A soil rich in roots indicates a good soil structure, which is described by the soil bulk density (Li and Shao, 2006), better discussed in Section 4.5.

### 4.3. Aboveground carbon stock

In this study, we assessed aboveground carbon stock divided into litter, herbaceous, trees, standing deadwood, and fallen dead wood pools. However, it was found by Gardon et al. (2020), reviewing forest-restoration studies in Brazil, that only 41% of the studies assessed other aboveground component rather than the tree pool. Aboveground carbon stock was higher on RF (54.719 Mg C $ha^{-1}$), followed by AP (7.003 Mg C $ha^{-1}$), AR (5.892 Mg C $ha^{-1}$), AsR (5.050 Mg C $ha^{-1}$), and P (4.917 Mg C $ha^{-1}$). In AP and CP, aboveground carbon stock was composed of herbaceous (3.198 and 2.088 Mg C $ha^{-1}$, respectively) and litter (3.805 and 2.829 Mg C $ha^{-1,}$ respectively) pools, which is not promising for the forest restoration progress and its future carbon stock. Abundant herbaceous cover can stagnate forest succession by impeding the establishment of propagules from other fragments, preventing seed germination and competing with seedlings, slowing their growth and even displacing native species (Campoe et al., 2014, 2010; Ferez et al., 2015), as evidenced by Ferez et al. (2015), Sansevero et al. (2017), and César et al. (2018).

On the other hand, AR and AsR showed relatively small aboveground carbon stock, but most of it was in the arboreal pool, which shows that the area is succeeding towards forest establishment. Other studies comparing the active and passive restoration methods in the Atlantic Forest also reached to similar conclusions. For example, César et al. (2018) studied seven to 20-year old Atlantic Forest restoration sites and found that the aboveground carbon stock in passive sites was 45% smaller than that of AR sites (91.3 Mg $ha^{-1}$ × 132.2 Mg $ha^{-1}$). Ferreira et al. (2015) found aboveground carbon stocks about three times larger in areas restored by the active restoration compared to the passive in an Atlantic Forest in Natal, RN. In accordance, Sansevero et al. (2017) studying the effect of passive restoration in Atlantic Forest sites with different past land-use also found that the active method may be preferable over the passive to speed up the regeneration process, even in resilient sites. The active restoration was the most studied method used to restore Atlantic Forest areas, present in more than half of the studies (Gardon et al., 2020), and that may be due to the low resilience in the area and the urgent need to recover it.

Thus, an important indicator that a restoration area is successfully becoming a mature forest is the increase of its carbon stock in the tree pool. Carbon stock on trees was significantly higher in RF (27.27 Mg C ha$^{-1}$, 17.9% TCS), followed by AR (2.65 Mg C ha$^{-1}$, 4% TCS) and AsR (2.33 Mg C ha$^{-1}$, 3.7% TCS), then followed by AP (0.28 Mg C ha$^{-1}$, 0.4% TCS) and P (0 Mg C ha-1, 0% TCS). It may indicate that planting trees was effective to increase carbon sequestration on the tree pool. Since a great part of this pool is composed of wood, carbon release through decomposition is slow, which makes it a key pool to increase local carbon stock sink capacity. Besides planting native seedlings, introducing N-fixing plants in the area was likely a positive factor for AR as well, as discussed for the roots pool. The positive effect of N-fixing plants was also observed by other researchers (Macedo et al., 2008; Nogueira et al., 2011). For example, Nogueira et al., (2011) and Macedo et al., (2008) also used leguminous trees to restore degraded Atlantic Forest areas and after 10 and 13 years, respectively, they found that it was useful to recover the areas, helping to reestablish the nutrient cycle. This shows that planting N-fixing species along with the practice of weed control are effective strategies to be used in restoration areas. Some studies have also shown that the mineral fertilization is effective to restore Atlantic Forest areas, as good as it is for the growth of commercial plantations, as claimed by Davidson et al. (2004), Campoe et al., (2010), Campoe et al., (2014), Ferez et al., (2015) and others. For example, Ferez et al., (2015) showed that 6 years after planting, the intensively-managed area (fertilization and weed control) stocked a 3-fold more carbon in trees than did the control (23.3 × 6.9 Mg C ha$^{-1}$), in the Tiete basin, SP, Brazil.

