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SOIL CARBON AND NITROGEN IN SILVOPASTORAL SYSTEMS.

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Highlights

Silvopastoral systems are a good alternative for the recovery of degraded areas.

TOC and TN tended to increase, while P, K and S decreased in the soil.

The Past treatment showed a higher N stock at depths up to 40 cm.

There was a shift in the C source in the soil surface (5 cm) in the Eucal and PE.

BNF in the treatments with legumes didn’t promote an increase in the soil.

Abstract

Silvopastoral systems are a good alternative for the recovery of degraded pastures. The objective of this study was to evaluate soil fertility, carbon and nitrogen stocks, the contribution of trees to carbon formation in the soil, and biological nitrogen fixation in silvopastoral systems in southern Espírito Santo. The experiment to evaluate the silvopastoral systems (SSP) was established in the municipality of Jerônimo Monteiro, ES, Brazil, with 5 treatments: Past: Pasture in monoculture; Eucal: Eucalyptus in monoculture; PE: Pasture and Eucalyptus in a Silvopastoral System; PEL: Pasture, Eucalyptus, and Leucaena in a Silvopastoral System; PA: Pasture and Araribá in a Silvopastoral System. Soil fertility, carbon and nitrogen stocks in the soil, the contribution of trees to carbon formation in the soil, and biological nitrogen fixation were analyzed. The treatments Past and PA showed the highest nutrient levels in the soil, attributed to low biomass absorption and composition above the soil in relation to the systems Eucal, PE, and PEL. Total organic carbon and total nitrogen levels tended to increase over time, while phosphorus, potassium, and sulfur showed a decrease in soil content. Carbon and nitrogen stocks in the soil did not show significant increases after 36 months of establishment in the silvopastoral systems. Only the Past treatment showed a higher nitrogen stock considering the depth up to 40 cm. Although no differences were found in the carbon and nitrogen stocks in the studied systems, a change in the surface soil (up to 5 cm) from a grass-derived C source to a tree-derived C source was observed in the Eucal and PE treatments. This was not observed in the nitrogen stock, where biological nitrogen fixation by legumes did not promote an increase in this nutrient in the soil.

Keywords

Soil Fertility, *Centrolobium tomentosum*, Stable Isotopes, Biological Nitrogen Fixation, Livestock-Forest Integration.

Introdution

The Atlantic Forest biome extends along much of Brazil's coastline and covers the entire territory of the state of Espírito Santo, and the most representative formations are the Dense Ombrophilous Forest and the Semideciduous Seasonal Forest (Garbin et al., 2017; MMA, 2015). With approximately 25% of its forests remaining (802 million hectares), livestock farming occupies 45% of the total area (1,473 million hectares), with the most adopted model by farmers is extensive cattle ranching in monoculture, due to the ease of implementation, management, and cultural practices (Abreu and Lopes, 2005; IBGE, 2019; Skroupa and Manzatto, 2019).

Intensive land use with monoculture pastures can lead to a loss of production capacity (Medeiros et al., 2022). Therefore, different management approaches can be applied to preserve and improve environmental conditions (Cary and Frey, 2020; Garrett et al., 2020; Wendling et al., 2021). Livestock-Forest Integration or Silvopastoral Systems (SPS) involves the combination of different production systems within the same area (Balbino et al., 2011; Gil et al., 2014) aiming for greater efficiency in the use of natural resources (Parra et al., 2022; Suárez et al., 2021). It is an alternative to achieve the goals of the Low Carbon Agriculture Program (ABC Program), which aims for sustainable agricultural production with a reduction in Greenhouse Gas Emissions (Bieluczyk et al., 2021).

However, the implementation of SPS requires knowledge of the synergy between the crops used and the best way to manage them (Bieluczyk et al., 2022; Wendling et al., 2021). Tree species from the *Eucalyptus* genus are widely used due to their adaptability in Brazil. Studies show that SPS with 250 to 350 trees per hectare are capable of sequestering 18 Mg ha^{-1} of CO_2 equivalent, which is sufficient to neutralize the emissions from 12 adult cattle (Almeida et al., 2011; Bieluczyk et al., 2022; Silva et al., 2021). Another strategy is to use tree legumes that fix nitrogen biologically, such as Leucaena (*Leucaena spp.*) and Araribá (*Centrolobium tomentosum*), which can provide up to 500 kg ha^{-1} year^{-1} of nitrogen (Carvalho, 2003; Pinheiro et al., 2021; Sujii et al., 2017).

