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Forest restoration rehabilitates soil multifunctionality in riparian zones of sugarcane production landscapes

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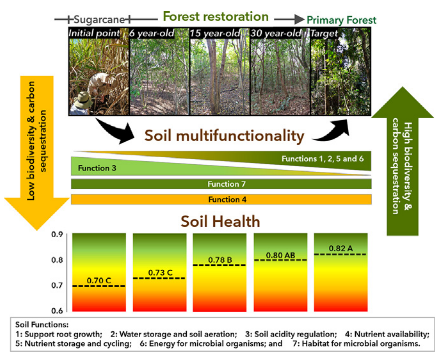
HIGHLIGHTS

- Riparian forest restoration increases soil C stocks and regenerates soil health.
- Forest to long-term sugarcane conversion reduced 30.6 Mg ha^{-1} of soil C stocks.
- Active forest restoration with 6–30 years increased 16–20 Mg C ha^{-1} stored in soils.
- Riparian forest restoration rehabilitated soil's multifunctionality with time.
- Forest restoration reached native forests' soil health and C stock levels in 30 years.

GRAPHICAL ABSTRACT



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ABSTRACT

Brazilian sugarcane plays a vital role in the production of both sugar and renewable energy. However, land use change and long-term conventional sugarcane cultivation have degraded entire watersheds, including a substantial loss of soil multifunctionality. In our study, riparian zones have been reforested to mitigate these impacts, protect aquatic ecosystems, and restore ecological corridors within the sugarcane production landscapes. We examined (i) how forest restoration enables rehabilitation of the soil's multifunctionality after long-term sugarcane cultivation and (ii) how long it takes to regain ecosystem functions comparable to those of a primary forest. We investigated a time series of riparian forests at 6, 15, and 30 years after starting restoration by planting trees (named 'active restoration') and determined soil C stocks, $\delta^{13}C$ (indicative of C origin), as well as measures indicative of soil health. A primary forest and a long-term sugarcane field were used as references. Eleven soil physical, chemical, and biological indicators were used for a structured soil health assessment, calculating index scores based on soil functions. Forest-to-cane conversion reduced 30.6 Mg ha^{-1} of soil C stocks, causing soil compaction and loss of cation exchange capacity, thus degrading soil's physical, chemical, and biological functions. Forest restoration for 6–30 years recovered 16–20 Mg C ha^{-1} stored in soils. In all restored sites, soil functions such as supporting root growth, aerating the soil, nutrient storage capacity, and providing C energy for microbial activity were gradually recovered. Thirty years of active restoration was sufficient to reach

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the primary forest state in overall soil health index, multifunctional performance, and C sequestration. We conclude that active forest restoration in sugarcane-dominated landscapes is an effective way to restore soil multifunctionality approaching the level of the native forest in approximately three decades. Moreover, the C sequestration in the restored forest soils will help to mediate global warming.

1. Introduction

Brazil is the world's leading sugarcane producer, accounting for 40 % of global production (FAOSTAT, 2022). The country has expanded over 10 million hectares of sugarcane cultivation in the past 22 years (CONAB, 2022). Most of the sugarcane landscapes are located in the Atlantic Forest region, which is the most environmentally devastated biome in Brazil. Agricultural activities, including sugarcane cultivation, have caused severe fragmentation of the remaining fragments of the Atlantic Forest. Despite this degradation, the biome remains to be one of the world's biodiversity hotspots (Myers et al., 2000; Laurance, 2009; Rezende et al., 2018). Currently, sugarcane field management is moving towards a more sustainable production by stopping burning prior to harvesting in combination with conservation tillage, crop residue maintenance, rational fertilization, and recycling of by-products, such as vinasse (Cherubin et al., 2021a). In addition, the sugarcane expansion is replacing degraded pastureland, which benefits the environment (Bordonal et al., 2018; Hernandes et al., 2022), while sugarcane-derived ethanol is fundamental for mitigating greenhouse gas emissions from fossil fuels in the energy matrix (Cerri et al., 2022; Maia and Bozelli, 2022). However, there are still challenges to further enhancing the sustainability of sugarcane production, as heavy machinery used for management and renewal of the plantations compacts and degrades the soil structure, which increases soil erosion (Gomes et al., 2019; Ogura et al., 2022).

The Brazilian landscapes dominated by sugarcane are located in a tropical climate with frequent and intense summer rains (>25 mm h^{-1}), which increase the soil erosion potential (Alvares et al., 2013; Medeiros et al., 2016; Youlton et al., 2016; Mello et al., 2020). Eroded particles consist of a mixture of soil, organic matter, fertilizers, nutrients, pesticides, and other solutes that may pollute the watersheds, often exacerbating in eutrophication and silting of rivers and lakes (Attanasio et al., 2012; Ogura et al., 2022). These impacts can be mitigated by restoring riparian forest buffers that prevent excessive runoff of eroded particles and filter the non-point source pollution (Gene et al., 2019). Restored forests in riparian zones also create ecological corridors and contribute to C sequestration in soil and vegetation (Dybala et al., 2019). This provides a wide range of ecosystem services (Brockerhoff et al., 2017), including the remediation of global warming (Zanini et al., 2021). Atlantic Forest restoration also helps Brazil to achieve the main goals for the global *Decade on Ecosystem Restoration* (2021 to 2030), contributing to reversing ecosystem degradation and mitigating climate change within its socio-environmental impacts (Brazil, 2015; UN, 2019).

Ecological restoration of forests in sugarcane landscapes requires intensive human intervention because these areas lack seed banks of trees from previous primary forests (Tambosi et al., 2014; Poorter et al., 2021). For this situation, the most cost-efficient alternative is active ecological restoration (Rodrigues et al., 2009a) through planting a diverse set of native tree species to speed up the recovery of a forest canopy, activate natural succession quickly, and later provide forest self-perpetuation (Rodrigues et al., 2009a; Brancalion et al., 2015, 2016). However, studies focused on ecosystem restoration are scarce compared to assessments of land-use change in Brazil (Rocha et al., 2015), and 59 % of the Atlantic Forest restoration assessments did not include soil analysis, despite its critical role in determining the success of restoration efforts (Mendes et al., 2019). This knowledge gap is particularly concerning given that healthy soil is widely recognized as an essential component for the establishment and long-term sustainability of functional forest ecosystems (Nolan et al., 2021).

Soil health has been defined as "the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans" (Lehmann et al., 2020). However, soil health analyses integrating chemical, physical, and biological indicators and soil functioning approaches are rare in the Atlantic Forest biome (Simon et al., 2022) and beyond. Soil health indicators should reflect soil multifunctionality (*i.e.*, the ability of the soil to deliver multiple functions or services simultaneously) while being sensitive to land-use changes and land management (Bünemann et al., 2018; Rinot et al., 2019; Hu et al., 2021). Furthermore, integrating these indicators into an overall soil health index (SHI) facilitates decision-making to enhance forest restoration management and agroecosystem sustainability (Schoenholtz et al., 2000; Cherubin et al., 2016a, b).

In the present study, we used soil health scores to assess the effects of active ecological restoration of riparian Atlantic forests on soil multifunctionality in sugarcane landscapes. Soil health analysis was based on a dataset of eleven indicators of chemical (pH, total N, available P and K, and cation exchange capacity [CEC]), physical (macroporosity [MaP], microporosity [MiP], and bulk density [Bd]), and biological (total C, fungal [fung-ITS], and bacterial [bact-16S] abundance) soil properties. These indicators are considered to represent essential soil functions well and are listed among the most used for Brazilian soil health integrative assessments (Simon et al., 2022). Soil health analyses have been reported in the literature (*e.g.*, Cardoso et al., 2013; Cherubin et al., 2016a, 2016b; Muñoz-Rojas, 2018; Rinot et al., 2019; Lehmann et al., 2020; Simon et al., 2022). In our study, we also assessed the restoration of carbon storage capacity of the soil, as this is a crucial ecosystem service to mitigate climate warming. In addition, we determined soil $\delta^{13}C$ to reveal the historical legacy of land use. Using $\delta^{13}C$ enables the quantification of C stocks derived from trees and other C_3 plants in the restoration, such as green manure leguminous plants (Balesdent et al., 1988), thus reflecting soil C accumulation by the restored forest (Smith and Epstein, 1971; Assad et al., 2013).

The present study aimed to evaluate the influence of long-term forest-to-sugarcane conversion and subsequent forest restoration on soil C storage, soil physical, chemical, and biological health indicators, and to develop a structured soil health assessment. To quantify the rate of restoration of soil functions, we examined a chronosequence of 6-, 15-, and 30-year-old restored forests, and compared those data with a long-term sugarcane area as the start of ecosystem restoration, and a primary semideciduous seasonal forest as the natural reference. We tested the hypothesis that riparian forest restoration in the sugarcane-dominated landscape would increase soil C storage and multifunctionality, by investigating at what time since restoration the soil properties would resemble those of a natural Atlantic Forest. In particular, we examined how: (i) the C stocks increase with age of restored forests and how this compares to reference forest; (ii) active riparian forest restoration influences the soil's physical, chemical, and biological properties; and (iii) restoration of riparian forests enhance soil's multifunctionality and health.

