



## Research article



# Linking above and belowground carbon sequestration, soil organic matter properties, and soil health in Brazilian Atlantic Forest restoration

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## ABSTRACT

Forest restoration mitigates climate change by removing CO<sub>2</sub> and storing C in terrestrial ecosystems. However, incomplete information on C storage in restored tropical forests often fails to capture the ecosystem's holistic C dynamics. This study provides an integrated assessment of C storage in above to belowground subsystems, its consequences for greenhouse gas (GHG) fluxes, and the quantity, quality, and origin of soil organic matter (SOM) in restored Atlantic forests in Brazil. Relations between SOM properties and soil health indicators were also explored. We examined two restorations using tree planting ('active restoration'): an 8-year-old forest with green manure and native trees planted in two rounds, and a 15-year-old forest with native-planted trees in one round without green manure. Restorations were compared to reformed pasture and primary forest sites. We measured C storage in soil layers (0–10, 10–20, and 20–30 cm), litter, and plants. GHG emissions were assessed using CH<sub>4</sub> and CO<sub>2</sub> fluxes. SOM quantity was evaluated using C and N, quality using humification index (H<sub>LIFS</sub>), and origin using  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . Nine soil health indicators were interrelated with SOM attributes. The primary forest presented the highest C stocks (107.7 Mg C ha<sup>-1</sup>), followed by 15- and 8-year-old restorations and pasture with 69.8, 55.5, and 41.8 Mg C ha<sup>-1</sup>, respectively. Soil C stocks from restorations and pasture were 20% lower than primary forest. However, 8- and 15-year-old restorations stored 12.3 and 28.3 Mg ha<sup>-1</sup> more aboveground C than pasture. The younger forest had  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of 2.1 and 1.7‰, respectively, lower than the 15-year-old forest, indicating more C derived from C<sub>3</sub> plants and biological N fixation. Both restorations and pasture had at least 34% higher H<sub>LIFS</sub> in deeper soil layers (10–30 cm) than primary forest, indicating a lack of labile SOM. Native and 15-year-old forests exhibited higher soil methane influx (141.1 and 61.9  $\mu\text{g m}^{-2} \text{h}^{-1}$ ). Forests out-performed pasture in most soil health indicators, with 69% of their variance explained by SOM properties. However, SOM quantity and quality regeneration in both restorations approached the pristine forest state only in

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the top 10 cm layer, while deeper soil retained agricultural degradation legacies. In conclusion, active restoration of the Atlantic Forest is a superior approach compared to pasture reform for GHG mitigation. Nonetheless, the development of restoration techniques to facilitate labile C input into deeper soil layers (>10 cm) is needed to further improve soil multifunctionality and long-term C storage.

## 1. Introduction

The Atlantic Forest biome is one of the world's biodiversity hotspots that faces severe forest fragmentation due to urbanization, industrialization, and agricultural expansion (Myers et al., 2000; Laurance, 2009; Rezende et al., 2018). Degenerative agricultural use has resulted in extensive areas of degraded pastures, which are a major liability in the region and in Brazil (Feltran-Barbieri and Féres, 2021). Within the Atlantic Forest, there are 11.6 Mha of pastures with an intermediate degree of degradation, and 4.1 Mha with severe degradation, encompassing 53% of the total pasture area (MapBiomass, 2020; Mello et al., 2020). Severe land degradation refers to the decline or deterioration of land health and productivity to a level of damage where the site can no longer support its intended function or regenerate naturally. Consequently, restoring forests or recovering degraded pastures in such land require intensive human intervention (Tambosi et al., 2014; Poorter et al., 2016). *Active forest restoration*, by planting diverse native plant species to rapidly cover the soil and rehabilitate ecological functions (Brancalion et al., 2015, 2016; Zanini et al., 2021), is the most cost-efficient method in such cases (Ribeiro et al., 2009). Recovering degraded pastures and restoring Atlantic forests are urgent and critical socio-environmental challenges for Brazil to fulfill the agreements to mitigate the climate crisis (Sá et al., 2017; Rezende et al., 2018), helping to re-establish environmental services (Lima et al., 2020) and reverse ecosystem degradation (Zanini et al., 2021), simultaneously contributing to global efforts on the UN Decade on Ecosystem Restoration (Bieluczyk et al., 2023).

Active forest restoration typically involves liming and soil fertilization, weed control, planting trees, and building fences for protection, among other techniques (Rodrigues et al., 2009). In other words, the active methodology is not a "fixed package" of techniques but comprises numerous possibilities of combinations based on tree planting (Brancalion et al., 2015, 2016), which have been continuously improved and implemented in the field (Di Sacco et al., 2021). For instance, a recent study tested a new management approach that involves dividing tree planting into two phases (Zanini et al., 2021). This approach includes planting functional tree groups to perform different ecological functions such as rapid soil coverage, provision of biodiversity, and attraction of seed-dispersing fauna. Strategically, trees are intercropped with green manure to suppress invasive grasses and provide nutrients to the soil (Rodrigues et al., 2009). This approach has been implemented since 2012, and the restoration sites are still young ( $\leq 10$  years), with much yet to be understood about their recovery effectiveness, emerging benefits, and ecosystem sustainability (Zanini et al., 2021).

Regenerating healthy soils is the foundation for restoring resilient forest ecosystems (Nolan et al., 2021). Soil health refers to the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans (Lehmann et al., 2020). However, about 60% of the studies on Atlantic Forest restoration have not included a single soil attribute (Mendes et al., 2019). In this context, soil organic matter (SOM) is a key indicator of soil health because it performs multiple functions, and through decomposition and transformation processes, influences other physical, chemical, and biological soil properties (Cherubin et al., 2016; Bieluczyk et al., 2020). Labile SOM provides energy for soil microbial activity (Kravchenko et al., 2019) and facilitates nutrient cycling (Banning et al., 2008). Non-labile (humified) SOM contributes to structuring the soil and increasing its cation exchange capacity, especially in tropical soils (Cotrufo et al., 2013; Tadini et al., 2021). Several methodologies have been developed to fractionate and

quantify these SOM forms related to the above-mentioned soil functions. A recently developed tool, laser-induced fluorescence spectroscopy (LIFS) has reliably characterized the SOM transformation qualitatively and, when associated with the SOM humification index ( $H_{LIFS}$ ), gathered the SOM compartment's balance into a unique index number (Segnini et al., 2013; Santos et al., 2015; Tadini et al., 2015, 2018, 2021; Xavier et al., 2019). In the present study, we intend to use the total soil C in parallel with  $H_{LIFS}$  to provide insights into whether SOM quantity and quality of restored forests are approaching the state of the native forest, which is a crucial goal of forest restoration.

We also aimed to determine the origin of C in the ecosystem as forest restoration progressed. Insights about the C origin can be achieved by using the isotopic composition of organic matter (e.g.,  $\delta^{13}C$  and  $\delta^{15}N$ ) in soils and plants (Zanini et al., 2021; Bieluczyk et al., 2020). The soil  $\delta^{13}C$  discriminates the SOM originated from  $C_3$  ( $\delta^{13}C = -24$  to  $-34\%$ , for example, planted trees) and  $C_4$  plants ( $\delta^{13}C = -9$  to  $-15\%$ , for example, previous pasture) (Smith and Epstein, 1971; Barros Ferraz et al., 2009). Including the  $\delta^{13}C$  analysis of SOM sources (e.g., litter and herbaceous biomass) alongside soil  $\delta^{13}C$  enables us to calculate the soil  $C-C_4/C-C_3$  proportion (Balesdent et al., 1988). Furthermore,  $\delta^{15}N$  allows for tracing some sources of N inputs into the soil (e.g., biological N fixation with  $^{15}N$  abundance around 0‰) and the transformations during SOM cycling within specific fractionation ranges (Szpak, 2014; Bieluczyk et al., 2020).

Lastly, several studies have investigated the effects of Atlantic Forest restoration on aboveground C storage (e.g., Nogueira Júnior et al., 2014; Shimamoto et al., 2014; Ferez et al., 2015; César et al., 2018; Azevedo et al., 2018; Brancalion et al., 2021; Poorter et al., 2021; Zanini et al., 2021). However, only a few studies have examined above and belowground C pools in tandem (e.g., Brancalion et al., 2021; Zanini et al., 2021; Poorter et al., 2021). Moreover, C-related field greenhouse gas (GHG) assessments as carbon dioxide ( $CO_2$ ) and methane ( $CH_4$ ) fluxes in restored forests are scarce (Bernal et al., 2018), despite their essentiality for understanding ecosystem C dynamics (Oertel et al., 2016). To our knowledge, no previous study has integrated soil, plant, and GHG flux data to understand the comprehensive process of C offset in Atlantic Forest restoration. Therefore, this study aimed to address this research gap as one of its primary objectives.

Given the above, we conducted an above-belowground assessment to evaluate C storage, GHG fluxes, and the quantity, quality, and origin of SOM in two sites with ongoing active forest restoration in the Atlantic Forest biome, comparing them with a reformed pasture and primary forest as references. Simultaneously, we examined the relations between SOM properties and soil health, using nine indicators representing crucial soil functions within the ecosystem. One of the restorations had 15 years and was implemented using an older approach of planting native trees in one go, without using green manure. In the younger 8-year-old restoration, native trees were planted in two phases and intercropped with green manure plants. Our hypothesis was that active forest restoration is more effective than pasture reformation in regenerating interconnected C storage, SOM properties, soil health, and GHG mitigation, thereby moving closer to the ecosystem services and functions provided by the native Atlantic Forest in Brazil. Providing this numerical and analytical information, encompassing soil-plant-atmosphere dynamics, can help ecologists in future restoration plans, as well as policymakers, in determining reliable actions to achieve the mitigation targets committed in international climate and environmental agreements.