The adoption of SPS alters the dynamics of Soil Organic Matter (SOM) formation due to the contribution of plant residues from the trees (Almeida et al., 2021; Cá et al., 2022). Using stable isotope analysis techniques, we can assess the contribution of different plants to the increase of carbon in the soil, where plants with a C_3 photosynthetic cycle have a depleted ^{13}C isotopic composition compared to plants with a C_4 photosynthetic cycle. This technique can distinguish the contribution of pasture (C_4) and trees (C_3) to SOM formation (Ngaba et al., 2019; Pinheiro et al., 2021). Similarly, Biological Nitrogen Fixation (BNF) increases the concentration of the stable isotope ^{15}N, generating differences in the concentration of ^{15}N from BNF compared to other nitrogen sources present in the soil (Bighi et al., 2021; Inácio and Urquiaga, 2017).

The implementation of SPS requires a deeper understanding of the species being introduced, as it can lead to both favorable and unfavorable interactions between the species used and with the local soil and climate conditions (Kruchelski et al., 2023). The objective of this study was to evaluate soil fertility, carbon and nitrogen stocks, and the contribution of trees to organic carbon formation in the soil and biological nitrogen fixation in silvopastoral systems in southern Espírito Santo.

Material and methods

Study area

The study area is located in the municipality of Jerônimo Monteiro (SIRGAS 2000 Coordinates: E 252842, N 7693711). According to the Köppen classification, the region's climate is of the Aw type, characterized by a dry winter and a rainy summer, with an annual average temperature of 23.1°C and an annual average precipitation of 1,341 mm (Alvares et al., 2013). The soil in the region is classified as “Argissolo vermelho Amarelo” by the Brazilian Soil Classification System (Cunha et al., 2016; Santos, 2018), equivalent to Utilsols in the Soil Taxonomy (Survey Staff, 1999). Figure

1 shows the monthly rainfall and average monthly temperature in the region, and Table 1 shows the soil characterization at the experimental site.

The area where the experiment was implemented has been economically explored through extensive cattle ranching since 1970. Between 2007 and 2016, a coffee plantation was established, and since 2016, the area has been used for pasture again. The experiment was designed in 2017 with the delineation of blocks and plots and began in January 2018 with the planting of seedlings and establishment of the pasture (Figure 2).

The experiment for the evaluation of silvopastoral systems (SSP) was implemented using a Randomized Block Design, consisting of 4 blocks and 5 treatments: **Past**: Pasture in monoculture (Marandu grass - *Urochloa brizantha* cv. Marandu); **Eucal**: Eucalyptus in monoculture (hybrid of *Eucalyptus grandis* x *Eucalyptus urophylla*); **PE**: Pasture and Eucalyptus in a Silvopastoral System; **PEL**: Pasture, Eucalyptus, and Leucaena (*Leucaena leucocephala*) in a Silvopastoral System; **PA**: Pasture and Araribá (*Centrolobium tomentosum* Guillemim ex. Benth.) in a Silvopastoral System.

Each experimental unit implemented has an area of 35 x 50 meters, and the blocks have different exposure faces. The Past treatment was implemented using seeds at a rate of 4 to 6 kg ha^{-1}. The seedlings of the tree species, with an average height of around 20 cm, were planted in holes measuring 30 × 30 cm. The Eucal treatment was implemented using eucalyptus seedlings spaced 2 x 3 meters, forming a stand of 1,667 plants ha^{-1}. In the PE, PEL, and PA treatments, the tree species were planted in two rows with three lines, spaced 2 x 3 meters between tree species and 17 meters between rows, forming a stand of 600 plants ha^{-1}. The Leucaena seedlings in the PEL treatment were planted within the 17 meter wide pasture strip, in groups of two rows with an initial spacing of 1 x 0.75 meters, spaced every 3.0 meters from the Marandu grass.

A base fertilization was applied to the planting hole and a lateral fertilization in the furrow immediately after planting, both with 150g of NPK (03-30-10) plus micronutrients. In the 3rd, 6th, and 12th months after planting, top dressing fertilization (NPK 20-00-20) with micronutrients was applied. No liming was carried out, as the pH value and the levels of Ca and Mg were satisfactory.