2. Material and methods

2.1. Study site

The study areas were located in Piracicaba and Ribeirão Preto mesoregions, São Paulo state, in the Atlantic Forest Biome in southeastern Brazil (Fig. 1). The climate is transitioning between the classes Cwa and Aw according to the Köppen classification, with dry winters and rainy summers (Alvares et al., 2013), where the annual rainfall ranges from 1400 to 1600 mm (INMET, 2022). The soil of all assessed areas was classified as

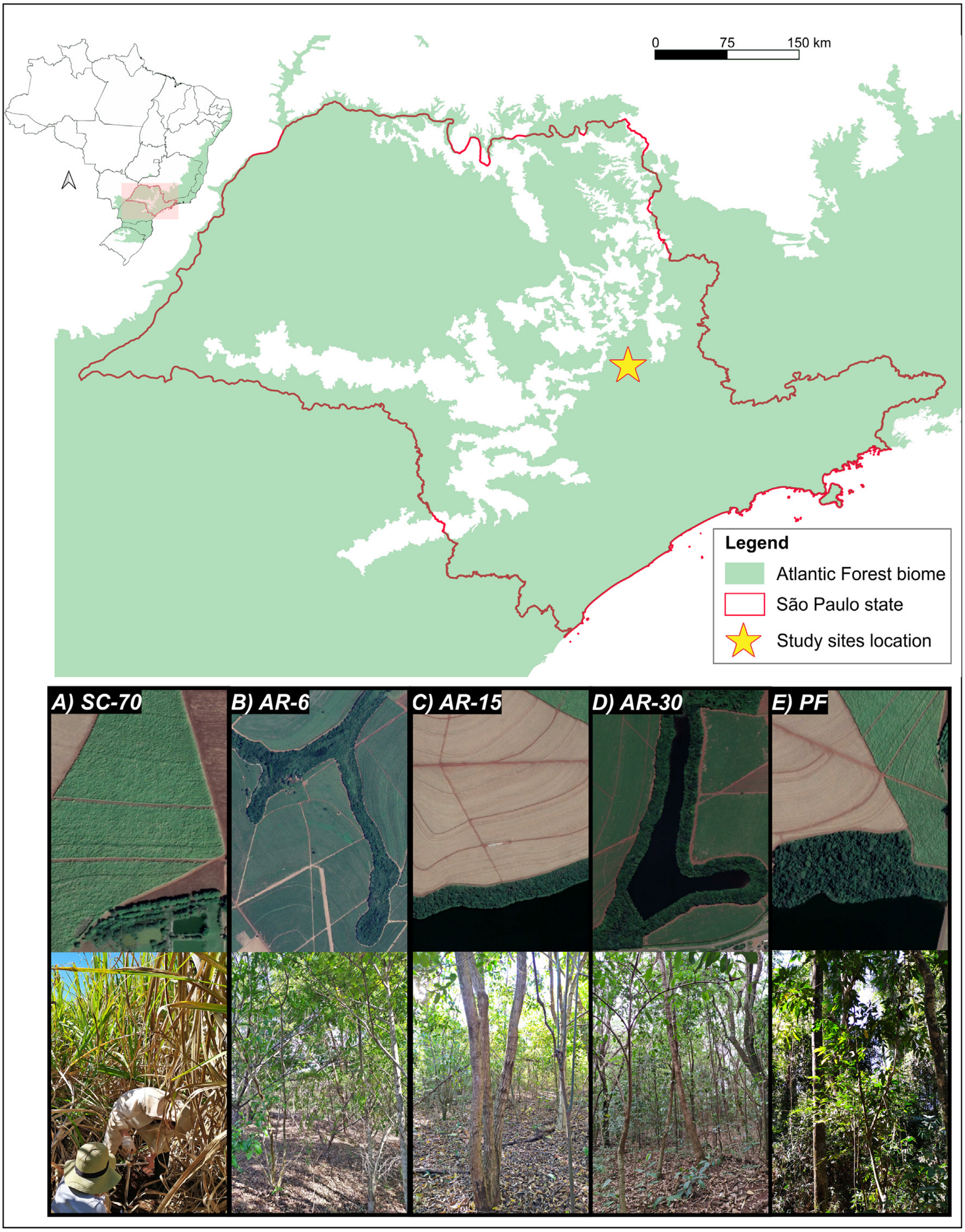

Fig. 1. Location of the study sites of long-term sucarcane production (SC-70), active forest restoration with 6 (AR-6), 15 (AR-15), and 30 (AR-30) years post-implementation, and primary forest (PF) within the Atlantic Forest biome and São Paulo state. The aerial photos were taken from 2020 and obtained online through Google Earth Pro. Terrestrial photos were taken by Wanderlei Bieluczyk.

Oxisol (Soil Survey Staff, 2014), *Latossolo Vermelho Distrófico típico*, and *Latossolo Vermelho Distroférrico típico* in Brazilian Classification (Embrapa Solos, 2013), with a clay texture (Fig. S1) and a maximum of 8 % slope of the landscape.

Most of the original Seasonal Semideciduous Forest area in the region, including riparian forests, has been cleared for agricultural use centuries ago (Fonseca, 1985), after which sugarcane has been cultivated until the present. Until 2005/2006, the sugarcane fields have been burned to facilitate the harvesting. Since 2006 the farms gradually began to keep the crop residues on the field, which has been the standard practice since 2012, when burning was banned in São Paulo State (Gomes et al., 2019). Meanwhile, riparian buffers began to be restored after 1980, and after numerous updates in Brazilian environmental laws, they are now protected by federal law N° 12,561 (Brazil, 2012). Currently, ecological restoration is mandatory for riparian areas still in use for agriculture.

We studied soil processes in three riparian buffer forests that have been restored on land previously used for sugarcane cultivation. Active methodology of ecological restoration was implemented following the techniques proposed by Rodrigues et al. (2009a, b) and Brancalion et al. (2015, 2016). Briefly, the procedures were as follows: (i) glyphosate (4 L ha^{-1}) and mechanized mowing were used to reduce competition with invasive exotic grasses; (ii) lime was superficially spread on the soil; (iii) to further plant the seedlings, the soil was mechanically prepared in lines performing minimal soil tillage plus fertilized down to 40 cm depth; (iv) >90 native tree species were planted in the rows, mixing fast-growing species for canopy cover (to shade the area quickly and suppress exotic grasses) with species from the functional diversity group (to guarantee the forest perpetuation); and, (v) up to two years after restoration implementation, pests such as ant infestation were chemically controlled, and nitrogen was applied by topdressing the young trees. Liming and fertilization were based on 0-40 cm soil layer analysis (van Raij et al., 1996, 2001). These forests are currently in different stages of development, 6-, 15- and 30 years after the start of the restoration (seedlings planting). Two areas were used as references near the forest restoration sites: a long-term sugarcane production area and a primary forest. The history of the areas is summarized in Fig. 2, and a further description of these lands is available in Table S1.

2.2. Soil sampling

Soil samples were collected in October and November 2020. Ten small trenches, each measuring approximately 50 × 50 × 50 cm (depth, width, and length), were excavated in each assessed site ($n = 10$), totaling 50 trenches across the five evaluated areas. Sampling points were located at least 30 m from forest edges (restoration-sugarcane), except for the primary forest, where the minimum distance was 80 m. Sampling points were distributed strategically to represent each assessed land area based on their respective sizes. The sizes of the sugarcane, 6-, 15-, and 30-year-old restorations, and the primary forest were 16, 30, 13, 25, and 13 ha, respectively. In the sugarcane area, we collected samples between the plant rows and avoided sampling inter-rows with recent and visible agricultural machinery traffic.

Disturbed soil samples were manually and carefully collected from the walls of the small trenches using a stainless-steel spatula and pedological knife to obtain soil from the 0–10, 10–20, and 20–30 cm layers for quantifying soil chemical and biological properties. In the same layers, undisturbed samples were collected for physical analysis in 5 × 5 cm volumetric rings using an Uhland-type auger. About 500 g of disturbed soil samples were packed in plastic bags for chemical and texture analysis. In addition, 50 mL Falcon tubes were aseptically filled with soil for further biological analysis. These samples were immediately placed in a thermal box, transported on ice to the laboratory, and stored at −20 °C.

2.3. Soil analyses

2.3.1. Soil texture and chemical properties

Soil samples for chemical and texture analyses were air-dried, ground, and sieved using a 2 mm mesh size. Soil chemical parameters (*i.e.*, the potential of hydrogen in $CaCl_2$ [pH_{CaCl2}], potential acidity [H + Al], available calcium [Ca], magnesium [Mg], aluminum [Al], phosphorus [P], and potassium [K]) were measured by analytical methods described in van Raij et al. (2001). Soil texture (*i.e.*, clay, silt, and sand contents) was quantified according to Teixeira et al. (2017). The texture of all areas was classified as clay according to the USDA Soil Texture Triangle (Fig. S1).

Soil subsamples, about 5 g previously air-dried and sieved at 2 mm, were macerated and then sieved at ≤149 μm to quantify soil C, N, and ^{13}C contents. These elements were analyzed with 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) at the Stable Isotope Laboratory of the Center of Nuclear Energy in Agriculture (CENA/USP). Isotope results were expressed as $\delta^{13}C$ (‰), using the international standards (Vienna PeeDee Belemnite – V-PDB for C [NBS19 and NBS22] as a reference for ^{13}C values. Delta values were based on standards, and the following equation was used for calculations: $\delta X = [(R_{sample} / R_{standard}) - 1]$ multiplied by 1000, where X refers to ^{13}C or ^{15}N and R_{sample}, and $R_{standard}$ are the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratios of sample and standard, respectively (Farquhar et al., 1982). Next, the proportion of C derived from C_3 plants (*e.g.*, trees and leguminous herbs) and C_4 plants (*e.g.*, tropical grasses such as sugarcane) was calculated using the mass balance equations as described in Balesdent et al. (1988).

Soil C stocks (Mg ha^{-1}) were quantified by multiplying the soil C contents (%), the soil density (Mg m^{-3}), and the thickness of the layer (cm). Considering that the soil density was higher in the sugarcane and restoration areas compared to the primary forest, uncorrected results would systematically overestimate soil C stocks in the managed areas. Thus, C and N stocks were corrected by the equivalent mass method, as described in Ellert and Bettany (1995), using the primary forest, with the lowest soil bulk densities, as a reference.