The other aboveground carbon pool is litter. Even though it is an essential part of the nutrient cycling process, since plants rely on its decomposition to access nutrients (Trumbore et al., 1996; Macedo et al., 2008; Vendrami et al., 2012), only 23% of the Brazilian forest-restoration related studies assessed this pool (Gardon et al., 2020). Litter stock is affected by environmental factors such as presence of trees, tree density, species richness, age (Carpanezzi, 1997), precipitation, temperature, soil moisture, soil organisms, degree of disturbance, and the degradation level of the area (Martins and Rodrigues, 1999), decomposition rate and growth rate (Machado et al., 2008). The litter carbon stock pool was larger in RF (13.87 Mg C ha$^{-1}$, 9.1% TCS), followed by AP (3.805 Mg C ha$^{-1}$, 5.8% TCS), AR (3.23 Mg C ha$^{-1}$ 4.6% TCS), P (2.829 Mg C ha$^{-1}$, 3.4% TCS), and AsR (2.719 Mg C ha$^{-1}$, 4.3% TCS), in this order. P and AP were dominated by grasses, so it was expected that they would not produce an abundant litter. AR and AsR did not produce litter abundantly as well. This can be related to the fact that trees in this phase may more likely use their resources to grow, rather than to drop material as litter. We do not know yet how litter production is related to age, restoration method, and the available resources in the area. Azevedo et al. (2018) found that litter production in Atlantic Forest restoration areas was not correlated to age: 3, 5, and 7 years after planting representing, litter proportions were 13%, 16%, and 11%, of TCS, respectively. Azevedo et al. (2018) also found that 5 years after the implementation of the restoration process, litter production was 2% of TCS on RF, while in this study litter production in RF was 9.1% of TCS, what could mean that our RF had more resources at the time to create conditions for the trees to grow more and produce more litter than Azevedo's RF area. However, more studies are necessary to confirm that.

Regarding the herbaceous pool, P (2.088, TCS 2.5%) and AP (3.198, TCS4.9%) had the largest stocks, followed by AsR (0.621, 0.1% TCS), AR (0.433, 0.7%TCS) and RF (0.111, 1%TCS). Deadwood and fallen dead wood were higher in RF (13.575 Mg Cha-1, 8.9% TCS) compared to the other restoration methods (AR = 0.281 Mg C ha-1, 0.4% TCS; AsR = 0.353 Mg C ha$^{-1}$, 0.6% TCS, AP = 0 and P = 0), because RF produced much more woody material than the restoration methods or P.

### 4.4. Total carbon stock

Carbon stock (Rodrigues et al., 2009; Shimamoto, 2014; Matos et al., 2020; Gardon et al., 2020; Safar et al., 2020), and the time elapsed between disturbance and the beginning of the restoration process (Crouzeilles et al., 2016) are the main drivers of the reestablishment of forest dynamics and ecosystem functions. Total carbon stock was significantly greater in RF (152.304 Mg C ha$^{-1}$) in comparison to the other treatments (AR: 66.414 Mg C ha$^{-1}$, AsR: 65.730 Mg C ha$^{-1}$, AP: 65.581 Mg C ha$^{-1}$, P: 84.378 Mg C ha$^{-1}$). There was no statistical difference amongst the restoration methods. Studies show that Atlantic Forest restoration areas need time for growth changes to be detected (Poorter et al., 2016; Azevedo et al., 2018; Jones et al., 2019; Matos et al., 2020; Safar et al., 2020). Gardon et al. (2020) found that most Brazilian forest restoration areas started to show significant differences between the passive and the active methods after the 5th to the 10th year after implementation. If differences between the methods start to be noticeable after 5 to 10 years, it can take much longer for the total carbon stock to be restored. Poorter et al., (2016) studied 45 tropical forests and found that they took 66 years to recover 90% of the original aboveground carbon, and Jones et al., (2019) also studied a tropical forest, in Panama, and estimated that it took about 120 years for the secondary forest to reach the original carbon stock. Safar et al., (2020) and Matos et al. (2020) studied different restoration areas in ES found that the fragments would need no less than 80 and 30 years to have 100% and 20% of their total carbon stock restored, respectively.