Soil Fertility

For soil fertility analysis, soil samples were collected from each plot at depths of 0.00 to 0.05 m, 0.05 to 0.10 m, 0.10 to 0.20 m, and 0.20 to 0.40 m, referred to as P1, P2, P3, and P4, respectively. The samples were collected by opening pits and taking samples from the side walls corresponding to each depth, as described by ALMEIDA et al. (2021), and at times before the implementation, referred to as 0 months, and at 6, 24, and 36 months after the start of the production systems installation.

The analyses for exchangeable calcium, exchangeable magnesium, exchangeable phosphorus, exchangeable potassium, and exchangeable sulfur were performed according to the routine laboratory methods described in the EMBRAPA Soil Analysis Manual (Teixeira et al., 2017). Total organic carbon (TOC) was determined by wet oxidation of organic matter using $K_2Cr_2O_7$ 0.167 mol L^{-1} in sulfuric acid medium, and total nitrogen (TN) was determined through sulfuric digestion followed by Kjeldahl distillation (Mendonça and Matos, 2005).

C and N stocks

For the quantification of C and N stocks, the same samples collected for soil fertility evaluation were used. For soil density analysis, undisturbed samples were collected in aluminum cylinders at 0 months and at 36 months.

Total organic carbon (TOC) was determined by wet oxidation of organic matter using $K_2Cr_2O_7$ 0.167 mol L^{-1} in sulfuric acid medium, and total nitrogen (TN) was determined through sulfuric digestion followed by Kjeldahl distillation (Mendonça and Matos, 2005).

The stocks were calculated using the formula:

$$Stock = content\ x\ \rho\ x\ thickness\ x\ 10000 \qquad \text{eq. 1}$$

in which:

Stock: Element content in a given area and soil thickness (g ha^{-1});
Content: Soil element content (g kg^{-1});
ρ: Soil density (kg m^{-3});
thickness: Soil thickness (m);
10000: Conversion of m² to ha.

Considering the need for stock correction, the correction calculations in relation to soil mass will follow the methodology by ELLERT and BETTANY (1995).

Stable Isotopes

For the quantification of ^{13}C and ^{15}N, soil was sampled at depths of 0 to 5 cm and 5 to 10 cm, and plant materials from the treatments were collected at 40 months from the beginning of the experiment. The soil was collected in the same manner as for soil fertility analysis. The plant material samples were composed of leaves collected from each plot using pruning shears, with the sample being made up of representative subsamples from each plot. The samples were dried in an oven at 65°C until they reached a constant weight, ground, and reserved for analysis.

The samples were analyzed using an Isotope Ratio Mass Spectrometer (IRMS). The isotopic ratio (δ) was calculated by comparing the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio of the sample with the reference standard Pee Dee Belemnite for C and the isotopic composition of the atmosphere for N (Caxito and Silva, 2015). The isotopic ratio was calculated using the following formula:

$$\delta = \frac{R_{sample} - R_{standard}}{R_{standard}} x1000 \qquad \text{eq. 2}$$

in which:
δ = isotopic ratio of the element (‰);
R_{sample}: ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ of sample; e
$R_{standard}$: ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ of standard.

The contribution of the treatments to the C and N input from BNF (Biological Nitrogen Fixation) was determined through the isotopic ratios $\delta^{13}C$ and $\delta^{15}N$ and calculated using the following formula:

$$CC_4 = \frac{(\delta^{13}C_{soil} - \delta^{13}C_{C3})}{(\delta^{13}C_{C4} - \delta^{13}C_{C3})} x100 \qquad \text{eq.3}$$

in which:
CC_4 = C derived from C_4 (%);
$\delta^{13}C_{soil}$ = isotopic ratio of the analyzed sample (‰);
$\delta^{13}C_{C3}$ = isotopic ratio of the plant tissue C_3 (‰); e
$\delta^{13}C_{C4}$ = isotopic ratio of the plant tissue C_4 (‰).

The isotopic ratio of the C3 plant tissue used was the average of the Eucal treatment, and the isotopic ratio of the C4 plant tissue was the average of the Past treatment.