## 2. Material and methods

### 2.1. Study site and treatments

The study areas were located at Capoava Farm (23°12'53"S, 47°10'45"W, 670 m asl) in Itu, São Paulo state, Southeastern Brazil (Fig. 1). The region's climate is Cwa, according to Köppen's classification, tropical with dry winters and warm-rainy summers, with 1299 mm of rainfall and 21.3 °C of average annual temperature (Alvares et al., 2013). Originally, all studied areas were covered by the semideciduous Atlantic Forest (Veloso et al., 1991). The soil was classified as a Ultisol (*Argissolo Vermelho-Amarelo distrófico* in Brazilian Classification) – with medium texture with clay contents ranging between 18 and 25% (Soil Survey Staff, 2014).

Initially, most of the farm's native forest area was converted to an extensive pasture of *Brachiaria decumbens* with one animal per hectare, no fertilizer input, and without soil acidity correction, leading to gradual land degradation. In 2006, Capoava Farm started implementing pasture soil fertility recovery and forest ecological restoration projects in the degraded areas. The study sites selection criteria are presented in the supplementary material, while land-use history and description of these areas with ongoing restoration are detailed below:

- (i) *Reformed pasture since 2006 (PA)*: liming and fertilization performed as recommended by Raji et al. (1996). In 2006, a new pasture with Tifton-85 bermudagrass (*Cynodon* spp) mixed with palisade grass (*Brachiaria brizantha*) was implemented. In the next five years (2006–2011), the soil fertility was recovered, and the pasture was renovated. From 2006 to nowadays, the pasture has been well-managed, with periods of grazing followed by pasture resting (rotational grazing).
- (ii) *Active restoration since 2012 (AR-8)*: Ten fast-growing, short-lived tree species were planted in the first phase in 2012 to shade the previously degraded area and reduce the weed population (especially *Brachiaria* sp.), quickly creating conditions for other regenerating species to establish. Four short life cycle green manure species (*Crotalaria breviflora*, *C. juncea*, *C. ochroleuca*, and *Cajanus cajan*) were seeded in this phase between the rows of native trees to supply nutrients and help weed control. Green manure plants senesced with time. Then, in the second phase, after a 1.5-year gap in 2013, eighty tree species were introduced to increase biodiversity and restore forest ecological dynamics. Therefore, ninety species were alternated and planted in a final density of 1666 trees ha<sup>-1</sup>.
- (iii) *Active restoration since 2005 (AR-15)*: In 2005, the pasture was mowed, and 110 different native tree species of the region were planted in the degraded area in one go. Seedlings of fast-growing species (pioneers) were alternated with successional species (early secondary and late secondary or climax), totaling 1666 trees ha<sup>-1</sup>. Mowing weeds and replanting dead trees were carried out only in 2006 and 2007.
- (iv) *Semideciduous primary forest (PF)*: Native forest fragment without signs of anthropogenic disturbance.

### 2.2. Soil assessment

#### 2.2.1. Soil sampling

The sampling was carried out in the second week of February 2021, under high air temperatures and a few days after heavy volumes of rain (Figure S1), aiming to represent the moment of most increased biological activity in these ecosystems (Kroeger et al., 2021; Venturini et al., 2022). Five small trenches, each measuring 50 × 50 × 50 cm (depth, width, and length), were excavated in each site. These sampling points were spaced at least 50 m apart, carefully distributed to capture spatial variability, and located 30 m from the area's limit to avoid edge effects.

Disturbed soil samples were collected using a stainless-steel spatula

and a pedological knife. Approximately 500 g of soil was manually obtained from the walls of each trench at depths of 0–10, 10–20, and 20–30 cm for chemical, texture, and microbiological analysis. In the same soil layers, undisturbed samples for physical analysis were collected in volumetric 5 × 5 cm rings using an Uhland-type auger. To preserve the soil structure during transportation, the soil-filled rings were wrapped with rubber and then surrounded by bubble wrap. Additionally, a portion of the disturbed soil was transferred to 50 mL Falcon tubes for subsequent microbiological analysis. These tubes were immediately packed in a thermal box, and transported on ice to the laboratory, where they were stored at –20 °C.

#### 2.2.2. Soil chemical and physical attributes

The soil for chemical and texture analyses was prepared by air-drying, grinding, and sieving (2 mm mesh). Soil fertility properties (the potential of hydrogen in water [pH], potential acidity [H + Al], mobile aluminum [Al], available phosphorus [P], and exchangeable calcium [Ca], magnesium [Mg], and potassium [K]) were measured by analytical methods described in Raji et al. (2001). The sum of bases (SB) in mmol<sub>c</sub> kg<sup>-1</sup> was calculated by adding the content of Ca, Mg, and K. Then, cation exchange capacity (CEC, mmol<sub>c</sub> kg<sup>-1</sup>) was determined by summing the SB and H + Al contents. Soil texture (clay, silt, and sand contents), microporosity, and soil bulk density were measured as described in Donagema et al. (2011). The soil's chemical and physical properties are presented in Table S2.

#### 2.2.3. Soil carbon and nitrogen stocks and their isotopic composition

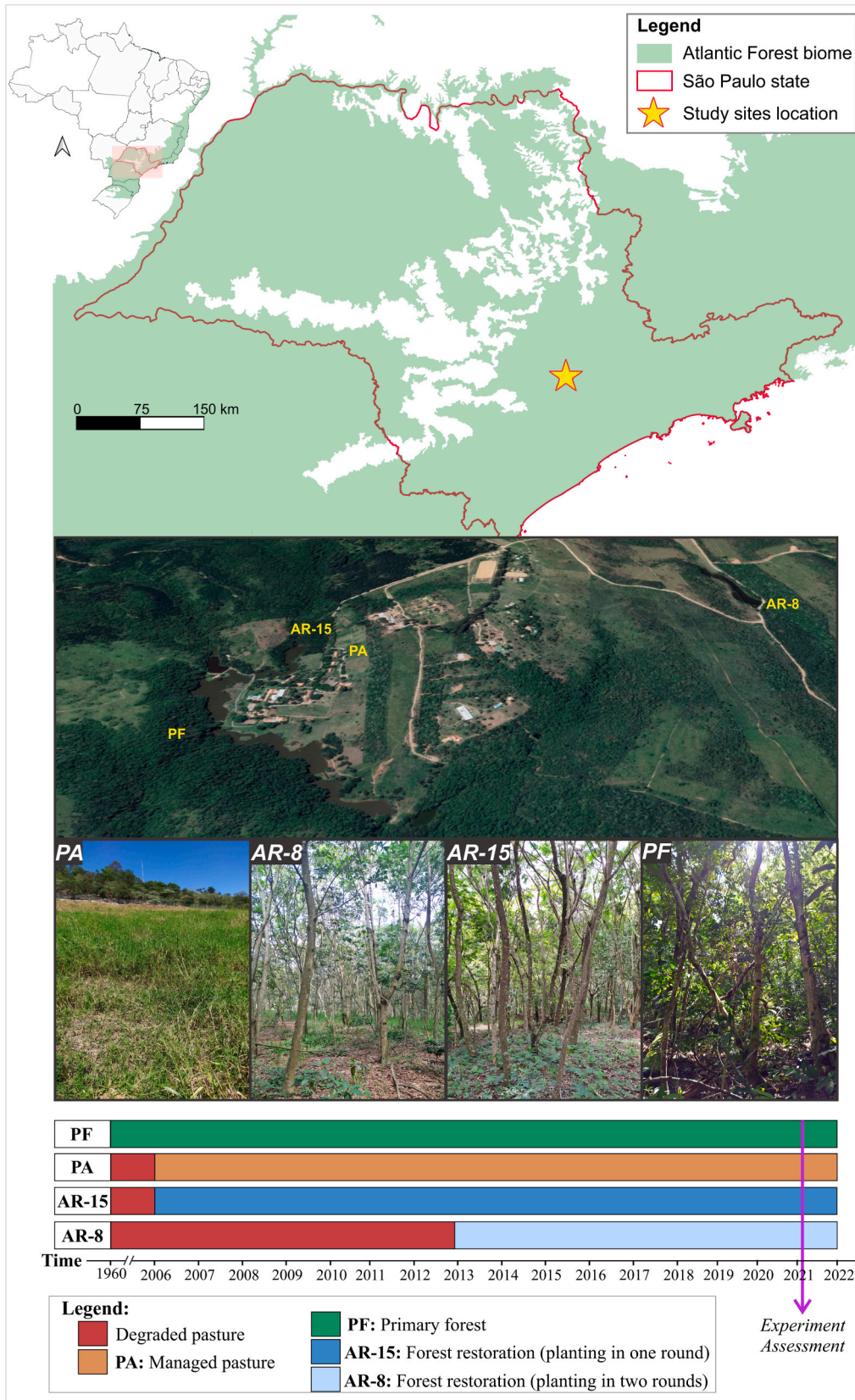
Soil subsamples (5 g) were air-dried, coarse roots were removed, and soil was sieved (2 mm). This soil was macerated and finer sieved (≤149 μm) to analyze C and N contents and <sup>15</sup>N and <sup>13</sup>C isotopic composition in an automatic nitrogen-carbon analyzer with a combustion interface to a continuous-flow isotope ratio mass spectrometer (Thermo Scientific, model Delta V Advantage; Milan, Italy). The stable isotopes' results were expressed as δ<sup>13</sup>C and δ<sup>15</sup>N (‰) using international standards (Vienna PeeDee Belemnite – V-PDB for C [NBS19 and NBS22] as a reference for <sup>13</sup>C values and composition of atmospheric air for N<sub>2</sub> [IAEA-N1 and IAEA-N2] as a reference for δ<sup>15</sup>N). The delta values were based on standards (Farquhar et al., 1982) and calculated using the following equation: δX = [(R<sub>sample</sub>/R<sub>standard</sub>) - 1] multiplied by 1000, where X refers to <sup>13</sup>C or <sup>15</sup>N and R<sub>sample</sub> and R<sub>standard</sub> are the <sup>13</sup>C/<sup>12</sup>C or <sup>15</sup>N/<sup>14</sup>N ratios of sample and standard, respectively.