Even though there was no significant difference in total carbon between the methods, it is already possible to see a trend: total carbon rises with management intensity (AR: 66.414 Mg C ha$^{-1}$ > AsR: 65.730 Mg C ha$^{-1}$ > AP: 65.581 Mg C ha$^{-1}$). This is in accordance with several studies showing that AR was the most appropriate method for the Atlantic Forest (Ferreira et al., 2015; César et al., 2018; Sansevero et al., 2017; Osuri et al., 2019; Poorter et al., 2016) because it minimizes the unfavorable biotic and or abiotic conditions, such as the presence of invasive weeds, lack of seed bank and seed dispersal, compensating the absence of a healthy neighboring fragment (Macedo et al., 2008; Campoe et al., 2010; Nogueira et al., 2011; Campoe et al., 2014; Osuri et al., 2019). In agreement, Osuri et al., (2019) assessed the active and passive restoration methods used in a tropical forest in India and concluded that the active method significantly contributed to the restoration of the area. Thus, planting trees is a way to speed up the restoration process and it is especially important for those areas that do not have a close forest fragment to provide propagules (Sansevero et al., 2017; Matos et al., 2020; Osuri et al., 2019), as it is, in general, the case for most of the Atlantic Forest due to fragmentation (Paula et al., 2011; Magnago et al., 2015; Rezende et al., 2018; Matos et al., 2020; Safar et al., 2020) and low resilience (Tambosi et al., 2014; Poorter et al., 2016; MMA, 2017).

The passive restoration method used in AP is a cheaper restoration method useful when the area can return easily to the original state. In our ap area, the major carbon stocks were in the soil (55.329 Mg Cha$^{-1}$, 84.4% TCS), litter (3.805 Mg C ha$^{-1}$, 5.8% TCS), and herbaceous (3.198 Mg C ha$^{-1}$, 4.9% TCS) pools. However, as previously discussed, AP's potential carbon stock is lower than in AR and AsR. Soil carbon stock takes many years, even centuries to change substantially (Don, 2011); litter is linked to the vegetation cover in the area; and herbaceous do not have the potential to increase carbon stocks as trees do, showing that if the area is not supplied with seeds from a fragment or does not have seedlings planted, its restoration potential is very limited. In this context, even though AP's total carbon stock average is similar to AR and AsR, it shows low potential to expand its stock, given the main pools where this carbon is found. It emphasizes the need to choose the most suitable restoration method for each situation. Correspondingly, Safar et al. (2020) restoring a degraded Atlantic Forest area in ES, Brazil, found that forest structure and tree composition would not be fully restored using natural regeneration, and stressed the need for the AR when trees (seeds or seedlings) are not naturally supplied by the surroundings.

P was similar to AP, in the sense that it presented high total carbon stock, mostly composed of the belowground pool (79.461 Mg C ha$^{-1}$, 90% TCS), which has low potential to increase over time. Indeed, as previously discussed, pastures can have high soil carbon stock, because of their soil's low temperature and high moisture created by a thick homogeneous grass cover, which decreases carbon mineralization (Bernoux et al., 2002; Marín-Spiotta and Sharma, 2013; Luo et al., 2020). Also, P's age is unknown, meaning that the soil has not been revolved recently for (grass) planting, as happened in AR and AsR, which had its soils revolved for tree planting. Thus, it is very likely that carbon was released from the soil during the implementation of AR and AsR, 5 years ago, what may have been a significant amount (Dhillon and Van Rees, 2017). In the temperate zone, it was estimated that it takes around 20 years for trees to offset carbon released by the soil revolved during planting (Dhillon and Van Rees, 2017).

### 4.5. Bulk density

Bulk density is used to assess soil structure and its degree of degradation (Li and Shao, 2006). Li and Shao (2006) studied the effects of the natural vegetation on areas under restoration in China and found that density was an important factor predicting several soil physical proprieties, such as total porosity, capillary porosity, gravimetric water content at the 0.03 and 1.5 MPa tensions, macro-aggregate contents at 41 mm depth, soil aggregate stability, and saturated hydraulic conductivity. In this study, as well in Liu and Shao's, density was significantly affected by vegetation cover. It was lower on RF (1.01 g cm$^{-3}$) compared to the other treatments (1.4 g cm$^{-3}$). As restoration was recently implemented, soil density was not affected by the restoration activities yet, which normally takes many years to happen (Fearnside and Barbosa, 1998; Desjardins et al., 2004; Nogueira et al., 2011). Nogueira et al. (2011) found that soil density decreased with increasing forest cover from passive restoration to the forest fragment, changing from low to high plant density (1.01–1.35 g cm$^{-1}$) 10 years after forest restoration was implemented, in an Atlantic Forest area. If implementing a forest can decrease soil density, the other way around is also true: Desjardins et al., (2004) found that soil density in a forest and pasture area in the Amazon region were 1.31 and 1.56 (g cm$^{-3}$), respectively, 15 years after pasture implementation. This makes clear the importance of vegetation cover for the health of the soil. Even though the restoration methods did not cause detectable differences in soil density yet, this happened for the C3/C4 proportion already, as explained ahead.