2.3.2. Soil physical properties

The 5 × 5 cm volumetric rings containing the sampled soil were saturated and placed on a tension table for 24 h under suction of 60 cm of the water column and weighed to quantify the macroporosity (MaP). Then, in sequence, they were dried in an oven at 105 °C for 48 h and weighed again to quantify the microporosity (MiP) and soil bulk density (Bd) according to the methods of Teixeira et al. (2017).

2.3.3. Soil biological properties

The soil DNA extractions were performed by weighing 0.25 g of soil, using the PowerLyzer PowerSoil DNA Isolation Kit (Qiagen, Hilden, German), and following the extraction procedures for tropical soils (Venturini et al., 2020). Briefly, the soil sample was homogenized, and the microbial cells were then lysed by mechanical and chemical methods. Next, the total genomic DNA was captured on a silica membrane in a spin column format, washed, and eluted from the membrane to obtain the extract. Finally, the DNA concentration was measured using a Qubit fluorometer

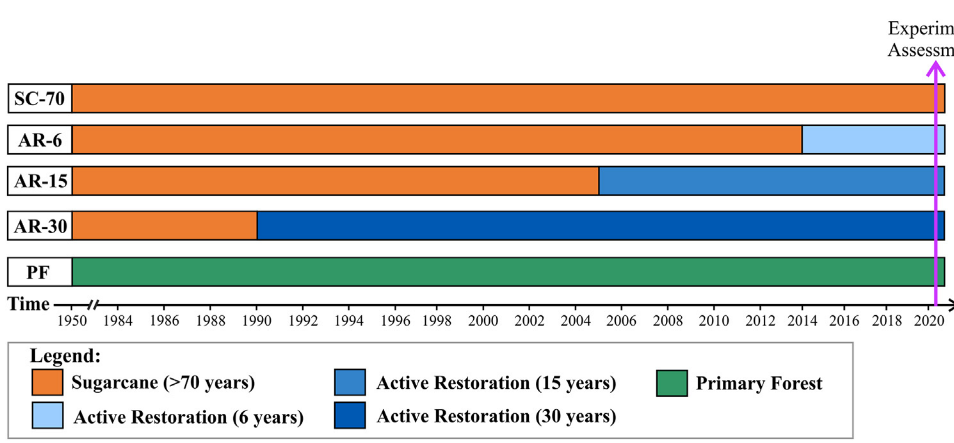

Fig. 2. Land use history of the evaluated study areas.

(Invitrogen, Carlsbad, USA), followed by electrophoresis analysis in 1 % sodium boric acid agarose gel (Brody and Kern, 2004).

The abundance (total number) of microbial communities was measured using the StepOnePlus™ Real-Time PCR System (qPCR) with 96-well plates (Applied Biosystems, Foster City, CA, USA). The qPCR analysis was performed for communities of bacteria (bact-16S), based on the bacterial 16S rRNA gene, and fungi (fung-ITS), based on the fungal ITS gene. Standard curves based on serial dilutions for each gene were created using DNA isolated from a pure culture. Melting curve analyses were performed from 68 to 95 °C. All standard curves showed R^2 >0.98. Results were analyzed using StepOnePlus™ Real-Time software version 2.2.2 (Applied Biosystems, Foster City, CA, USA). The results were exported to Excel (Microsoft) and converted to the number of gene copies per gram of soil. The strains used to construct the standard curves, primers, and reaction conditions for gene amplification are described in Table S2.

2.4. Soil health index calculation

The soil health index (SHI) framework calculation (Table 1) followed three steps previously outlined in the literature (Cherubin et al., 2016a, 2016b; Bünemann et al., 2018; Rinot et al., 2019; Simon et al., 2022).

In STEP I (*selection*), we selected appropriate soil attributes to represent seven critical soil functions: f(i) support root growth; f(ii) water and aeration supply for plants and fauna; f(iii) soil acidity regulation; f(iv) nutrient availability; f(v) nutrient storage and cycling; f(vi) energy provision for biological activity; and f(vii) habitat for microbial organisms. Based on soil health assessments in the literature, a minimum dataset of eleven indicators of chemical (pH, N, P, K, and CEC), physical (MaP, MiP, and Bd), and biological (C, fung-ITS, and bact-16S) soil properties were used to determine how multifunctionality was being performed under sugarcane, forest restorations, and primary forest reference. These indicators have been previously used to represent the soil function scoring in literature (Cherubin et al., 2016a, 2016b; Rinot et al., 2019; Simon et al., 2022). Briefly: (i) soil pH reveals possible soil acidity restrictions for nutrient uptake by plants or soil microbial survival; (ii) N, P, and K indicate soil nutrient availability; (iii) CEC corresponds to the soil nutrient storage and release; (iv) an equilibrated Bd is fundamental to supporting root growth; (v) MaP and MiP are directly associated with soil aeration and water retention, as tolerance to erosive processes; and, (vi) soil C is considered a key soil health parameter, supporting multiple processes, being the energy source for soil biological functional diversity. Furthermore, our assessment included new biological soil health indicators, as suggested by Lehmann et al. (2020).

Table 1
Model[a] of the soil functions framework and indicators for developing the soil health index (SHI).

Index	Component	Weight I	Soil functions	Weight II	Indicators	Weight III
SHI	Physical	0.33	Support root growth	0.50	Bd	1.00
			Water storage and soil aeration	0.50	MaP	0.50
					MiP	0.50
	Chemical	0.33	Soil acidity regulation	0.33	pH	1.00
			Nutrient availability	0.33	N	0.33
					P	0.33
					K	0.33
			Nutrient storage and cycling	0.33	CEC	1.00
	Biological	0.33	Energy for biological activity	0.50	C	1.00
			Habitat for microbial organisms	0.50	fung-ITS	0.50
					bact-16S	0.50

[a] Adapted structure from previous outlines (Cherubin et al., 2016a, 2016b; Bünemann et al., 2018; Rinot et al., 2019; Simon et al., 2022). **Bd:** bulk density, **MaP:** macroporosity, **MiP:** microporosity, **pH:** pH_{CaCl2}, **N:** total nitrogen content, **P:** available phosphorus, **K:** available potassium, **CEC:** cation exchange capacity in pH = 7, **C:** total carbon content, **fung-ITS:** total fungal community and, **bact-16S:** total bacterial community.

We added bact-16S and fung-ITS that mediate nutrient cycles and soil structuring, among other soil processes (Cardoso et al., 2013; Fierer, 2017).

STEP II (*indicator interpretation*) transformed each indicator into a unitless value by standardization, which linearly ranged from 0 to 1, to be later included in the SHI calculation. All the soil layers (0–10, 10–20, and 20–30 cm) were independently considered but also averaged in the 0–30 cm layer to represent the full assessed soil profile. The transformation was performed by ranking the values of indicators in ascending or descending order, depending on if the higher value was declared “good” or “bad” for soil functioning (Rinot et al., 2019). The pH, N, P, K, CEC, MaP, MiP, C, fung-ITS, and bact-16S were included in the ‘more is better’ approach, where each observation was divided by the highest observed value. Therefore, the highest observed value received a score of 1. For the ‘less is better’ indicators (only Bd), the lowest observed value (score = 1) was divided by each observation.

Lastly, in STEP III (*integration*), we used the weighted additive integration strategy (Rinot et al., 2019). Therefore, some indicators had a more significant influence than others in the final index because they were used individually, while others were combined to represent a soil function. First, each indicator was multiplied by weight to represent the seven soil-assessed functions. Then, soil functions were distributed in equal weights of 0.33 for each component of soil health (*i.e.*, chemical, physical, and biological sectors). The complete framework of calculation and integration is presented in Table 1.

2.5. Data analysis

The statistical analyses were performed using the R platform v. 4.1.2 (RStudio Team, 2022). Levene's and Shapiro–Wilk tests were applied to the results of each parameter to validate the requirements of variance (ANOVA), homogeneity of variance, and normality of errors. When necessary, the data were transformed using the Box-Cox technique (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, means were compared by the Tukey test (p < 0.05). The PCA analysis and Spearman correlations were performed for the soil function scores, which were calculated according to the Table 1 Model.

3. Results

3.1. Soil carbon stocks

The primary forest (PF) showed the highest soil C stocks for the 0–30 cm layer when compared to the other treatments (Fig. 3). The sugarcane area had lower soil C storage (65.5 Mg C ha^{-1}) when compared to the native forest (96.1 Mg C ha^{-1}) or the development of active forest restoration (78.7–85.7 Mg C ha^{-1}). After 6- (AR-6), 15- (AR-15), and 30 years (AR-30) after the start of forest restoration, the soil of these areas showed 25, 20, and 31 % greater C stocks than in sugarcane, respectively. Although no differences were found for soil C accrual between the three restoration areas (AR-6, AR-15, and AR-30), the oldest (30-year-old) restoration approached the C stocks level of the primary forest.

More than 90 % of the soil C accumulated in the primary forest (88.6 Mg C ha^{-1}) originated from plants with a C_3 (C—C_3) photosynthetic mechanism. Even with different ages, the 15- and 30-year-old restorations were not different for C—C_3 in the soil, accumulating 50.3 and 51.5 Mg ha^{-1}, respectively. The six-year-old restored forest had 4.6 Mg ha^{-1} more C—C_3 in the soil than the sugarcane area, but this difference was not statistically significant. C stocks originated from C_4 plants (C—C_4) were highest in AR-6 and sugarcane fields. Unexpectedly, the AR-30 contained 20 % greater soil C—C_4 stocks than the AR-15, although that site was restored twice as long ago.