The soil C and N stocks (Mg ha<sup>-1</sup>) were calculated by multiplying the contents of soil C or N (%), bulk density (Mg m<sup>-3</sup>), and the thickness of the soil layer (cm). Since the soil bulk density was higher in pasture and restored areas compared to the native forest (Table S2), non-corrected C stocks would be systematically overestimated in these managed areas. Then, the stocks were corrected by an equivalent soil mass method, as Ellert and Bettany (1995) described, using the primary forest area as a reference. Also, to not overestimate the soil C and N stocks, we discounted the mass occupied by the gravel in each soil layer, representing 28–36% of the soil mass (Table S2).

#### 2.2.4. Soil humification index

Part of the soil subsamples sieved to ≤149 μm (approximately 0.5 g) was pelletized (1 cm × 2 mm pellets) in a hydraulic press before a laser-induced fluorescence spectroscopy (LIFS) analysis (Tadini et al., 2021). A continuous-wave laser (20 mJ power) with excitation at 405 nm was used to acquire the LIFS spectra in a mobile prototype system (Santos et al., 2015). The wavelength ranged from 420 to 800 nm, and the maximum emission intensity was 4096 counts. The integration time, mean, and selected boxcar was 900 ms, 3, and 7, respectively.

We confectioned four pellets for each field sample. After the LIFS analysis, we calculated the averages among them to represent the spectrum. The humification index (H<sub>LIFS</sub>) was determined as described in Milori et al. (2006) and calculated using the following equation: H<sub>LIFS</sub> = ESA/C, where ESA refers to emission spectrum area (arbitrary units or



**Fig. 1.** Location of the study sites of pasture (PA), active restoration with eight (AR-8) and fifteen (AR-15) years of growth, and a semideciduous primary forest (PF), as well as the land-use history of these sites. \*The aerial photo is from April 2019 and was obtained through Google Maps 3D feature. Terrestrial photos were taken by Wanderlei Bieluczyk.

a. u.) at 405 nm with 420–800 nm excitation, and C to the total amount of carbon (%) in the soil sample.

### 2.2.5. The abundance of soil microorganisms

We used quantitative PCR (qPCR) targeting functional marker genes to reveal the abundance of methanogenic (methyl coenzyme-M reductase – *mcrA*), methanotrophic (particulate methane monooxygenase – *pmoA*), and nitrogen-fixing (nitrogenase reductase – *nifH*) microorganisms in the soil (Supplementary Material). The *nifH*, *pmoA*, and *mcrA* genes were used as complementary information to support our discussion.

## 2.3. Plant assessment

### 2.3.1. Carbon stocks from trees

Three plots (30 × 10 m) were randomly delineated at each site, with a minimum distance of 30 m from the area boundaries. All living trees containing at least one stem with a diameter at breast height (DBH, 1.3 m) ≥ 5 cm were assessed. Then, if one tree's stem met this inclusion criteria, all its stems were measured. DBH was measured using tape, and the trees were classified at a species level. The wood density (WD) of the species was retrieved from several sources, including online databases (ICRAF database [2022] and Chave et al., [2006]), as botanical books (Lorenzi, 2011, 1998, 1992). Tree biomass was determined differently for restoration plantations (AR-15 and AR-8) and mature forest (PF) areas and subsequently multiplied by 0.47 (Parry et al., 2007) to obtain the tree C stocks (Mg ha<sup>-1</sup>). In the restoration areas, we used the allometric equation developed by Nogueira Júnior et al. (2014) for actively restored Atlantic forests:

$$\log(AGB) = -0.970 + 1.1421 \log(DBH^2 \times WD) \quad (1)$$

Since the native forest structure differs from restorations throughout the advanced stage of succession (Ferez et al., 2015), we used an equation developed by Chave et al. (2005) for the mature semideciduous forest, recommended as one of the best predictive models for mature moist forest stands:

$$AGB = WD \times \exp(-1.499 + 2.148 \ln(DBH) + 0.207(\ln(DBH))^2 - 0.0281(\ln(DBH))^2) \quad (2)$$

In the above equations (1) and (2), AGB is the aboveground biomass (kg per tree), DBH is the diameter at breast height (m), and WD is the wood density (g cm<sup>-3</sup>).

### 2.3.2. Herbaceous and litter carbon and nitrogen stocks

Litter and herbaceous pools were sampled in a 25 × 25 cm square frame in the five points where the soil was taken. These samples were oven-dried at 50 °C for 96 h until weight stabilization. Then, the dry biomass (g) was weighed and calculated for Mg ha<sup>-1</sup>. The dry biomass was ground (0.25 mm) in a micro mill and analyzed for total C and N, as <sup>15</sup>N and <sup>13</sup>C isotopic composition, with the same equipment used to analyze the soil. The litter and herbaceous C and N stocks (Mg ha<sup>-1</sup>) were calculated by multiplying their content (%) by the total biomass (Mg ha<sup>-1</sup>).

## 2.4. Atmosphere: greenhouse gases assessment

Higher GHG emissions in tropical land uses typically occur during the rainy season, related to higher soil moisture and weather temperatures (Keller and Reiners, 1994; Steudler et al., 1996; Verchot et al., 2000; Fernandes et al., 2002; Keller et al., 2005; Carmo et al., 2012; Bento et al., 2018; Venturini et al., 2022). Therefore, we strategically assessed methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>) fluxes after torrential rains and during the hottest period of the year (Figure S1). We also removed 10 and 20 cm of the topsoil to investigate possible changes in patterns between areas, possibly eliminating compacted layers that

cause impediments to water infiltration and favor methane production.

The evaluations were carried out on four sequent sunny days, standardizing the exact incubation times of the chambers throughout the day for the different areas. Gas measurement was performed close (~2 m) to each soil sampling point (n = 5). The base dimensions of the static chambers were 19.5 cm (diameter) × 20 cm (height). This base was inserted 2–3 cm in the soil and coupled with a lid with 2 L volume, totalizing approximately 7 L of air when the chamber was closed. The chamber lid contained two inlets for flexible tubes (6 mm diameter), which were connected, for 10 min incubation time, to a portable GHG analyzer for humid conditions (model DX4015, Gaset Technologies, Finland) (Figure S2). During the incubation, the vacuum of the analyzer moved the air from the chamber in a closed circulation continuous flow. CH<sub>4</sub> and CO<sub>2</sub> concentrations were measured every 5 s *in situ* and in real-time. The output of the portable gas analyzer provided the gas parameters needed for calculating the total C-CH<sub>4</sub> (μg) and C-CO<sub>2</sub> (mg) inside the chamber. The calculation was done using the Clapeyron ideal gas law:  $pV = nRT$ , which relates absolute pressure  $p$  to absolute temperature  $T$ , with volume  $V$  of the container holding the gas and the amount  $n$  (in moles) of gas contained in there, and  $R$  is the molar gas constant. Then, the C-CH<sub>4</sub> and C-CO<sub>2</sub> fluxes were quantified by the first derivative relating concentrations and times, excluding the measurements of the first 60 s of incubation to avoid stabilization biases. Using the extrapolation from second to hour and from the chamber area (0.0266 m<sup>2</sup>) to a square meter, we obtained the fluxes in μg C-CH<sub>4</sub> m<sup>-2</sup> h<sup>-1</sup> and mg C-CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup>.

## 2.5. Data analysis

Most statistical analyses were performed in the R platform v. 4.1.2 (RStudio Team, 2022). Levene's and Shapiro-Wilk tests were applied to each attribute to check and validate the analysis requirements of variance analysis (ANOVA): homogeneity of variance and normality of errors. When necessary, the data were Box-Cox transformed (Box and Cox, 1964). With all requirements satisfied, ANOVA was performed using the level of 5% significance to test treatments' influence on the attributes evaluated in the study. When significant, the Tukey test ( $p < 0.05$ ) was used to compare the means. Univariate analyses compared sites within each assessed layer of the ecosystems, including soil (0–10 cm, 10–20 cm, and 20–30 cm), litter, herbaceous plants, and trees. Exceptionally, for GHG, we also compared the soil layers. In all cases, we provided the errors, calculated as the standard deviation divided by the square root of the sample size.

To verify the correlation between SOM properties (C, N, H<sub>LIFS</sub>, δ<sup>13</sup>C, δ<sup>15</sup>N, and C:N relation) and soil health, we selected the following attributes that indicate soil functions and health: physical (bulk density, macroporosity, and microporosity) and chemical properties (pH, cation exchange capacity, sum of bases, and available P), and soil CO<sub>2</sub> and CH<sub>4</sub> fluxes. The soil functions indicated by these properties are as follows: (i) soil bulk density is associated with the support for root growth; (ii) jointly, macroporosity and microporosity represent the soil aeration and water retention capacity; (iii) soil pH reveals soil acidity regulation for nutrient uptake; (iv) available P and the sum of bases demonstrate the soil nutrient availability for weathered tropical soils; (v) cation exchange capacity corresponds to the nutrient storage and release in the soil; and (vi) collectively, CO<sub>2</sub> and CH<sub>4</sub> fluxes reflect biological activity in the soil. These soil health indicators and their represented soil functions are widely recognized in the literature (e.g., Rinot et al., 2019; Lehmann et al., 2020; Simon et al., 2022; Bieluczyk et al., 2023). Therefore, these parameters were used to perform a distance-based redundancy analysis (db-RDA) based on Bray-Curtis dissimilarity matrices. Forward selection and Monte Carlo tests were applied with 1000 random permutations to investigate the significance of SOM properties as explanatory variables upon soil health indicators. The RDA plot was generated using Canoco 4.5 (Biometrics, Wageningen, The Netherlands) and PERMANOVA using the PAST software v.3.0 (Hammer et al., 2001).