The contribution of Biological Nitrogen Fixation will be calculated using the following formula:

$$Ndfa = \frac{(\delta^{15}N_{ref} - \delta^{15}N_{leg})}{(\delta^{15}N_{ref} - B)} x100 \qquad \text{eq. 4}$$

in which:
Ndfa = N derived from atmospheric fixation via BNF;

$\delta^{15}N_{ref}$ = Natural enrichment of a non-N2 fixing species;
$\delta^{15}N_{leg}$ = Natural enrichment of the legume evaluated in the system;
B = Natural abundance of 15N (δ15N) in the legume grown with exclusive dependence on N2.

The isotopic enrichment of the non-N2 fixing species used was the average of the Eucal treatment, and the B value was defined as -2.5 (Araujo et al., 2019; Favero et al., 2022).

Statistics

The experimental design used was a Randomized Complete Block Design (RCBD), consisting of 5 treatments and 4 blocks, totaling 20 plots. The results were compared between treatments at each time and depth, and each treatment over time and depth. All results were initially subjected to normality testing (Shapiro-Wilk test) and homogeneity of variance testing (Levene's test), followed by statistical tests for analysis of variance and mean comparisons using parametric tests.

The data on soil fertility levels and stable isotopes were subjected to analysis of variance for comparison of treatment means, and when a significant difference was found at the 10% significance level, Tukey's test was performed for mean comparison.

The data on C and N stocks at each depth and in total were subjected to analysis of variance to compare the treatment means. When a significant difference was found at the 10% significance level, Tukey's test was performed for mean comparison. To compare the values obtained within each treatment over time, the results were subjected to a Student's t-test at the 10% significance level, comparing the means of 0 months and 36 months for each treatment.

The isotopic ratios ($\delta^{13}C$ and $\delta^{15}N$), the proportion of C derived from plants with C assimilation via C_4 photosynthesis (CC_4) in the soil, and the N derived from atmospheric fixation via BNF (Ndfa) in plant materials were subjected to analysis of variance to compare the treatment means. When a significant difference was found at the 10% significance level, Tukey's test was performed for mean comparison.

For the execution of statistical analyses and the creation of graphs, the software R version 4.3.3 (R Core Team, 2024) was used.

Results

Soil fertility

The exchangeable P content between treatments showed a significant difference at the 6 month, at the P2 depth, with the Eucal treatment being higher than the Past and PA treatments (Figure 3a). There was an increase in exchangeable P levels over time, with the 6 month values being higher compared to the 0 month values, followed by a decline in subsequent analyses, particularly in the more superficial layers (P1 and P2). This pronounced increase at 6 months is possibly due to the planting and cover fertilizations carried out.

The exchangeable K content in the Past treatment was higher compared to the others at 24 months in the P1 depth and at 36 months in the P1 and P2 depths, as well as in the Past and PA treatments at 36 months and the P3 depth (Figure 3b). No differences were observed at the P3 and P4 depths over time, except in the PE treatment at the P4 depth between 6 and 24 months. In the more superficial layers, the Eucal treatment showed a decline over time.

The exchangeable Ca content did not show any significant difference between treatments or between sampling times. However, the exchangeable Mg content showed a significant difference only in the PEL treatment at the 6 month in the P4 depth, which was lower than all other treatments, and in the Past treatment, which was higher than the Eucal and PEL treatments at the 36 month in the P1 depth (Figure 3c). Over time, the Eucal treatment showed a significant difference between 0 and 6 months, which were higher than the 36 month values in the P1 and P2 depths, and between the 0 month and 36 month values in the P4 depth.

There was no significant difference between treatments for exchangeable S content (Figure 4a). When comparing between time, no differences were observed during the first two collections (0 and 6 months), however, at the 24 and 36 month, a significant decrease in levels was observed across all treatments.

COT showed significant differences in its levels at the 24 and 36 month, with the PE treatment having lower values compared to the other treatments (Figure 4b). The temporal variation in COT levels showed significant differences in the P3 and P4 depths in the Eucal and PA treatments, which were higher at 36 months compared to the 24 month.

NT content showed a significant difference at the 0 month in the P2 depth, with the PE treatment being lower than the others, and in the P3 depth, where the PE and PEL treatments were lower than the others (Figure 4c). It also showed differences at 24 months in the P1 cm depth, where the Past treatment was higher than the others, and at

36 months in the P2 and P4 depths, where the Past treatment was superior to the others. When comparing over time, significant differences were found only in the P3 and P4 depths.