The soil C—C_3 proportion increased with the age of forest restoration, mainly in the 0–10 cm soil layer (Fig. 4A). This layer showed the most significant effect on soil C additions by trees in the restored forests, with a

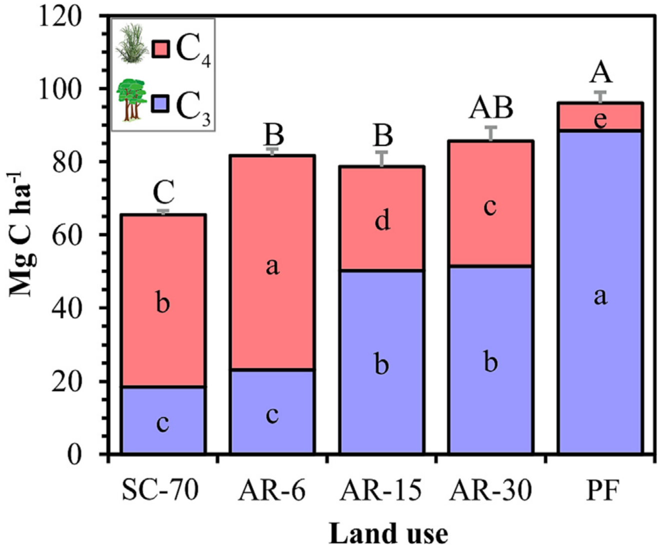

Fig. 3. Soil carbon stocks (0–30 cm layer) originated from C_3 and C_4 plants in the treatments assessed in the study. **SC-70:** 70 years of sugarcane production, **AR-6:** active restoration with six years post-implementation, **AR-15:** active restoration with 15 years post-implementation, **AR-30:** active restoration with 30 years post-implementation, and **PF:** primary forest. Uppercase letters compare treatments for total soil C stocks, and lowercase letters compare treatments for soil C stocks originating from C_3 and C_4 plants according to the Tukey test with 95 % confidence.

higher C_3/C_4 ratio when compared to the subsurface layers (Fig. 4B and C). The pattern was inversed in sugarcane with soil deepening, increasing the C—C_3 proportion from 20.2 % in 0–10 cm to 43.5 % in 20–30 cm.

3.2. Physical soil health indicators

In all evaluated soil layers, the highest soil bulk density (Bd) values were found in the sugarcane area (Fig. 5A). Bd did not differ between areas covered by forests (the primary and restored). However, it increased with soil depth in all treatments, smoothly in the primary forest (6 % increase) and more intensively in the 15-year restoration (18 % increase). The Bd of the younger restoration resembled the values of the sugarcane area in 10–20 and 20–30 cm soil layers, showing remnants of the previous agricultural management.

Macroporosity (MaP) and microporosity (MiP) must be interpreted in combination because the soil must simultaneously provide aeration (MaP) and water storage (MiP) for plant growth and soil biological activity. Although the six-year restoration was one of the greatest areas in MaP (Fig. 5B), the volume of soil occupied by micropores (Fig. 5C) was the smallest. Therefore, there were limitations to soil water retention. The sugarcane soil also presented imbalances, as it showed suitable MiP (Fig. 5C), but the macropore volume was at least 60 % lower than the other areas, with values between 0.05 and 0.08 $m^3\ m^{-3}$ (Fig. 5B). Generally, Bd and MaP of the 15- and 30-year restorations were not different from the primary forest, however, with some non-standardized differences in the soil profile.

3.3. Chemical soil health indicators

Soil pH ranged from 4.1 to 4.5 in the primary forest, which was well below the other areas (Fig. 6A). The soil pH was altered in all other treatments because of superficial liming for sugarcane production and forest restoration. Therefore, soil pH did not differ in the superficial layer of all these areas (*i.e.*, SC-70, AR-6, AR-15, and AR-30), ranging from 5.2 to 5.6. However, the sugarcane soil pH was higher at 10–20 and 20–30 cm compared to the forest restoration areas.

In the primary forest, soil N contents were at least 1.7, 1.4, and 1.2 times higher in the 0–10, 10–20, and 20–30 cm layers, respectively, than the contents of other areas (Fig. 6B). Sugarcane showed the lowest soil N content, mainly in the 0–10 and 10–20 cm soil layers, with values at least two times lower than in the primary forest. In the 0–10 cm layer, AR-6, AR-15, and AR-30 contained soil N contents 22, 72, and 70 % higher than in the sugarcane fields. N contents in the restoration areas decreased with depth and did not differ between forest ages in the 20–30 cm soil layer.

Soil P availability decreased from AR-15 > SC-70 > AR-30 > AR-6 = PF in all evaluated soil layers (Fig. 6C). Even with the periodic P replenishment in the sugarcane soil, the 15-year restoration site had higher soil P levels. Soil P levels in the two other restorations were considerably lower and did not differ from the primary forest. Soil available K did not follow the same pattern as P, and among all areas, K contents were highest in the six-year-old forest restoration (Fig. 6D).

In the 0–10 cm layer, the soil potential CEC (pH = 7.0) was 89, 98, 124, 151, and 152 $mmol_c\ kg^{-1}$ for SC-70, AR-6, AR-15, AR-30, and PF areas, respectively (Fig. 6E). The 30-year-old restored forest was the only one that reached the CEC level of the primary forest, specifically in the 0–10 and 10–20 cm soil layers. In 10–20 and 20–30 cm soil layers, the CEC from the two youngest forest restoration areas (six and fifteen years old) did

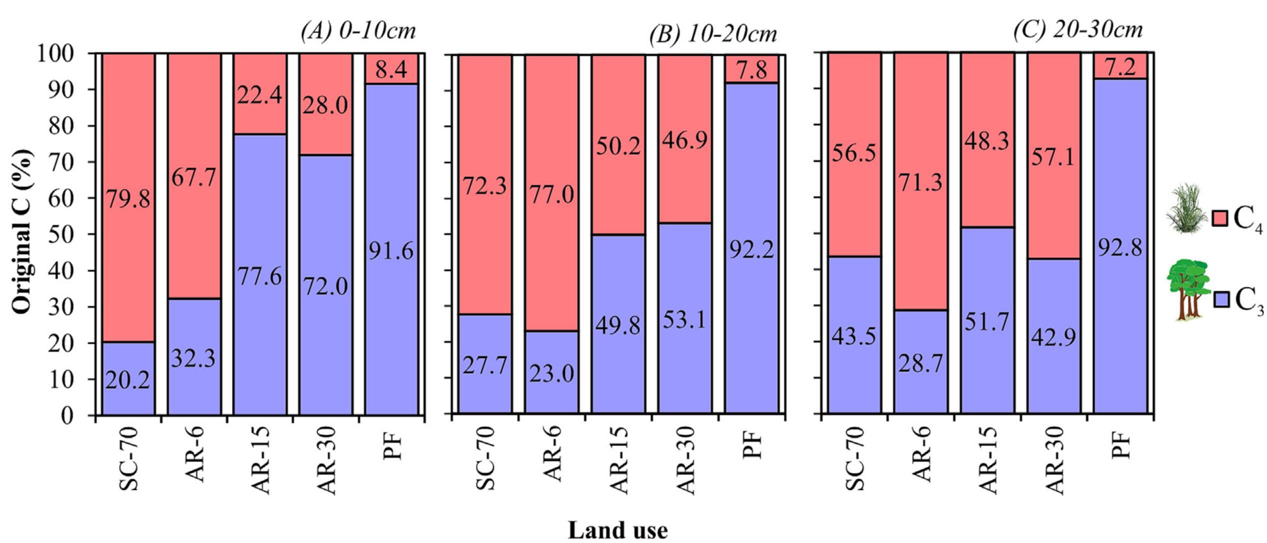

Fig. 4. Proportion of carbon from C_3 and C_4 plants in 0–10 (A), 10–20 (B), and 20–30 cm (C) soil layers in the treatments assessed in the study. **SC-70:** 70 years of sugarcane production, **AR-6:** active restoration with six years post-implementation, **AR-15:** active restoration with 15 years post-implementation, **AR-30:** active restoration with 30 years post-implementation, and **PF:** primary forest.

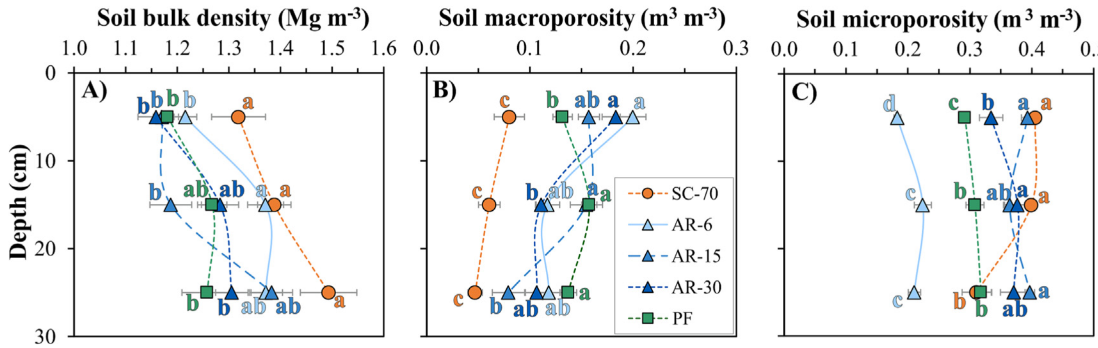

Fig. 5. Soil bulk density (A), microporosity (B), and microporosity (C) composing the physical soil quality indicators group in 0–10, 10–20, and 20–30 cm soil layers under the treatments of the study. **SC-70:** 70 years of sugarcane production, **AR-6:** active restoration with 6 years post-implementation, **AR-15:** active restoration with 15 years post-implementation, **AR-30:** active restoration with 30 years post-implementation, and **PF:** primary forest. The letters compare the treatments using the Tukey test with 95 % confidence.

not differ from sugarcane, but its values were at least 25 % lower than in the 30 years old restored forest.