### 4.6. C3/C4

Isotope analyses allowed a good assessment of the effect of restoration treatments in the studied site. Low $\delta^{13}$C indicates that the main source of carbon in the soil pool was C3 plants (trees), and one can infer that a given area is evolving into a forest. High $\delta^{13}$C, on the other hand, indicates that the main source of carbon is C4 plants (grasses), suggesting that the area is still at an early stage of the restoration process (Troughton and Card, 1975; Desjardins et al., 2004; Auerswald et al., 2009). Several factors affect the C3/C4 ratio, such as temperature (−), fertility (−), CO2 concentration (+), and precipitation (−) (Auerswald et al., 2009; Von Fischer, 2008). However, in areas under restoration, such as the area we studied, the main factor affecting this ratio is the presence of trees.

In this study, RF was the treatment with the lowest $\delta^{13}$C (−26‰), indicating that carbon came predominantly from C3 plants (92%). AR (−17‰) and AsR (−19‰) also had carbon stocks mainly originated from C3 plants (38% and 51%) in comparison with the AP (−15‰) and P (−14‰) treatments (19% and 17%, respectively). This indicates that planting trees in AR and AsR quickly affected carbon dynamics in the soil in comparison to P and AR.

### 4.7. Carbon content (%)

Carbon content in trees was retrieved from Zanini (2019), who studied the same plant community and found no difference in arboreal carbon pool or amongst tree species, averaging 45.3%. A similar value (46.35%) was pointed out by Ma et al., (2018), who reviewed carbon content (%) from 4.318 tropical species (45% in reproductive organs, 47.9% in stems, 46.9% in foliage, and 45.6% in roots). Considering all the carbon pools, there was no difference in carbon content between treatments for in herbaceous, but there were differences in the litter, fine roots, and soil pools. In all cases, carbon content was highest in RF and higher in P compared to the other treatments. It can be related to the fact that RF and P are older and well-established areas, while AR, AsR, and AP are still developing. Also, it may be related to the higher volume of woody material in RF than in the other treatments given its higher proportion of carbon in the tree pool (Ma et al., 2018). Fonseca et al. (2011) also related the carbon content to age and the presence of lignin, studying a humid forest in Costa Rica.

Information on carbon content is missing for many forest areas, and this is especially true for tropical forests in Latin America (Fonseca et al., 2011; Gardon et al., 2020). The importance of precise carbon content for carbon stock estimates is frequently underestimated. Only 25% of the Brazilian forest-restoration related studies measured it (Gardon et al., 2020). In general, studies have considered carbon content from 45% (Gardon et al., 2020) to 47% (IPCC, 2007) to 50% (Fonseca et al., 2011; Li and Shao, 2006; Gardon et al., 2020). However, this value can diverge much more than that: studies on tropical forests have pointed that carbon content varied from 37.3% (Fonseca et al., 2011) to 51.6% (Thomas and Martin, 2012). Unfortunately, due to the use of inaccurate carbon content values, carbon stock estimates can be seriously biased. For example, by varying carbon content by 1% on the standard value (50%), it yields a difference of 7 Pg C on the global carbon stock (Jones and O'Hara, 2016). Biased carbon stock estimates, even on a local basis, can seriously distort the carbon sequestration mitigation plans, which are crucial to avoid the disastrous climate change outcomes.

### 4.8. Implications and future directions

Besides deforestation and fragmentation, climate change also threatens the Atlantic Forest (Scarano, 2015). As a global pattern, forest mortality is rising while trees are growing shorter, reducing their carbon stocking capacity, despite $CO_2$ fertilization (McDowell et al., 2020). Also, the increasing average temperature is increasing mineralization of labile carbon (Vieira et al., 2011; Marín-Spiotta and Sharma, 2013). To offset it, it is necessary to restore forest areas, since trees shade the soil, which reduces temperature, and consequently the rate of mineralization and carbon release from the soil to the atmosphere (Marín-Spiotta and Sharma, 2013; Azevedo et al., 2018). Besides keeping carbon in the soil, restoring areas locks a great amount of carbon within trees, litter, and herbaceous, since secondary forests have a high growth-rate and sequester carbon faster (Poorter et al., 2016; Osuri et al., 2019), which is especially true for tropical forests, such as the Atlantic Forest. Besides growth-rate, the Atlantic Forest restoration potential lies in the huge amount of areas to be reforested: if land-owners restore their lands to comply with the Brazilian legislation, the Atlantic Forest could have 35% of its original area recovered (Rezende et al., 2018). It means a 9% increase in comparison to what we have today (28%), achievable just by getting landowners to follow the legislation (Rezende et al., 2018).