### 3. Results

#### 3.1. C and N stocks

The primary forest contained the highest C stocks in both aboveground and belowground pools and the highest soil N stocks at the 0–30 cm layer (Table 1). Herbaceous plants were present in the eight-year-old restoration but absent in the primary forest and 15-year-old restoration areas. The reformed pasture had the highest C stocks in herbaceous plants, three times higher than the younger restoration.

The 15-year-old restoration had more aboveground C than the younger restoration. The tree and litter C stocks were 2.1 and 1.6-fold higher, respectively. The forest growth in 15 and 8-year-old restorations accumulated on average 1.8 and 1.5 Mg C ha<sup>-1</sup> y<sup>-1</sup>, respectively, in the tree biomass. The C and N stocks in the litter pool were highest in the primary forest and active restoration with 15 years, with both sites showing greater stocks than in the younger forest. The younger forest, in turn, sequestered more C and N stocks in litter than the pasture. However, there were no differences in soil C and N stocks between the forest restoration sites and the pasture (Table 1).

Sequestered C (the sum of soil, litter, herbaceous, and tree pools) was the highest in the primary forest (108 Mg ha<sup>-1</sup>), followed by the 15-year-old (70 Mg ha<sup>-1</sup>) and 8-year-old (56 Mg ha<sup>-1</sup>) restorations, and the reformed pasture (42 Mg C ha<sup>-1</sup>) (Fig. 2A). The inverse order was observed for the soil C stocks' relative contribution to the total measured amount of C, with 44, 53, 70, and 89% in primary forest, 15-year-old and 8-year-old restorations, and pasture systems, respectively (Fig. 2B).

#### 3.2. Herbaceous and litter C and N

Herbaceous C contents were similar in pasture and 8-year-old restoration, but N contents were higher in the restoration, reducing C:N ratio by eight units (Table 2). The herbaceous pool in the 8-year-old restoration had lower isotopic abundance (around -2 and -3‰ for δ<sup>13</sup>C and δ<sup>15</sup>N, respectively) than in pasture, indicating mixtures of C<sub>3</sub> plants in the C<sub>4</sub> material.

Litter C contents did not differ across sites, but N increased from pasture to forest restorations and native forest, with 7.8, 16.1, 17.4, and 21.6 g kg<sup>-1</sup>, respectively (Table 2). The litter in the 8- and 15-year-old forest restoration areas originated from C<sub>3</sub> plants, as the δ<sup>13</sup>C ranged from -28.7 to -28.9‰, similar to that of the native forest (δ<sup>13</sup>C = -28.9‰). Pasture and older restoration sites had higher δ<sup>15</sup>N in the litter, while the primary forest and 8-year-old restoration showed lower values (difference of ~1‰).

#### 3.3. Soil C and N contents

At the topsoil layer (0–10 cm), there were no differences in soil C between the assessed sites (Fig. 3A). However, the native forest showed the highest C at deeper soil layers, at least 1.2 and 1.5-fold higher than the pasture and restorations. The δ<sup>13</sup>C in native forest soil ranged from

-26.2 to -26.7‰, characteristic of soils under C<sub>3</sub> plants. In contrast, the pasture area ranged from 15.5 to 15.6‰, reflecting the long-term C<sub>4</sub> grasses' organic matter inputs (Fig. 3D). A surprising result was the decreased soil δ<sup>13</sup>C in the younger restoration area (8 years old) compared to the older (15 years old) in all soil layers, showing closer C isotopic signals to those of the native forest area. Given these results, the new restoration approach seemed to play an essential role in changing the dynamics of cycling and the origin of the soil organic matter.

The native forest had at least 21, 40, and 17% higher soil N contents (0–10, 10–20, and 20–30 cm soil layers, respectively) than the other sites (Fig. 3B). Further, there were no differences between the two forest restoration areas and the pasture in the 0–10 and 10–20 cm soil layers. However, in 20–30 cm, the N levels of pasture approached those of the native forest. N was heavier in pasture for all soil layers, showing higher δ<sup>15</sup>N ranging from 7.9 to 8.7‰ (Fig. 3E). The 8-year-old restoration had the lowest δ<sup>15</sup>N in the soil, ranging from 4.0 to 6.4‰. Primary forest and 15-year-old restoration showed intermediate δ<sup>15</sup>N values in the 10–20 and 20–30 cm soil layers. Additionally, forest restoration sites presented a higher soil C:N relation than the native forest (Fig. 3C), probably reflecting the litter inputs, which also contained higher C:N under restoration (Table 2). Even if the pasture biomass presented the highest C:N relation (Table 2), its soil C:N ratio was intermediate between the restoration areas and native forest, probably related to frequent herbivory and direct changes in its N cycling.

#### 3.4. Proportion of soil carbon from C<sub>4</sub> and C<sub>3</sub> plants

The native forest had at least 93% of its soil C originating from C<sub>3</sub> plants in all soil layers (Fig. 4). In contrast, at least 88% of soil C under the pasture was derived from C<sub>4</sub> plants (i.e., forage grass). After eight years of implementation, the younger restoration's soil showed 51.8–66.4% of the C originating from C<sub>3</sub> plants (i.e., trees or green manure). These values were lower in the 15-year-old restoration, ranging from 38.3 to 50.3%. Moreover, in both areas, the proportion of C<sub>3</sub>-derived SOM reduced from the topsoil to the deepest layer, decreasing by 12 and 14% in forests with 15 and 8 years of growth, respectively.

#### 3.5. SOM humification index

The assessed sites' fluorescence emission spectra (Fig. 5) showed a wide band with a maximum intensity of around 525 nm and distinct peaks between 550 nm, 580 nm, and 620 nm in all soils. The presence of aromatic structures in SOM was indicated by peaks in longer spectral wavelengths, between 550 and 580 nm. Moreover, the change in fluorescence to longer wavelengths reflects a higher concentration of even highly condensed aromatic chains (Tadini et al., 2021, 2015; Tivet et al., 2013), especially with a shoulder around 620 nm observed in the deepest assessed soil layer (20–30 cm) (Fig. 5C).

The pasture's fluorescence peaks were higher than the forest in all soil layers (Fig. 5A, B, and 5C). The soil in both forest restorations had

**Table 1**

Carbon (C) and nitrogen (N) stocks (Mg ha<sup>-1</sup>) in above and belowground-assessed pools in a semideciduous forest (PF), reformed pasture (PA), and active forest restoration with eight (AR-8) and fifteen (AR-15) years of growth.

	Pools	PF	AR-15	AR-8	PA				
<i>Carbon stocks</i>									
Aboveground	Tree	54.06 ± 1.48	a <sup>a</sup>	26.31 ± 3.23	b	12.29 ± 1.48	c	0 ± 0	d
	Herbaceous	0 ± 0	b	0 ± 0	b	0.55 ± 0.42	b	2.00 ± 0.24	a
	Litter	6.19 ± 0.48	a	6.40 ± 0.53	a	3.92 ± 0.79	b	2.44 ± 0.57	c
Belowground	0–30 cm soil	47.44 ± 2.92	a	37.11 ± 2.96	b	38.76 ± 2.99	b	37.38 ± 3.76	b
<i>Nitrogen stocks</i>									
Aboveground	Herbaceous	0 ± 0	b	0 ± 0	b	0.02 ± 0.01	b	0.06 ± 0.02	a
	Litter	0.34 ± 0.04	a	0.27 ± 0.02	ab	0.16 ± 0.04	bc	0.05 ± 0.01	c
Belowground	0–30 cm soil	4.93 ± 0.23	a	3.27 ± 0.20	b	3.22 ± 0.23	b	3.62 ± 0.33	b

<sup>a</sup> Letters compare values in the row according to the Tukey test with 95% confidence.

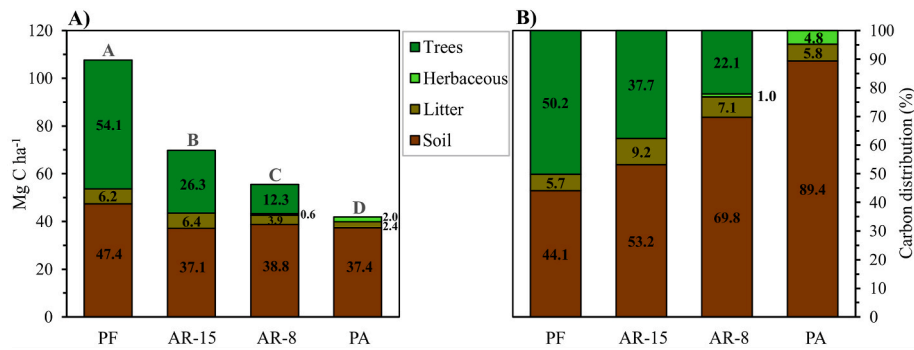


Fig. 2. Carbon stock accumulation (A) and proportion (B) of the different ecosystem pools in a semideciduous primary forest (PF), active forest restoration with fifteen (AR-15) and eight (AR-8) years of age, and a reformed pasture (PA). Letters compare total C stocks according to the Tukey test with 95% confidence.

Table 2

- Carbon (C) and nitrogen (N) contents, C/N ratio, and abundance of the stable isotopes of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) in herbaceous leaves and litter biomass in areas of semideciduous primary forest (PF), reformed pasture (PA), and active forest restoration with eight (AR-8) and fifteen (AR-15) years of growth.

Areas	C (g kg <sup>-1</sup> )	N (g kg <sup>-1</sup> )	C/N	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
<i>Herbaceous</i>					
PA	408.3 ± 6.2	11.61 ± 0.82	35.78 ± 2.26	-14.06 ± 0.16	2.64 ± 0.25
AR-8	409.2 ± 11.4	14.92 ± 0.98	27.82 ± 1.63	-16.04 ± 0.35	-0.14 ± 0.32
<i>Litter</i>					
PA	395.5 ± 6.5	7.79 ± 0.38	51.20 ± 2.33	-15.36 ± 0.58	2.13 ± 0.34
AR-8	398.7 ± 13.9	16.12 ± 1.95	26.30 ± 3.25	-28.93 ± 0.55	1.06 ± 0.14
AR-15	413.3 ± 6.4	17.45 ± 1.09	24.06 ± 1.54	-28.74 ± 0.32	2.23 ± 0.78
PF	392.0 ± 15.3	21.60 ± 1.48	18.40 ± 1.06	-28.85 ± 0.41	0.96 ± 0.39

<sup>a</sup> Letters compare treatments for each pool in the column according to the Tukey test with 95% confidence.