3.4. Biological soil health indicators

Significant differences in soil C contents occurred in the superficial soil layer (0–10 cm), and ranged from 20.2 to 40.2 g kg^{-1}, depending on the treatment (Fig. 7A). In this layer, soil C increased from sugarcane, increased ages of forest restoration, to the primary forest. The sugarcane area showed the lowest soil C content, regardless of soil depth. However, soil C contents and their variation among treatments sharply decreased with soil depth (variation of 20, 4.6, and 1.7 g kg^{-1} in the 0–10, 10–20, and 20–30 cm soil layers, respectively).

The size of the fungal community (fung-ITS) was the highest in the sugarcane area in the 0–10 and 10–20 cm soil layers (Fig. 7B). In the superficial layer of the forests the fung-ITS values were not different (Fig. 7B). The same pattern was observed for the community of bacteria (bact-16S) (Fig. 7C). The size of the fungal and bacterial communities declined with soil depth, however, most smoothly in the soil profile of the primary forest and the 30-year active restoration forest.

3.5. Soil health index (SHI) scores

In the 0–10 cm soil layer, the overall (physical + chemical + biological) soil health index (SHI) scores were increased in the 15- and 30-year-old restoration areas, showing no statistical differences from the primary

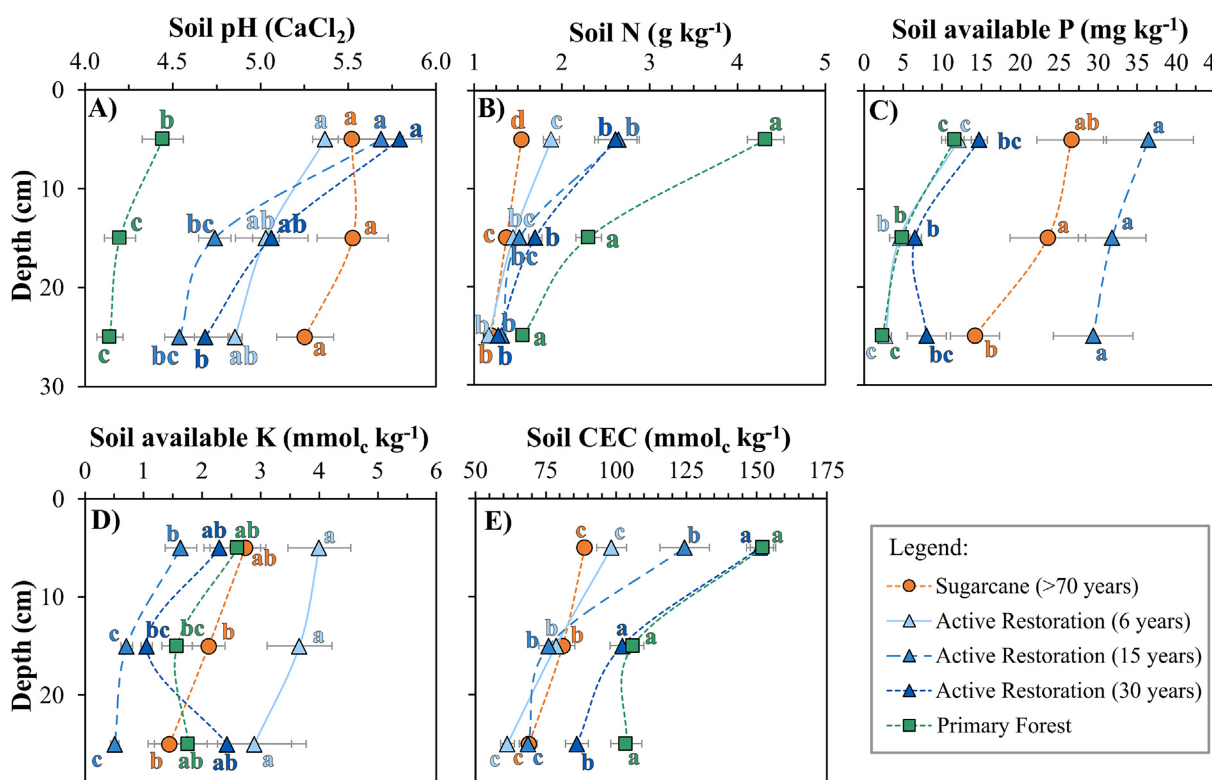

Fig. 6. Potential of hydrogen in calcium chloride [pH_{CaCl2}] (A), total soil nitrogen [N] (B), available phosphorus [P] (C) and potassium [K] (D), potential cation exchange capacity [CEC] (E) composing the chemical soil quality indicators group in 0–10, 10–20 e 20–30 cm soil layers under the treatments of the study. **SC-70:** 70 years of sugarcane production, **AR-6:** active restoration with 6 years post-implementation, **AR-15:** active restoration with 15 years post-implementation, **AR-30:** active restoration with 30 years post-implementation, and **PF:** primary forest. The letters compare the treatments using the Tukey test with 95 % confidence.

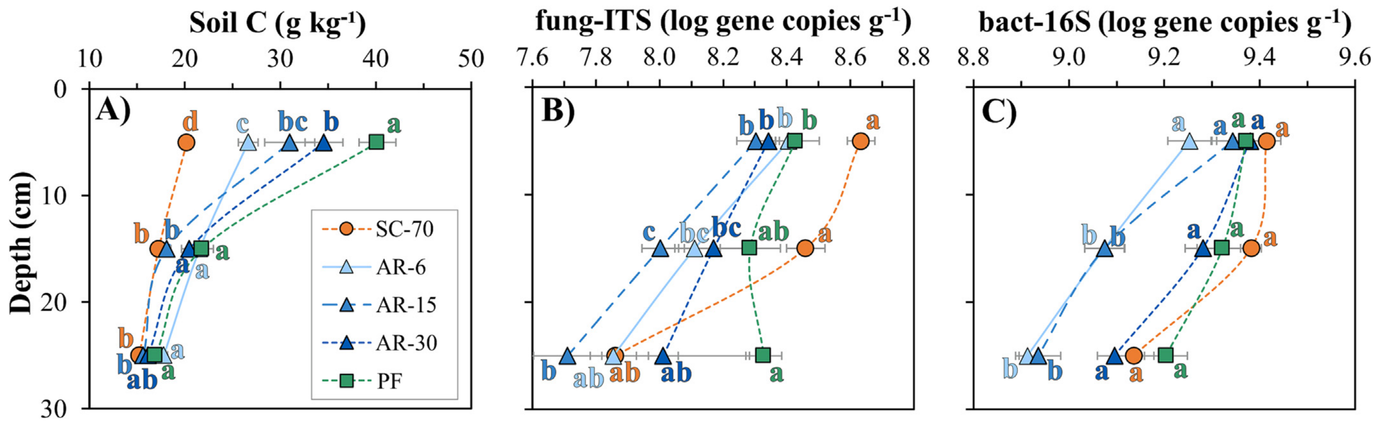

Fig. 7. Soil carbon contents (A), and size of fungi [fung-ITS] (B) and bacteria [bact-16S] (C) communities composing the biological soil health indicators group in 0–10, 10–20 e 20–30 cm soil layers under the treatments of the study. **SC-70:** 70 years of sugarcane production, **AR-6:** active restoration with 6 years post-implementation, **AR-15:** active restoration with 15 years post-implementation, **AR-30:** active restoration with 30 years post-implementation, and **PF:** primary forest. The letters compare the treatments using the Tukey test with 95 % confidence.

forest (Fig. 8A). However, sugarcane and AR-6 areas contained lower SHI scores for all individual components (*i.e.*, physical, chemical, and biological). In the 10–20 cm soil layer, there were no differences among treatments for $SHI_{chemical}$ scores (Fig. 8B). However, $SHI_{physical}$ scores were lower in SC-70 and AR-6. Specifically, the AR-6 showed the highest biological SHI scores in this layer, reaching levels that were observed in the primary forest. Furthermore, overall SHI scores in AR-15 and AR-30 were no different from the highest scores in PF and the lowest in AR-6 and SC-70. An ascending overall SHI gradient in the 20–30 cm soil layer was found, as follows: SC-70 < AR-6 < AR-15 < AR-30 < PF (Fig. 8C). Generally, the primary forest and 30-year-old restored forest scored better in all components in this soil layer. An exception was the AR-6 which, as in the upper contiguous soil layer, showed greater scores for the biological component.

The primary forest showed lower soil acidity regulation and low nutrient availability, which was common for all assessed areas (Fig. 9A; Table S3). Overall SHI scores (0–30 cm) suggest that the soils of 6-, 15-, and 30-year-old restorations performed at 73, 78, and 80 % of their potential soil health, respectively (Fig. 9B). Soil functioning in riparian restorations was greater when compared to sugarcane (SHI = 70 %), but it was inferior to that observed in the primary forest (SHI = 82 %). Overall, SHI scores were higher as older the forest restoration, and the oldest area (AR-30) was statistically similar to the primary forest.

The physical and biological components showed consistent differences between the treatments up to 30 cm soil depth (Fig. 9C). The soil's physical quality was at least 10 % lower in SC-70 and RA-6 if contrasted with the AR-15, AR-30, and PF. On the other hand, biological quality was 14 % higher in the soil of the primary forest when compared to sugarcane. In the 0–30 cm soil layer, there were no differences between the three restorations for the $SHI_{biological}$ scores. However, the 30-year-old area was the only one that did not differ from the primary forest in $SHI_{biological}$ scoring. Finally, given the inconsistencies in variations for the different individual chemical indicators (*i.e.*, pH, N, P, K, and CEC) (Fig. 6), the chemical component of the 0–30 cm soil layer did not differ among the assessed areas (Fig. 9C).