To be able to properly restore those lands and count on them for global warming mitigation plans, the effect of the different restoration methods on secondary forest growth needs to be fully understood (Jones et al., 2019; Osuri et al., 2019). However, studies comparing the methods and properly assessing them in all pools are scarce, as was pointed by many researchers (Fonseca et al., 2011; Poorter et al., 2016; Jones et al., 2019; Lyu et al., 2019; Osuri et al., 2019; Luo et al., 2020; Gardon et al., 2020), which makes forest restoration a much more complex science than it already is. To the best of our knowledge, it was the first time that an Atlantic Forest restoration area had all its carbon stock pools assessed to compare the active, assisted, and passive restoration methods.

The most suitable restoration method varies from case to case, depending on environmental conditions, local and landscape resilience, goals, time, and resources available. It is crucial to study the methods and know better how they affect all carbon pools under the influence of these different factors since these pools diverge regarding the residence period of carbon, which affects global carbon stock estimates. For example, trees contain more lignin than grasses, so carbon stock is more stable on trees, and stays for longer locked within the ecosystem (Novaes et al., 2010; Cunningham et al., 2015). Also, organic matter with higher C:N ratios in the woody pools slows down decomposition rate and increases carbon stability in the pool as well, in comparison to carbon from non-woody materials (Desjardins et al., 2004; Novaes et al., 2010; Cunningham et al., 2015). This was shown by Mosquera et al. (2012a), who found that that, 30 years after a pasture implementation in the Amazon, more than 50% of the carbon in the topsoil was still originated from the previous vegetation cover; and Mosquera et al. (2012b), who found that 40 years after forest clearing, roughly 80% of the organic carbon was still forest-derived.

It is not known yet if the forests can be restored to exactly what they were before the disturbance. However, some researchers have reached conclusions that bring hope (Crouzeilles et al., 2016; Matos et al., 2018; Safar et al., 2020). It was pointed out by a global-wide meta-analysis encompassing 221 studies that restoration enhances biodiversity by 15–84% and vegetation structure by 36–77% in comparison to degraded areas (Crouzeilles et al., 2016). Matos et al. (2018) found that 30 years after implementation, an Atlantic Forest being restored had 76%, 84%, and 96% of its taxonomic, phylogenetic, and functional diversity reestablished. Similarly, Safar et al (2020) studied the resilience of restoration areas in ES, Brazil, and found that the area could still have its richness and carbon stock recovered within a landscape that had only 5.53% of its forest cover. They also found that it would need 80 years to be fully restored, which is a short time considering natural processes. This shows that Atlantic Forest areas can still be recovered using the most suitable methods for each case. Also, it is crucial to protect this entire ecosystem by building corridors to link fragments and stopping deforestation (Paula et al., 2011; Magnago et al., 2015; Matos et al., 2020; Crouzeilles et al., 2016; Rezende et al., 2018; Safar et al., 2020), otherwise many endemic and threatened species may not be recovered (Crouzeilles et al., 2016; Matos et al., 2018; Safar et al., 2020).

## 5. Conclusion

All the restoration methods evaluated, AR, AsR, and AP, have the potential to restore degraded areas in the Atlantic Forest. However, the most suitable restoration method varies from case to case, considering local and landscape resilience, time, and available resources. Total carbon stock was not significantly different amongst the restoration methods yet, as the sites are too young. However, carbon stock and carbon content differed in the pools according to the restoration method used, and in all cases the largest carbon pool was soil. This shows the importance of sampling every pool for unbiased carbon stock estimates. The source of soil carbon, C3 or C4 plants, also differed among the restoration methods, as shown by the isotope analysis. Thus, even though the restoration methods did not result in different total carbon stock yet, the predominant source of carbon in the soils already differed, which will potentially be amplified in the future. We conclude that all restoration methods tested are already effectively providing environmental services including carbon sequestration, which is essential to mitigate climate change.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2020.118734.

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