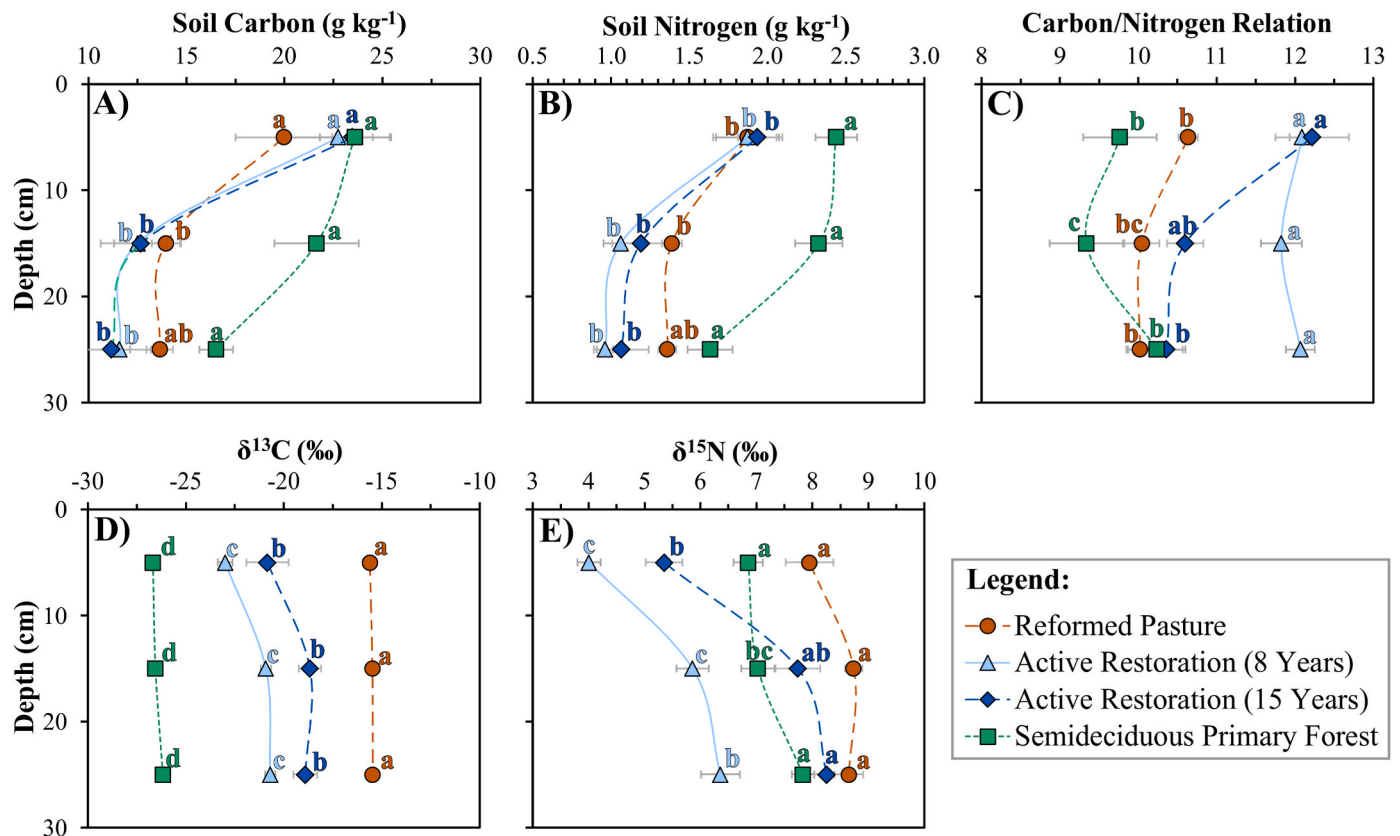


Fig. 3. Soil carbon (A) and nitrogen (B) contents, C/N ratio (C), and abundance of the stable isotopes of carbon ( $\delta^{13}\text{C}$ ) (D) and nitrogen ( $\delta^{15}\text{N}$ ) (E) in semideciduous primary forest, reformed pasture, and forest restoration areas. Letters compare treatments for each depth according to the Tukey test with 95% confidence.

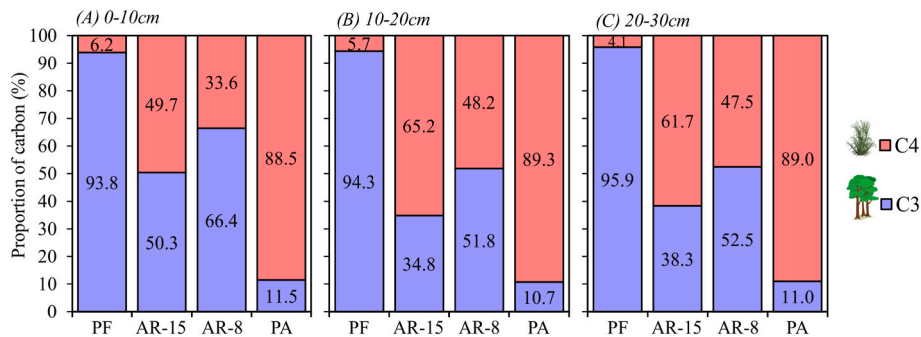


Fig. 4. Proportion of carbon from C<sub>3</sub> and C<sub>4</sub> plants in soil (0–10, 10–20, and 20–30 cm layers) under a semideciduous primary forest (PF), active forest restoration with fifteen (AR-15) and eight (AR-8) years of age, and a reformed pasture (PA).

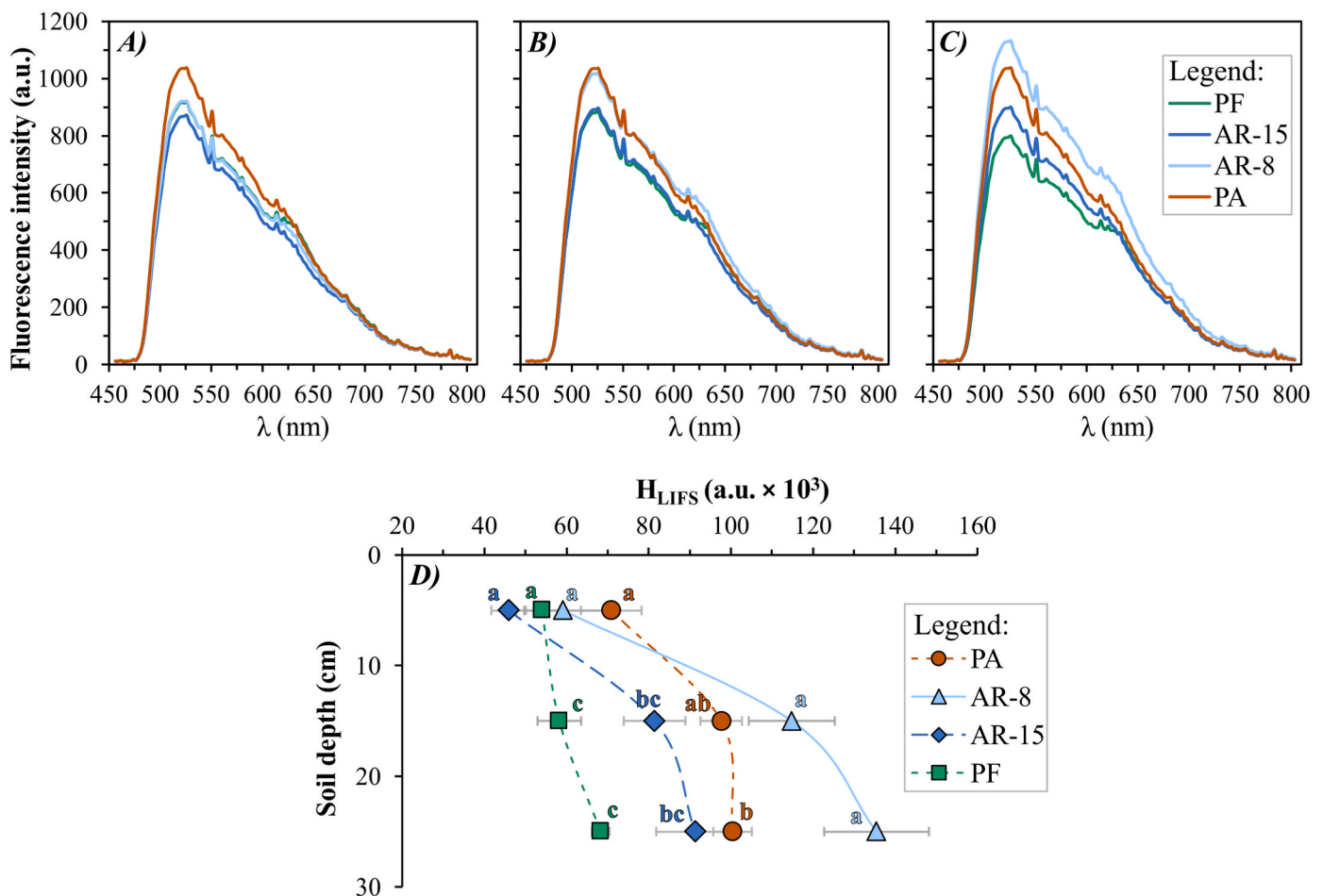


Fig. 5. Average laser-induced fluorescence spectra (LIFS) in 0–10 cm (A), 10–20 cm (B), and 20–30 cm (C) soil layers and soil organic matter humification index ( $H_{LIFS}$ ) for all soil layers (D) under a semideciduous primary forest (PF), active forest restoration with fifteen (AR-15) and eight (AR-8) years of age, and a reformed pasture (PA). Letters compare treatments for each depth according to the Tukey test with 95% confidence.

similar spectra as the native forest in the topsoil layer (Fig. 5A), but emission intensity increased with soil depth, especially in the younger restoration. Then, in the 20–30 cm soil layer, the restoration areas showed higher peaks than the native forest, indicating a higher predominance of aromatic SOM.