Greater soil functioning scores were preferentially related to the primary forest and the 30-year-old restoration area (Fig. 10). The PCA of the scores for soil functions showed that these areas provided more energy for microbial activity, nutrient storage and cycling, and support for plant growth (Fig. 10A). However, sugarcane and AR-6 were more associated with soil acidity regulation. The scores for this function did not follow the same trend as for the other functions, showed by negative correlations with support for plant growth ($r = -0.58$), water storage and aeration ($r = -0.43$), nutrient storage and cycling ($r = -0.31$), and energy for biological activity ($r = -0.34$) (Fig. 10B). As expected, we found positive correlations between the two physical functions ($r = 0.87$), showing an interdependency of the support for plant growth and water storage-soil aeration. Additionally, energy for

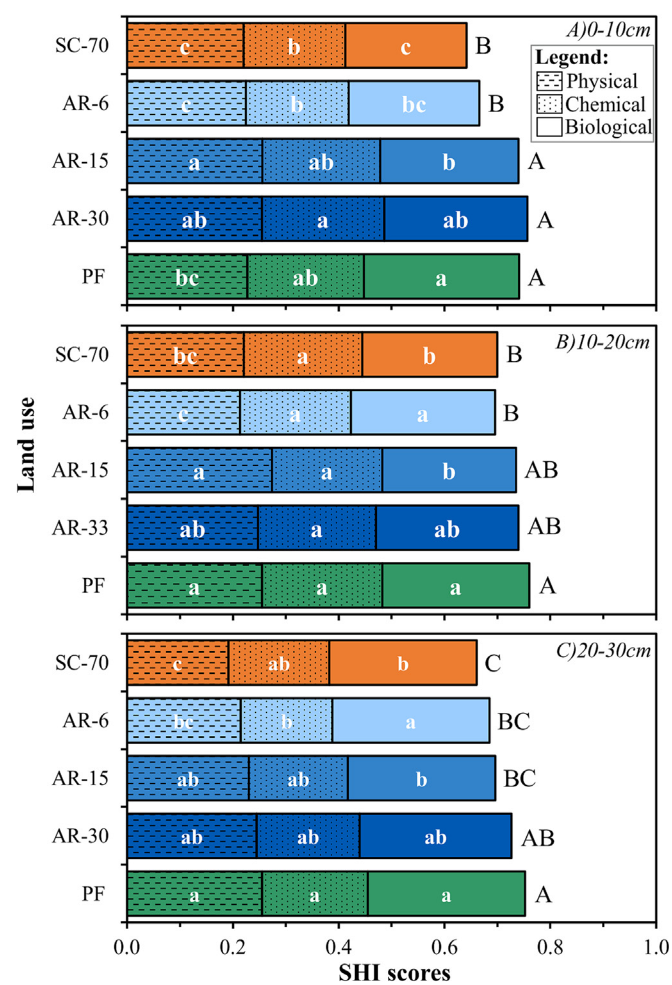

Fig. 8. Contribution of each sector of attributes (*i.e.*, physical, chemical, and biological) to the overall soil health index (SHI) scores under sugarcane in long-term cultivation (SC-70), active restoration with 6 (AR-6), 15 (AR-15) and 30 (AR-30) years post-implementation, and a primary forest (PF) in 0–10, 10–20 e 20–30 cm soil layers. Uppercase letters compare treatments for overall SHI scores, and lowercase letters compare treatments for each sector (physical, chemical, and biological) according to the Tukey test with 95 % confidence.

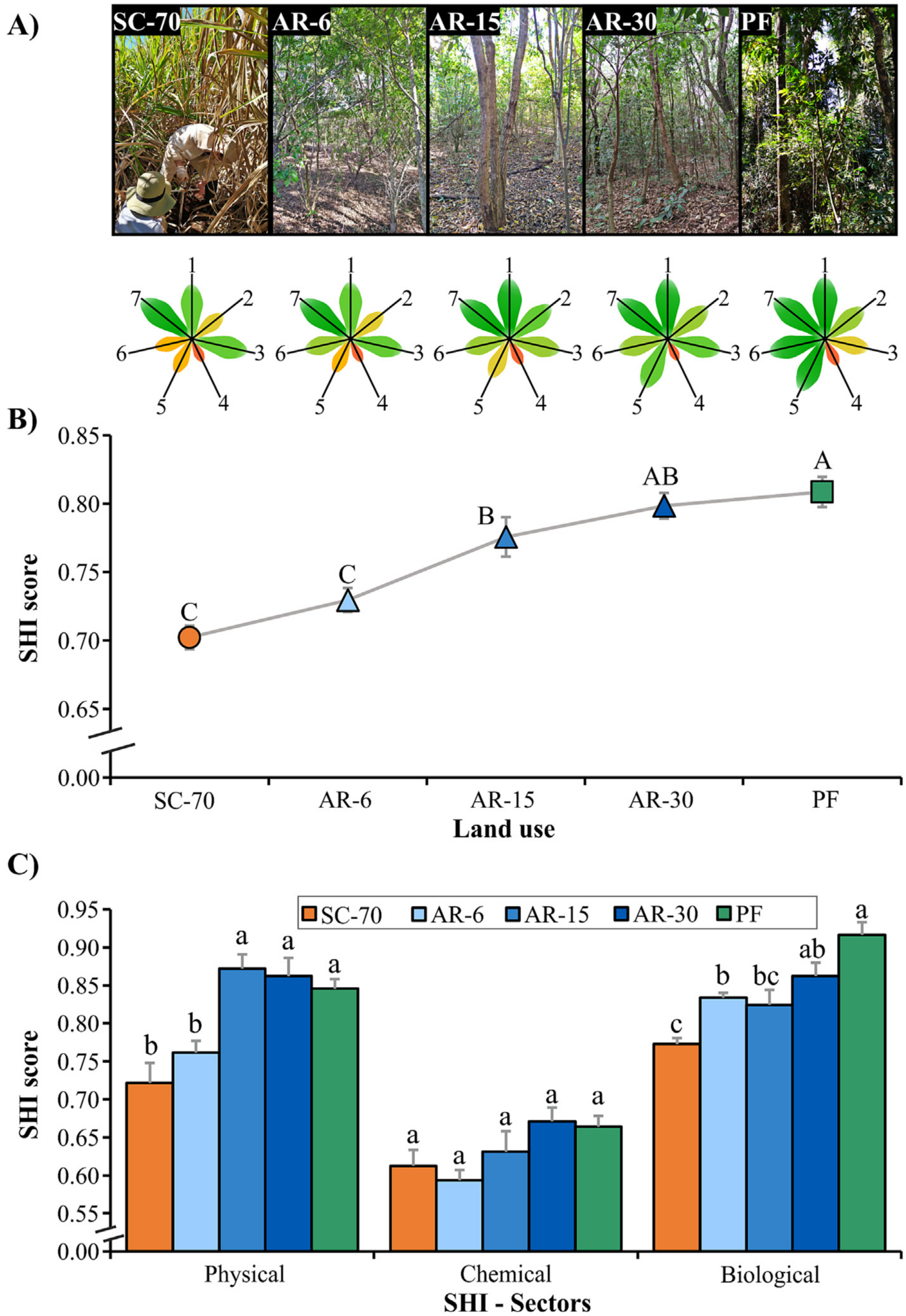

Fig. 9. Soil functional performance (A), overall soil health index (SHI) scores (B) and scores by chemical, physical and biological sectors (C) under sugarcane in long-term cultivation (SC-70), active restoration with 6 (AR-6), 15 (AR-15) and 30 (AR-30) years post-implementation, and a primary forest (PF) in 0–30 cm soil layer. Soil functions - **1:** Support root growth, **2:** Water storage and soil aeration, **3:** Soil acidity regulation, **4:** Nutrient availability, **5:** Nutrient storage and cycling, **6:** Energy for biological activity, and **7:** Habitat for microbial organisms. Uppercase letters compare treatments for overall SHI scores, and lowercase letters compare treatments for each sector (physical, chemical, and biological) according to the Tukey test with 95 % confidence.

microbial organisms was positively correlated ($r = 0.76$) with nutrient storage and cycling, suggesting their feedback in this weathered tropical soil. Lastly, the microbial organism's habitat function showed the lowest contribution for the PCA variation (smaller vector arrow).