C and N stocks

The C stock up to a depth of 40 cm at the 0 month was estimated at 29.255 Mg ha^{-1}, and at 36 months, it was estimated at 30.926 Mg ha^{-1}, representing a 5.7% increase in the carbon content stored. No significant differences were observed between the treatments studied or between time points within each treatment (Figure 5a).

The N stock up to a depth of 40 cm at the 0 month was estimated at 4.991 Mg ha^{-1}, and at 36 months, it was estimated at 5.108 Mg ha^{-1}, representing a 2.3% increase in the N content stored (Figure 5b). The Past treatment at 36 months was higher than the other treatments. When comparing between time, no significant differences were found in the stored content of nitrogen in the Eucal and PA treatments. The PEL treatment showed a lower N content compared to the start of the treatment. In the other treatments, there was an increase in nitrogen content in the soil at 36 months compared to the initial values.

The carbon stock is lower than that found by DAMIAN et al. (2023) for C in pasture (87.88 Mg ha^{-1}) and silvopastoral system (93.58 Mg ha^{-1}), but is comparable for N stock in pasture (6.06 Mg ha^{-1}) and silvopastoral system (5.32 Mg ha^{-1}).

The C stock at each depth studied showed a significant difference after 36 months of experiment installation in the surface layer (P1) between the Past and PE treatments, with contents of 6.355 and 4.462 Mg ha^{-1}, respectively (Figure 5c), indicating a 28% and 20% increase for the PE and Past treatments, respectively. The C stock in the Past treatment at the P3 depth at 0 months was higher than the PE treatment (7.305 and 5.281 Mg ha^{-1}, respectively). However, no significant difference was observed after 36 months of system installation. No significant difference was found between the same treatment at the 0 and 36 month for C stock.

The N stock showed no significant difference at the P1 depth (Figure 5d). At the P2 depth, the Past treatment showed a significant difference compared to the PE treatment at 0 months and to the PA treatment at 36 months. At the P3 depth and at 0 months, the Eucal treatment showed a significant difference compared to the PE and PEL treatments, but no differences were found at 36 months. The Past treatment was superior to the other treatments at 36 months in the P4 depth. Analyzing the data over time, the N stock at the P3 depth in the Eucal treatment decreased by 18% after 36 months of the experiment. At the P4 depth, there was a decrease in the PEL treatment (26%) and an increase in the Past (47%) and PE (51%) treatments.

Stable isotopes

The isotopic ratio of ^{13}C determined in the plant materials showed the expected significant difference between plants with C_3 photosynthetic cycle (Eucalyptus and Araribá) and plants with C_4 photosynthetic cycle (Marandu grass), with an isotopic ratio of -13.5 ‰ for the Past treatment and between -30.1 and -31.0 ‰ for the other treatments with C3 species (Figure 6a). No differences were observed between the C_3 treatments, indicating that the adoption of different systems does not affect the way plants absorb CO_2 from the atmosphere. In the soil, a significant difference was observed at the P1 depth, where Past and PA treatments had less negative values, and Eucal and PE treatments had more negative values. The PEL treatment did not show a significant difference compared to any other treatment. At the P2 depth, only the Eucal treatment showed a significant difference compared to the Past and PA treatments.

The contribution of C in the soil at the P1 depth in the Eucal (42.4%), PE (47.5%), and PEL (50.2%) treatments showed the lowest participation from C_4 photosynthetic cycle plants (Figure 6b). At the P2 depth, the Eucal (46.2%) and PE (47.3%) treatments showed the lowest participation of C originating from C_4 plants. The PA treatment showed little change in the participation of C in the soil. Considering the C values in the Past treatment (59.6% and 57.0% at the P1 and P2 depths, respectively), after 40 months of implementation, only the silvopastoral system PE showed a significant difference, with a reduction of 12.1% and 9.7% at the P1 and P2 depths, respectively.

The isotopic ratio of ^{15}N did not show significant differences in the soil (Figure 6c). The isotopic ratio in the Araribá plant material showed a significant difference compared to the other treatments.

The contribution of BNF in the plant material indicated that 73.2% of the nitrogen in the Araribá plant material comes from atmospheric nitrogen fixed through biological nitrogen fixation (Figure 6d). This value is higher than those found for native species of the Atlantic Forest in Agroforestry systems in the Zona da Mata of Minas Gerais, such as Mulungu (Erythrina verna), Fedegoso (Senna macranthera), and Ingá

(Inga subnuda), where Ndfa represented 22%, 16%, and 20%, respectively (Duarte et al., 2013).