The SOM humification index ( $H_{LIFS}$ ) showed similarities between sites in the topsoil layer (Fig. 5D). The main observations for  $H_{LIFS}$  were: (i) the native forest showed constant  $H_{LIFS}$  values with soil depth, lower than anthropized areas in deeper layers, suggesting chemical stability of SOM (Bayer et al., 2006); (ii) the 8-year-old restoration had the highest  $H_{LIFS}$  in deeper soil layers (10–20 and 20–30 cm), the most distanced

indices from the native forest, indicating an unbalance in SOM fractions with the predominance of a higher degree of aromaticity and complexity of humified compounds (Tivet et al., 2013); and (iii) in soil layers of 10–20 and 20–30 cm,  $H_{LIFS}$  mean values decreased as follows: 8-year-old restoration > pasture > 15-year-old restoration > primary forest.

### 3.6. Fluxes of methane and carbon dioxide

Methane (C-CH<sub>4</sub>) fluxes showed the same pattern when measured on the soil surface and after removing 10 and 20 cm topsoil (Fig. 6A). On the surface, the native forest showed the highest consumption of C-CH<sub>4</sub>



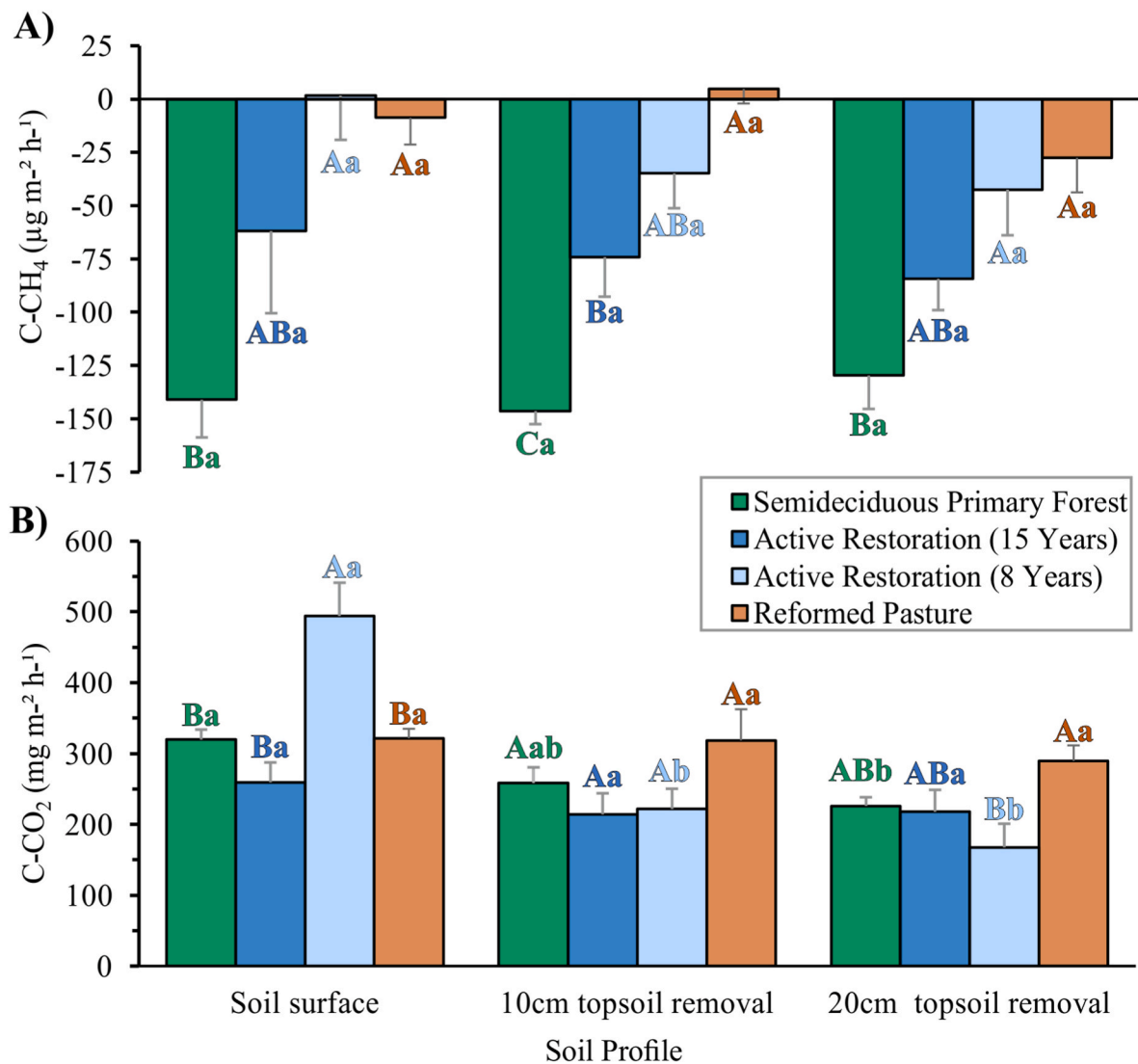


Fig. 6. Methane (A) and carbon dioxide (B) fluxes in the soil surface and with 10 cm and 20 cm topsoil removal in the native semideciduous forest, reformed pasture, and forest restoration areas. Uppercase letters compare treatments for each depth, and lowercase letters compare depths according to the Tukey test with 95% confidence.

( $-141.1 \mu\text{g m}^{-2} \text{h}^{-1}$ ), followed by the older restoration, with  $-61.9 \mu\text{g m}^{-2} \text{h}^{-1}$ . In the pasture, the C-CH<sub>4</sub> consumption was lower ( $-8.8 \mu\text{g m}^{-2} \text{h}^{-1}$ ), and in 8-year-old restoration, there was a slight production ( $1.7 \mu\text{g m}^{-2} \text{h}^{-1}$ ). When removing 20 cm of soil, the pasture and the younger restoration consumed about 28 and  $42 \mu\text{g C-CH}_4 \text{ m}^{-2} \text{h}^{-1}$ , respectively.

Carbon dioxide (C-CO<sub>2</sub>) emissions were at least 30% higher on the soil surface of the younger restoration compared to the other areas, but it was reduced by more than half with the topsoil removal (Fig. 6B). The pasture showed no change in C-CO<sub>2</sub> fluxes with soil removal but produced more CO<sub>2</sub> in deeper layers when compared to the other areas. Meanwhile, the CO<sub>2</sub> emissions in the native forest and 8-year-old restoration areas decreased significantly with the soil removal.

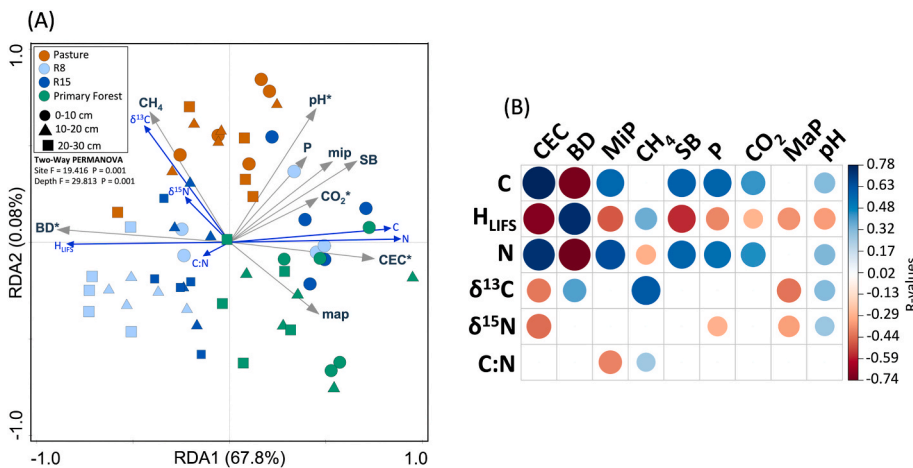
### 3.7. Relationship between SOM fractions and other soil health indicators

RDA analyses and the two-way PERMANOVA showed that the assessed sites ( $F = 19.4$ ,  $P < 0.0001$ ) and the soil layers ( $F = 29.8$ ,  $P < 0.001$ ) were markedly different (Fig. 7A). RDA1 explained the most significant variation (67.8%), while RDA2 explained 0.8%. The samples clustered according to the land-use systems, with primary forest and

pasture in separate clusters. Forest restoration areas presented similarities, and some points crossed with the primary forest.

We used Spearman's correlations to investigate the relationship between SOM properties and other soil health indicators (Fig. 7B). Soil C and N were positively correlated ( $r > 0.5$ ) with cation exchange capacity (CEC), the sum of bases (SB), and microporosity (MiP), and negatively with bulk density ( $r \sim -0.7$ ) and CH<sub>4</sub> emissions ( $r \sim -0.3$ ). The primary forest area was more likely associated with higher soil C, N, microporosity, and CEC (Fig. 7A). H<sub>LIFS</sub> was negatively correlated with soil CEC ( $r = -0.71$ ), SB ( $r = -0.56$ ), P ( $r = -0.39$ ), and MiP ( $r = -0.48$ ), while positively correlated with the bulk density ( $r = 0.75$ ) and CH<sub>4</sub> emissions ( $r = 0.36$ ). H<sub>LIFS</sub> was more likely associated with the pasture and the eight-year-old restoration, showing opposite arrows to soil C and N contents and a positive association with higher bulk density values.