4. Discussion

4.1. The losses of carbon and soil health due to forest-to-sugarcane conversion

Land-use conversion from native forest to agriculture on which sugarcane has been produced >70 years promoted significant soil C losses of 30.6 Mg C ha^{-1} and soil health decline (reducing 12 % of the overall SHI scores). It is relevant to consider that more sustainable sugarcane management with unburned mechanical harvesting, crop residue maintenance, and vinasse soil fertilization has been applied in the sugarcane fields from 2006 to 2020, thus benefitting soil health (Jiang et al., 2012; Trivelin et al., 2013; Cherubin et al., 2021b). However, these practices were still insufficient to recover the soil's physical and biological functions to the previous level of the primary forest. With this, we can also infer that previous losses in soil health and carbon stocks, until sugarcane crops were burned, could have been even higher than the current situation. Overall, SHI for the physical and biological sectors was at least 13 % lower in sugarcane than in

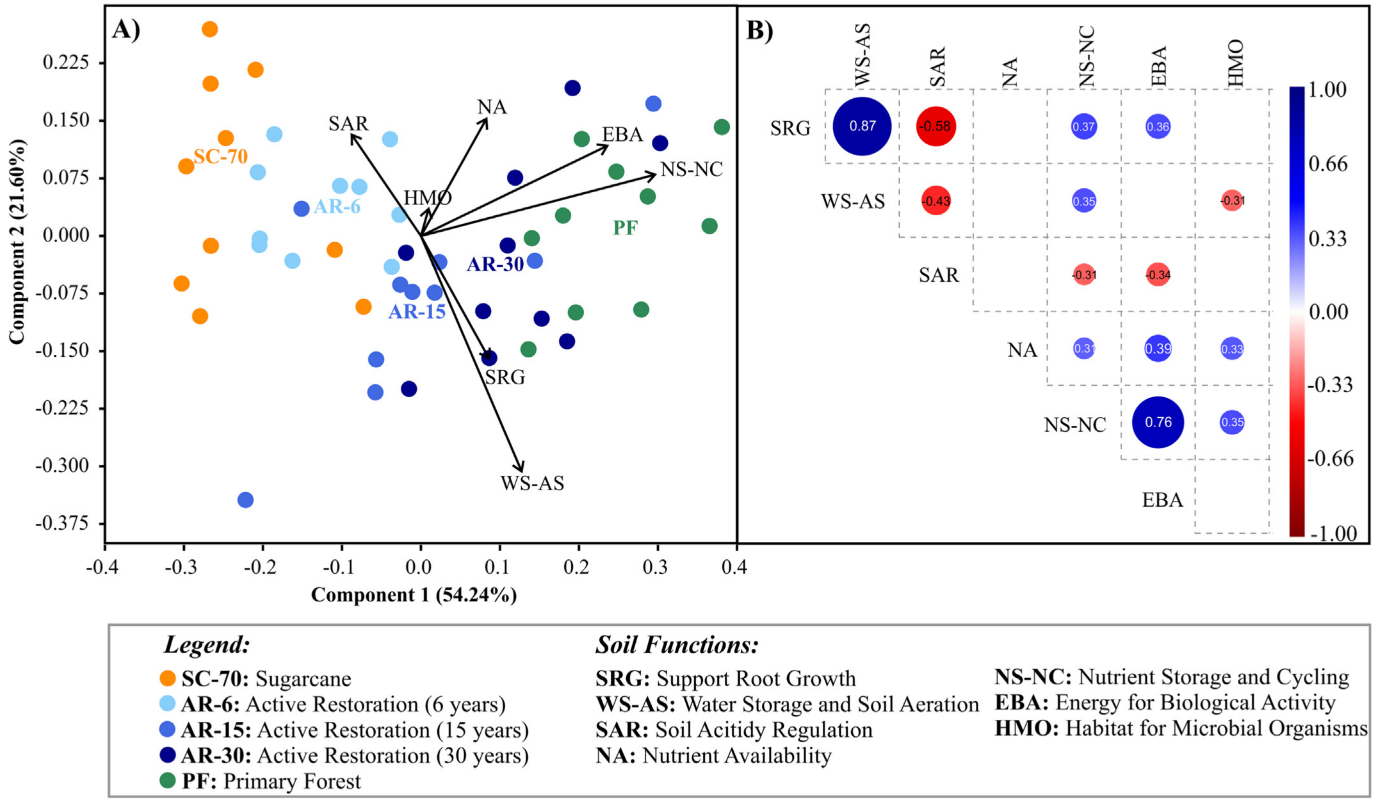

Fig. 10. Principal component analysis of soil health index (SHI) scores (A) and correlations [$p < 0.05$] (B) of the soil functions assessed in the study.

the primary forest. The sugarcane soil is still compacted, increasing soil bulk density by 10 to 20 % depending on the soil layer and reducing >60 % of the soil macroporosity. Soil bulk density under sugarcane was mainly above the critical value of 1.35 Mg m^{-3} for root growth when considering the clay contents (Reichert et al., 2009). The macroporosity was also below the critical limit of 0.10 m^3 m^{-3} for soil aeration, water infiltration, and root penetration (Reichert et al., 2007; Souza et al., 2014). Furthermore, our results are consistent with several studies that reported deleterious impacts of large-scale sugarcane cultivation on impairing soil physical and biological functions (*e.g.*, Cherubin et al., 2016a, 2016b, 2017, 2021b; Ortiz et al., 2017; Luz et al., 2019; Cavalcanti et al., 2020).

Using fire for over five decades and exposing soil during tillage until the present was the most likely driver of soil structure degradation and soil C depletion, increasing soil bulk density associated with soil C ($r = -0.48$) and CEC ($r = -0.45$) reductions. Previous studies have shown that soil tillage exposes SOM causing accelerated decomposition, oxidation, and loss (Segnini et al., 2013; Tenelli et al., 2019; Guo and Gifford, 2002; Don et al., 2011; Lal, 2005). Another insight was that lowering soil C, CEC, and N was reduced most for the topsoil layer of the sugarcane area. While the forest showed a downward depth curve for these properties, as expected, these "in-depth" changes were much smoother in sugarcane soil (see Figs. 6B, E, and 7A). These effects probably resulted from mixing soil layers due to tillage and exposing the topsoil C to erosion (Youlton et al., 2016).

Sugarcane cultivation increases nutrient availability due to the application of lime, fertilizers, and organic soluble compounds through vinasse application. These favorable soil chemical conditions promote the growth of microbial communities (Lauber et al., 2008; Yang et al., 2013). Therefore, in our study, vinasse application could have positively influenced the microbial abundance in the sugarcane area, especially fungi, which was the highest along the first 20 cm of the soil profile. However, the fungal abundance in sugarcane decreased when deepening into the soil (*i.e.*, 20–30 cm layer), where the effect of nutrient availability (Fig. 5C and D) and C from root exudates (Esteban et al., 2019) are lower, especially because the soil was compacted (Fig. 5). On the other hand, deeper intact soil layers under forests are benefited from the constant release of nutrient- and C-rich exudates by diverse roots of trees. Tree roots grew deeper into the soil than sugarcane and higher macroporosity was observed under the forest stands, which creates suitable conditions for a richer mycobiome (Yamauchi et al., 2021). This could explain the higher fungal and bacterial abundance in the primary forest in the 20–30 cm soil layer.

4.2. Forest restoration and soil C sequestration

We expected that the older the restoration, the higher the soil C stocks would be. For instance, the 6-, 15- and 30 years post-implementation restorations increased the soil C stocks by 25, 20, and 31 % compared to sugarcane. However, there was no significant difference in total soil C stocks between restoration areas of different ages. Furthermore, we found an age gradient for soil C derived from trees or herbaceous leguminous plants ($C—C_3$), where the two older restoration areas showed at least double the stocks compared to the younger restoration and sugarcane areas. In this case, our results suggest that soil $C—C_3$ may be a more appropriate indicator for quantifying the restorative effect of forest vegetation regarding C drainage and storage in soils when sugarcane has been previously cultivated over the long term. Previous studies also have shown no clear trends in forest age regarding total C storage in soils (*e.g.*, Jones et al., 2019; Brancalion et al., 2021). However, Silver et al. (2000) and Poorter et al. (2021) have demonstrated that soil C accumulation in forest restoration follows an asymptotic curve, with higher annual rates in the early years that decrease exponentially until approximately 20 years of age and then further stabilize at much lower rates. These findings are consistent with our results. Considering sugarcane as a reference starting point, the ecological restoration areas would have accumulated, on average, 2.71, 0.88, and 0.67 Mg C ha yr^{-1} after 6-, 15- and 30 years post-implementation, respectively. The time limitation occurs due to the reduced amount of C that can accumulate per unit area in aboveground C pools, which also reflects in the soil (Silver et al., 2000).

Herbaceous plants, including invasive grasses, can compete with regenerating tree species in young Atlantic Forest restorations (Brancalion et al., 2016; Oliveira et al., 2021). However, their abundant root growth and renewal can also contribute significant amounts of labile SOM in a partially shaded environment, temporarily increasing soil C stocks (Bieluczyk et al., 2020). Consequently, SOM derived from herbaceous plants may have contributed to C stocks in the 6-year-old restoration, reaching levels comparable to those of the 15-year-old area. In older restoration areas (*i.e.*, 15- and 30-year-old), the suppression of these plants increases due to more intense shading from trees, while part of the previous herbaceous SOM is lost through decomposition. In our study, the C_4-derived C (from sugarcane or grasses) was reduced by half from the 6- to the 15-year-old restoration. Nevertheless, SOM can accumulate with forest aging and succession due to protection from the denser forest stand and soil stable aggregates (Lugato et al., 2021; Shi et al., 2023). This may explain why C stocks increased in the 30-year-old restoration. These findings suggest that herbaceous plants can play a significant role in SOM dynamics in forest restoration areas and can have implications for the accumulation of C stocks over time. Although we used literature to support our evidence, SOM fractionation, and root growth analysis in different forest ages would be valuable to validate these dynamics in future restoration chronosequence studies.

Generally, the 15- and 30-year-old restoration areas exhibited similar levels of C_3-derived C in different soil depths but had higher contents than the 6-year-old restoration. The youngest restoration also showed higher soil C—C_4 than in the sugarcane area, which suggests that C_4-derived C was more efficiently incorporated into the soil during the early stages of active forest restoration. Further, our restoration method used high-diversity semideciduous native tree species that naturally shed a substantial proportion of leaves during the dry season (Bianchini et al., 2001). During this period, sunlight penetrates the canopy and triggers the germination of seed banks of exotic grasses, such as *Brachiaria* species, which may remain dormant for decades in the understory soil and litter (Garwood, 1989; Sorreano, 2002; Weidlich et al., 2020). As a result, considerable time (*e.g.*, decades) is needed to completely suppress grass growth in areas under seasonal forest restoration. Despite these challenges in interpreting the origins of C in soil, our study yielded some interesting insights. For example, 20 % less soil C—C_4 in the 15-year restoration than the restoration with twice its age (30) could indicate that the 15-year restoration was more efficient in suppressing the exotic grasses. Additionally, the same levels of C—C_3 stocks in these two areas are evidence that this restoration, based on functional groups, accruals faster the soil C originated from trees than the older approach based on modules of species.

4.3. Forest restoration and the soil health

The 6-, 15- and 30-year-old restorations increased soil overall potential multifunctionality by 3, 8, and 10 %, respectively, compared to sugarcane. The forest restoration led to significant improvements in the performance of soil functions, increasing the support for root growth, water storage, and aeration, more capacity for storing and cycling nutrients, and provision of more available energy for biological activity (see Fig. 10 and Table S3). These same soil functions were previously degraded by long-term sugarcane production. Then, our soil functional assessment revealed that soil multifunctionality is being recovered, as indicated by the upward trend observed with increasing restoration age post-implementation (Fig. 9B). However, the younger six-year-old restoration still exhibited a significant legacy of sugarcane cultivation, while the older 30-year-old restoration performed more similarly to the primary forest reference.

The improvements in soil health indicators resulting from forest restoration generally followed a top-to-bottom effect intensity in the soil profile, with more pronounced changes in the surface layer. The same pattern was found for the proportion of C—C_3 in the soil, diminishing C from trees (or leguminous herbs) mixed in SOM with increasing depth in the soil profile. Therefore, adding soil C from trees during forest restoration may facilitate the simultaneous recovery of other soil health attributes, as Poorter et al. (2021) suggested. In addition to the quantity of SOM, the presence of diverse tree species may be improving SOM quality (*i.e.*, providing a variety of organic compounds for short-, medium- and long-term turnover) and consequently enhancing the soil's biological structure for ecosystem functioning (Hoffland et al., 2020; Lavallee et al., 2020). Our results suggest that soil C accrual influenced other soil health indicators because soil C was positively correlated with N ($r = 0.88$), CEC ($r = 0.75$), and available K ($r = 0.44$) (Fig. S2). Furthermore, soil functions and C contents were associated more with the older restorations and the primary forest (Fig. 10A), except for the higher soil pH and P availability in sugarcane soils, which reflected the constant fertilizing and liming of the soil (Cherubin et al., 2016a, 2016b, 2016c; Dotaniya et al., 2016; Soltangheisi et al., 2019).

After 15 years of forest restoration, physical soil health was recovered in all soil layers (Figs. 8 and 9). Suspending heavy mechanization and the growth of invasive grasses may have quickly benefited the soil structure under forest restoration. The weathered Brazilian Oxisols typically show a high physical resilience after compaction and structure destruction, especially when they contain high clay proportion (such as in our assessed areas) within high aluminum and iron oxides, facilitating the binding of soil particles and promoting soil aggregation and strengthening (Bonetti et al., 2017). Furthermore, in a dichotomous way, while invasive grasses compete with understory regenerating tree species, their abundant root system in the soil profile help to alleviate soil compaction, even under partial shading (Bieluczyk et al., 2021), which improves soil aggregation (Batista et al., 2013). Additionally, the decomposition of roots from grasses and green manure plants opens bio pores that promote soil macroporosity enhancement (Galdos et al., 2020). Lastly, as soil C contents correlated negatively with soil bulk density ($r = -0.76$) and positively with macroporosity ($r = 0.49$), and all restorations increased soil C sequestration, soil organic matter accrual was crucial and strategic for recovering soil functions and gaining soil health, especially for the physical and biological sectors.

4.4. Did the ecological restoration reach the primary forest's soil health level?

Assessing soil indicators and analyzing them only individually in the restoration of tropical forests has been common in the Brazilian tropics (*e.g.*, Rocha et al., 2015; Koryś et al., 2021; Cabreira et al., 2021; Ribeiro et al., 2021), which is risky because it often leads to unclear conclusions, not aligned with a holistic soil health assessment. Therefore, we integrated our dataset into SHI scores based on soil multifunctionality, evidencing the stage of soil health recovery under forest restoration. With the integration, we could indicatively rank the overall SHI scores in ascendent order 0,70 < 0,73 < 0,78 < 0,80 < 0,82 in sugarcane, 6-, 15- and 30 years restorations, and primary forest, respectively. Fundamentally, all restorations were intermediate between the references of intensive agriculture and native forest. Still, statistically, the 30-year-old restoration did not differ from the native forest in overall soil health (Fig. 9B) and in its three components (Fig. 9C), as in all the soil function scores (Table S3) and soil C stocks (Fig. 3). Thus, our analysis strongly suggests that 30 years of active riparian forest restoration, surrounded by extensive sugarcane production areas, may be sufficient to recover soil multifunctionality, health, and C sequestration to a degree comparable with a remaining fragment with primary forest in the same (riparian) landscape position. Previous studies have also utilized chronosequences to investigate the time required of soil restoration in various ecosystems, including mangroves (*e.g.*, Osland et al., 2012; Salmo et al., 2013), tropical secondary forests (*e.g.*, Poorter et al., 2021; Van Der Sande et al., 2022), semi-arid secondary forests (*e.g.*, Zethof et al., 2019; Van Der Sande et al., 2022) and natural grasslands (*e.g.*, De et al., 2020). Such studies are fundamental because they contribute to discovering the trajectory of soil health recovery over time, providing crucial knowledge for planning human interventions and public policies on ecosystem restoration.

The primary forest area showed the highest C, N, and CEC_{pH7} compared to sugarcane and ecological restorations to the depth of 20 cm. This pattern is typically reported when comparing native forests with converted land for agriculture (*e.g.*, Galdos et al., 2009; Cherubin et al., 2016a, 2016b, 2016c;

Oliveira et al., 2016) and with different ages of forest restoration (*e.g.*, Nogueira Jr et al., 2011; Ferez et al., 2015; Brancalion et al., 2021; Zanini et al., 2021). The conserved forest is a resilient environment that prevents soil erosion, reduces soil temperature variation, maintains soil moisture, improves litterfall rates, and protects SOM (Silver et al., 2000; Lal, 2005). Consequently, nutrient cycling is efficient, soil C residence time is prolonged, and soil conservation benefits soil functions and health (Chen et al., 2016). Our data are consistent with these dynamics, as the primary forest showed the highest soil C stocks (96.1 Mg C ha^{-1}), as the physical ($SHI_{physical}$ = 0.85) and biological ($SHI_{biological}$ = 0.92) SHI scores. However, naturally, the primary forest area showed chemical ($SHI_{chemical}$ = 0.66) limitations. As mentioned above, only using chemical attributes may lead to inconclusive or misunderstood interpretations of soil health. For example, lower nutrient availability and lower chemical soil health indexes have been previously reported for native forests compared to managed land in weathered Brazilian Oxisols (Cherubin et al., 2016a, 2016b; Luz et al., 2019).

The active restoration method provided initial acidity correction and localized fertilization of tree seedlings to promote early forest growth. This contributed to the higher soil pH in restoration areas compared to the native forest. In the youngest restoration, additional herbaceous green manure fertilization was used to enrich soil nutrients, provide primary N through biological fixation, and enhance soil biological activity (Araújo Neto et al., 2014). This process plays an essential role in the early incorporation of C into the soil (Mayer et al., 2020), which could have benefited soil C accumulation in the 6-year-old restoration. Moreover, the 15-year-old restoration achieved physical and chemical soil functions comparable to the primary forest's (Table S3). In this context is important to mention that active restoration management and techniques are continually evolving through ongoing scientific research and implementation in the Brazilian Atlantic Forest biome fields (Rodrigues et al., 2009a; Brancalion et al., 2015). Therefore, we believe that our young, actively restored forests are speeding up soil health restoration, as authors have observed for above-ground forest restoration (Zanini et al., 2021). These forests may reach the overall soil health levels of the native forest earlier than the 30-year post-implementation mark. However, long-term data are necessary to confirm this hypothesis.

5. Conclusions

Our study showed that the conversion of Atlantic forests to long-term sugarcane cultivation caused the loss of 30.6 Mg ha^{-1} of soil carbon combined with deterioration of soil health. Even with the implementation of more sustainable sugarcane management practices for 14 years, the soil structure remained unsuitable for healthy plant growth, with impediments to water and air fluxes within the edaphic environment. However, the restoration of riparian forests for 6–30 years restored 53–66 % of lost carbon and improved soil health indicators such as cation exchange capacity, microbial abundance, porosity, and bulk density. Additionally, in terms of soil health indexes, these restored soils gained 3–10 % of their full potential multifunctionality. Therefore, our findings demonstrate that restoring riparian forests in sugarcane-dominated landscapes is an effective strategy for enhancing belowground carbon sequestration and soil functioning towards healthy native forest ecosystems. We conclude that forest restoration in sugarcane production landscapes helps to mitigate global warming, restore important ecosystem functions, and improve soil health. Furthermore, our study suggests that active restoration of riparian forests can restore soil health, multifunctional performance, and carbon sequestration levels close to those of native forests within approximately three decades.

CRediT authorship contribution statement

Wanderlei Bieluczyk: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Visualization, Writing – original draft, Writing – review & editing. **Luis Fernando Merloti:** Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing. **Maurício Roberto Cherubin:** Conceptualization, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing. **Lucas William Mendes:** Conceptualization, Methodology, Investigation, Validation, Writing – review & editing. **José Albertino Bendassolli:** Conceptualization, Resources, Funding acquisition. **Ricardo Ribeiro Rodrigues:** Conceptualization, Methodology, Resources, Funding acquisition, Investigation, Writing – review & editing. **Plínio Barbosa de Camargo:** Conceptualization, Resources, Funding acquisition, Supervision, 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.

Data availability

Data will be made available on request.

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 data

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