Other SOM properties showed lower correlations with soil health variables. For example,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were negatively correlated with CEC ( $r \sim -0.4$ ) and microporosity ( $r \sim -0.3$ ). CO<sub>2</sub> emissions positively correlated with soil C ( $r = 0.42$ ) and N ( $r = 0.44$ ). Finally, CH<sub>4</sub> emissions were correlated positively with  $\delta^{13}\text{C}$  ( $r = 0.61$ ) and H<sub>LIFS</sub> ( $r = 0.34$ ) and negatively with total soil C ( $r = -0.22$ ) and N ( $r = -0.29$ ) contents.



**Fig. 7.** Redundancy analysis (RDA) of soil organic matter parameters and soil chemical, physical, and biological related properties (A). The heatmap shows Spearman correlations between the RDA parameters (B). Arrows indicate the correlation between selected soil health properties (chemical, physical and biological) and soil organic matter parameters. The significance of these correlations was evaluated via the Monte Carlo permutation test and is indicated by an asterisk ( $P < 0.05$ ). Analysis of permutation (PERMANOVA) is indicated in the upper left corner of the RDA graph. **C**: total soil carbon, **H<sub>4F5</sub>**: soil organic matter humification index, **N**: total soil nitrogen,  $\delta^{15}\text{N}$ : natural soil abundance of  $^{15}\text{N}$ ,  $\delta^{13}\text{C}$ : natural soil abundance of  $^{13}\text{C}$ , **pH**: the potential of hydrogen in water; **CEC**: potential cation exchange capacity of the soil, **SB**: sum of bases in soil, **P**: soil available phosphorus content, **BD**: soil bulk density, **MaP**: soil macroporosity, **MiP**: soil microporosity, **CO<sub>2</sub>**: carbon dioxide flux from the soil. **PA**: reformed pasture, **R8**: active restoration with eight years, **R15**: active restoration with 15

years, and **PF**: primary forest.

## 4. Discussion

### 4.1. The carbon sequestration

The trees of the primary forest stored  $54.06 \text{ Mg C ha}^{-1}$ , within the typical range of  $27\text{--}56 \text{ Mg C ha}^{-1}$  observed in previous studies of native semideciduous forests fragments (Villanova et al., 2019; Zanini et al., 2021). Moreover, the primary forest had aboveground C stock (trees + herbaceous + litter) at least 28% higher than forest restoration and reformed pasture sites. The incomplete recovery of C storage in young regrown Atlantic forests has been recently and often reported (e.g., Brancalion et al., 2021; Zanini et al., 2021; Poorter et al., 2021). Our findings revealed that soil C and N sequestered by 8–15-year restorations were at most 78% of the amount stored in the primary forest. These results evidence the critical importance of preserving native forests, as they have achieved ecosystem stability and C storage potential through thousands or even millions of years (FAO and UNEP, 2020). Importantly, our results also support how imperative is to include the soil in C storage inventories, as soil C accounted for 44–70% of the total C stocks within our native forest and ongoing restorations. Regrettably, quantifying belowground C is often neglected in tropical forest restoration studies (Jones et al., 2019), leading to biased estimations of ecosystem-level C storage (Zanini et al., 2021). Therefore, soil C stands as an indispensable component that must be considered in reliable plans for ecological forest restoration and global warming mitigation.

Our findings demonstrate that 15- and 8-year-old restorations had similar soil C ( $37.1$  and  $38.8 \text{ Mg C ha}^{-1}$ , respectively) and N ( $3.3$  and  $3.2 \text{ Mg N ha}^{-1}$ , respectively) stocks, despite their age difference. However, the older restoration had over 60% more aboveground C and a higher average rate of aboveground C accumulation, storing  $0.3 \text{ Mg C ha}^{-1} \text{ y}^{-1}$  more than the younger restoration. Brancalion et al. (2021) identified that forest age was the main driver of aboveground C accrual in planted forest restorations in the Atlantic. In their study, the authors also verified that soil and landscape variation obscured the relationship between soil C stocks and forest age. Our study resolved this issue by standardizing soil and landscape position. Surprisingly, we found equivalent soil C stocks in both the 8- and 15-year-old restorations, possibly due to differences in forest restoration management. For example, green manure such as *Crotalaria* sp. was planted in the younger restoration, which is known to fix atmospheric N in the soil and benefit soil biological activity (Araújo Neto et al., 2014). Such symbiosis is essential for incorporating C into the soil (Ros et al., 2003) and can speed up soil C accumulation rate in ecological restorations (Mayer et al., 2020).

The reformed pasture showed soil C stocks at the same level as the forest restoration areas. Indeed, previous studies have shown that well-managed pastures can accrue significant amounts of C in the soil's upper layers, many times showing C stocks equal to or even higher than forests (Guo and Gifford, 2002; Segnini et al., 2019). However, our reformed pasture agroecosystem stored only 11% of its C ( $4.4 \text{ Mg C ha}^{-1}$ ) in aboveground pools. In the same pools, the 8- and 15-year-old restorations accumulated  $16.8$  and  $32.7 \text{ Mg C ha}^{-1}$ , respectively. Furthermore, when considering the sum of above and belowground C pools, the younger and older forest restorations accumulated  $14$  and  $28 \text{ Mg C ha}^{-1}$ , respectively, more than the reformed pasture. Reforming and intensifying pasture for cattle ranching, which is the main purpose in Brazil, also increases GHG emissions by animals and fertilizer production chain (Batista et al., 2019), which were not accounted in our study. As C sequestration was higher under our active forest restoration than in the reformed pasture, restoring the Atlantic Forest should be preferred to mitigate GHG emissions on severely degraded land instead of reforming pastureland.

### 4.2. Soil organic matter: quantity, quality, and origin

Our study provides a compelling demonstration that variations in the C dynamics of the assessed systems started with differences in aboveground biomass sources, influencing the soil's organic matter properties. Herbaceous and litter pools from different areas showed variations in N contents, C:N relation,  $\delta^{13}\text{C}$ , and  $\delta^{15}\text{N}$  (Table 2), exhibiting differences in organic matter inputs across the sites. The litter in the primary forest had the highest N content, while the pasture litter had the lowest. Additionally, herbaceous and litter biomass in the pasture presented  $\delta^{13}\text{C}$  typical of  $\text{C}_4$  grasses, ranging from  $-14.06$  to  $-15.36\%$ , while the litter under primary forest and restorations showed  $\delta^{13}\text{C}$  ranging from  $-28.74$  to  $-28.93\%$ , typical of  $\text{C}_3$  tree plants.

The effects of forest restoration in soil were more evident in the top layer (0–10 cm), where C contents of the 8- and 15-year restorations reached the level of the primary forest. Moreover, restorations showed at least 15% more C derived from  $\text{C}_3$  plants (trees or green manure) in the upper 0–10 cm layer than in deeper soil. These findings suggest that both forest restoration sites follow a top-to-bottom effect intensity in the soil profile for recovering SOM quantities. Several other studies on forest restoration have also shown that SOM properties of the upper soil layers are primarily regenerated due to adjacency to litter deposition and higher root concentration, benefiting biological activity and the incorporation of organic matter (Don et al., 2011; Medeiros et al., 2017).

Our  $\delta^{13}\text{C}$  results validated that over 93% of the soil C of the primary forest originated from  $\text{C}_3$  plants. In contrast,  $\delta^{13}\text{C}$  values of the pasture reflected its long-term cultivation, with all soil layers containing more than 88% of C originated from  $\text{C}_4$  plants (grasses). Unexpectedly, the younger forest restoration (8-year-old) displayed 14.2–17.0% more C from  $\text{C}_3$  plants than the older restoration (15-year-old), depending on the soil layer. These results suggest the effectiveness of improved ecological restoration techniques employed in the younger forest. Rapid growth and efficient control of invasive grasses were achieved through the initial planting of trees and green manure. As a substantial portion of green manure plants senesced with time, they contributed to the accrual of soil C through the decomposition of their biomass. Additionally, the presence of green manure plants increased N-fixation (as indicated by the lower  $\delta^{15}\text{N}$  and higher *nifH* gene abundance [see Figure S3]), which improves biological activity, C cycling efficiency, and soil C accumulation rates (Mayer et al., 2020).

We assessed the quality of SOM using laser-induced fluorescence spectrum (LIFS) and the humification index ( $\text{H}_{\text{LIFS}}$ ) for the first time in forest restoration.  $\text{H}_{\text{LIFS}}$  balances SOM fractions (a division of LIFS by the C content), so caution is required when interpreting it. If  $\text{H}_{\text{LIFS}}$  is significantly higher than the local primary forest, which is a reference for a functionally stable edaphic ecosystem (Teixeira et al., 2020), it suggests excessively accelerated SOM transformation or a poor recent addition of organic material. Our forest restorations and pasture sites' deeper soil layers (10–30 cm) had at least 34% higher  $\text{H}_{\text{LIFS}}$  values than the primary forest. Thus, SOM fractions balance was not restored to a degree comparable to a native environment, showing a lower presence of metabolizable organic compounds (Bayer et al., 2006) that are fundamental for biological activity and nutrient cycling (Kravchenko et al., 2019). Based on our  $\text{H}_{\text{LIFS}}$  results, the take-home message is that 8–15 years of active forest restoration and reforming pasture have fully restored SOM fractions balance only in the topsoil layer. However, in deeper layers, legacies of land degradation remain, and improvements are still needed to increase labile SOM.

#### 4.3. Relations between soil organic matter fractions and other soil health indicators

RDA and correlation heatmap visually re-emerge and summarize evidence that the primary forest accumulated higher levels of C and N in the soil and improved SOM quality by achieving a better balance of SOM fractions, resulting in lower  $\text{H}_{\text{LIFS}}$  values. The forest's higher SOM quantity and quality reflected increased nutrient storage capacity (indicated by increased CEC), enhanced soil structure (suggested by lower bulk densities and higher macroporosity), and higher  $\text{CH}_4$  consumption (which helps to mitigate GHG emissions). While the forest showed positive associations with various soil health indicators, including CEC, macroporosity,  $\text{CH}_4$  consumption, and lower soil bulk density and  $\text{H}_{\text{LIFS}}$ , these indicators were interrelated with SOM properties (see Fig. 7B). Our findings regarding SOM and soil health align with other studies (e.g., Steudler et al., 1996; Lal, 2005; Cherubin et al., 2016; Pedrinho et al., 2019; Bieluczyk et al., 2020, 2021), highlighting the impact of converting forests into pastures on physical, chemical, and biological soil properties. These researchers emphasize that undisturbed forests harbor an environment that protects and accumulates high-quality SOM, supporting critical ecosystem functions such as nutrient storage and biodiversity. However, the forest-to-pasture conversion degenerates the properties of SOM and the potential of soils to fulfill its functions.

Although the pasture had a negative impact on most soil health indicators compared to the native forest, the management improved some chemical attributes, showing higher pH values (indicating better regulation of soil acidity) and higher levels of P and soil bases (indicating higher nutrient availability). These improvements were a result of liming and fertilization, as shown in previous studies (e.g., Pedrinho et al., 2019; Bieluczyk et al., 2020). However, in the pasture, soil  $\text{CH}_4$

consumption was lower or even emitted depending on the soil profile position, likely due to a broader amplitude of soil temperature and moisture that impairs methanotrophy (Fernandes et al., 2002; Carmo et al., 2012), or limited oxygen diffusion caused by compaction, creating suitable conditions for methanogenic activity (Steudler et al., 1996). Our findings are consistent with the literature because air and soil temperatures were 16 and 6 °C higher in the pasture than in the primary forest (refer to Figure S5) during gas sampling at the same moment of the day. Additionally, the pasture soil exhibited a higher abundance of *mcrA* than forests (see Figure S4), indicating higher methanogenic activity. Several studies in the Amazon biome have previously reported higher soil *mcrA* abundance in pastures compared to forests (e.g., Meyer et al., 2017, 2020; Kroeger et al., 2021; Venturini et al., 2022), and this pattern seems to hold true for the Atlantic Forest as well.

The two forest restorations presented similarities in SOM and soil health attributes and were in an intermediate position between the primary forest and pasture, indicating partial rehabilitation of forest-like soil multifunctionality. Based on RDA and correlations, the key changes that must still take place in restorations towards the soil health of the forest, mainly in the 10–30 cm layer, include: (i) recovering soil structure to reduce bulk density and increase macroporosity, and (ii) increasing labile SOM to achieve balance in  $\text{H}_{\text{LIFS}}$  and gain CEC. Inter-cropping a mixture of complementary non-invasive functional herbaceous plants, either prior to or during the initial tree planting, is a potential approach to facilitate labile C input into deeper soil layers. Diversifying cover plants to grow deep and spatially abundant roots produces exudates and root litter, stimulating biological activity within its functional benefits (Lange et al., 2015; Dessureault-Rompré, 2022). However, the viability and compatibility of these herbaceous species with the forest are factors that still require further study. Despite this, based on our results, we can state that enhancing forest management to provide a variety and quantity of organic compounds for short-, medium-, and long-term turnover holds the potential to facilitate soil health regeneration and ecosystem functioning.

#### 4.4. Greenhouse gas fluxes

We evidenced that the undisturbed forest soil is a significant  $\text{CH}_4$  sink ( $141.1 \mu\text{g m}^{-2} \text{h}^{-1}$ ) due to the abundance of soil methanotrophs. Our primary forest area showed at least 30% higher *pmoA* gene abundance in the topsoil layer than other sites (Figure S4). Our findings are consistent with other studies showing that undisturbed upland tropical forests serve as substantial and persistent  $\text{CH}_4$  sinks (Fernandes et al., 2002; Zhao et al., 2019; Siqueira-Neto et al., 2021; Mombrini et al., 2022). However, the non-native vegetation areas are also oxidizing C- $\text{CH}_4$  in the soil, with emphasis on the 15-year-old restoration ( $-61.9 \mu\text{g m}^{-2} \text{h}^{-1}$ ). A lower potential was observed in the well-managed pasture ( $-8.8 \mu\text{g m}^{-2} \text{h}^{-1}$ ). While we corroborate the results of studies that have shown that secondary forests gradually bring back soil  $\text{CH}_4$  influx strength (e.g., Kroeger et al., 2021; Meyer et al., 2020), the  $\text{CH}_4$  consumption in well-managed pastures in the Atlantic Forest biome was also recently evidenced, sinking between  $-1.82$  and  $-0.87 \text{ kg C-CH}_4 \text{ ha}^{-1} \text{ yr}^{-1}$  (Amadori et al., 2022). On the other hand, pasture soils have also emitted significant amounts of  $\text{CH}_4$  (e.g., Fernandes et al., 2002; Meyer et al., 2020) because of their higher soil methanogenic activity (Kroeger et al., 2021; Venturini et al., 2022). Given this contrast, we understand that pasture management is decisive to the area's role in  $\text{CH}_4$  fluxes. Unmanaged or overgrazed pastures degrade the soil, which acts as a source of  $\text{CH}_4$ , while pastures conducted under conservation principles (such as our reformed pasture) reduce emissions or even sink  $\text{CH}_4$  in the soil.

The  $\text{CO}_2$  emission is associated with SOM decomposition and oxidation, heterotrophic microorganisms' activity, and plant root respiration, simultaneously modeled by soil environmental conditions such as temperature, moisture, oxygen, and nutrient availability (Wang et al., 2019; Siqueira-Neto et al., 2021). For example, a recent global

meta-analysis showed that converting forests to other land uses decreased soil C–CO<sub>2</sub> effluxes, a direct consequence of reducing soil microbial biomass and its respiration (Han and Zhu, 2020). Here, we did not find differences between primary forest, pasture, and 15-year-old restoration for C–CO<sub>2</sub> emissions on soil surface incubation, except for 30% higher effluxes for the 8-year-old restoration. Increased C–CO<sub>2</sub> emissions have been used as ecosystem productivity and belowground carbon allocation indicators (Raich and Nadelhoffer, 1989; Salimon et al., 2004) since they reflect the total biological activity in the soil (Carmo et al., 2012). In this context, we did find positive correlations between C–CO<sub>2</sub> efflux with soil C ( $r = 0.42$ ) and N ( $r = 0.44$ ). However, higher C–CO<sub>2</sub> in the 8-year-old restoration and mostly in pasture soil profiles can also be related to higher air and soil temperatures (Figure S5). Rising air and soil temperature with enough available soil moisture (as was the case in this study) multiply microbial populations, which degrade organic compounds and increase C–CO<sub>2</sub> emissions (Souza et al., 2019). As we can see, soil management and environmental factors which alter soil microbial communities also change CO<sub>2</sub> fluxes. Therefore, CO<sub>2</sub> emissions result from complex interactions of different elements in space and time, and it is challenging to trace straight-line explanations (Siqueira-Neto et al., 2021). However, unlike CH<sub>4</sub> emissions, CO<sub>2</sub> effluxes in our systems could have a positive relationship with a healthy functioning ecosystem because it embraces the respiration of soil organisms and plant roots, essential for performing soil functions.

## 5. Conclusions

Planting trees to restore the Atlantic Forest in severely degraded pastureland is a better option than reforming the pasture to mitigate GHG emissions. Our young 8–15 years restored sites captured at least 14 Mg C ha<sup>-1</sup> more than the pasture, particularly storing more C in aboveground pools. Forests in restoration are progressively regenerating C storage, soil organic matter properties, and soil health towards the primary forest state. However, as these forests are still young, they have only reached this state in the topsoil layer (0–10 cm), while the aboveground environment and deeper soil still carry legacies of previous historic degradation. Introducing green manure and dividing the plantation of native trees into two rounds during restoration management brings new benefits, such as increased biological nitrogen fixation and faster soil C accrual. However, the performance of aboveground C storage, GHG emission mitigation, quality of soil organic matter, and soil health seemed to be more influenced by the age of active restoration than by the implementation management. We conclude that both the forest restoration sites are efficiently regenerating C and N stocks towards a native healthy ecosystem, benefiting nature and humans with essential ecosystem services, such as mitigating GHG emissions. Simultaneously, our findings show that integrating quantity, quality, and origin of soil organic matter can predict restoration success as their conjunction is strongly related to soil health and functional ecosystems.

## Credit to authorship contribution statement

**Wanderlei Bieluczyk:** Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Visualization, Writing – original draft, Writing – review & editing. **Fernanda Ometto Asselta:** Conceptualization, Methodology, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Deisi Navroski:** Conceptualization, Investigation, Data curation, Writing – review & editing. **Júlia Brandão Gontijo:** Conceptualization, Methodology, Investigation, Data curation, Writing – review & editing. **Andressa Monteiro Venturini:** Conceptualization, Methodology, Investigation, Data curation, Writing – review & editing. **Lucas William Mendes:** Conceptualization, Funding acquisition, Methodology, Investigation, Formal analysis, Data curation, Writing – review & editing. **Carla Penha Simon:** Investigation, Data curation, Writing – review & editing. **Plínio Barbosa de Camargo:**

Conceptualization, Resources, Funding acquisition, Supervision, Writing – review & editing. **Amanda Maria Tadini:** Conceptualization, Investigation, Methodology, Data curation, Writing – review & editing. **Ladislau Martin Neto:** Conceptualization, Resources, Writing – review & editing. **José Albertino Bendassolli:** Resources, Funding acquisition, Writing – review & editing. **Ricardo Ribeiro Rodrigues:** Conceptualization, Methodology, Resources, Funding acquisition, Investigation, Writing – review & editing. **Wim H. van der Putten:** Conceptualization, Project administration, Funding acquisition, Resources, Supervision, Writing – review & editing. **Siu Mui Tsai:** Conceptualization, Project administration, Funding acquisition, Resources, Supervision, Writing – review & editing.

## 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.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jenvman.2023.118573>.

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