Discussion

Soil fertility

Macronutrients (N, P, K, Ca, Mg, and S) are required in large quantities by plants as they participate in key biochemical components and physiological processes (Fernandes et al., 2018). The proper maintenance of soil fertility, combined with good agricultural practices, is essential for the nutritional improvement of plants (Suárez et al., 2021). This is why fertilization was carried out during the installation of the plots. The effect of fertilization can be seen in the increase in P levels at 6 months compared to the initial time (0 months). Exchangeable P levels at 36 months in silvopastoral systems are similar to those found in 12 and 20 years old Pinus plantations, but the levels in pasture systems are higher than those in native fields (Mafra et al., 2008). However, this increase was not observed for N and K levels. One of the reasons for the lack of an increase in N and K levels after the installation is the split application of doses over time. The quantities of K absorbed by the plants are quite significant, and it can be observed at 36 months after planting that K levels were lower compared to the Past treatment.

The fertilization recommendation for P is in high doses and localized near the plant's root system, due to the fertilizer's lack of salinity, its low mobility in the soil, and the adsorption of this nutrient by Fe and Al oxides, making it unavailable for plant absorption (Fernandes et al., 2018). DAMIAN et al. (2020) observed an increase in labile P levels in agroforestry systems compared to conventional pastures. P and K levels decreased over time, as also observed by ARÉVALO-GARDINI et al. (2015), who found a decline within 6 years of implementing agroforestry systems with cacao.

The tendency for the Past treatment to show higher soil fertility compared to the treatments with tree planting can be attributed to the extraction of nutrients by the trees to form above-ground biomass. This does not occur to the same extent in pasture, which produces less biomass than the forest species and has a faster rate of biomass return to the soil (Kotowska et al., 2016; Primavesi et al., 2006; Wink et al., 2018). However, this is not observed in systems with greater ages of establishment, as noted by SUÁREZ et al. (2021), who found higher levels of exchangeable nutrients in agroforestry systems with cacao compared to monoculture pastures. The extraction in the PA treatment was not significant, as this tree species grows slowly compared to eucalyptus, resulting in lower nutrient accumulation in its biomass during the early years.

The Ca content in the soil did not change between treatments. An accumulation in the surface layer was expected due to its low mobility within the plant and, with the input of leaves from forest species, a deposition on the surface. However, this increase was not observed, which can be attributed to the fact that the leaf deposition was still not quantitatively sufficient to cause noticeable accumulation. REGO et al. (2023) found that integrated crop-livestock systems with 8 years of establishment had higher exchangeable Ca, Mg, and K levels compared to the same systems at 5 years. On the other hand, the Mg levels in the soil showed a trend of lower values across all depths in the Eucal, PE, and PEL treatments, indicating that in soils under eucalyptus, the high nutrient demand results in the depletion of this nutrient. The S content at all depths and treatments decreased at the 24 and 36 months, which may be attributed to the extraction of this nutrient by the plants or losses from the system. This could be linked to the source of S in the soil, which comes from organic forms with high mobility, either being absorbed by plants or leached (Fernandes et al., 2018).

Fertilization recommendations cease after 3 years of establishing tree species, justifying that the nutrient input through leaf deposition becomes more important for nutrient supply than the soil itself. It is precisely at this stage of forest plantations that fertilization ends, and there is no longer any external nutrient input, marking the beginning of biogeochemical cycling (Kotowska et al., 2016). Due to the sampling period in this experiment, the nutrient input may still not be sufficient for full nutritional support. However, we can observe, even though not statistically significant, an increase in the COT content in the surface layer of the soil.

It was expected that the treatments involving legumes, which form symbiotic relationships with BFN, would show higher N levels in the soil (Bighi et al., 2021). However, the opposite was observed, with treatments showing low N values, including the PA treatment, which was significantly lower at 36 months after the establishment of the experiment. The biomass production and leaf litter input from Araribá may not have been sufficient to increase the nitrogen levels, or the low nitrogen levels may be meeting only the needs of the tree species. Since N is mobile within the plant, the senescent material deposited may not contain high nitrogen concentrations (Jiang et al., 2024).

C and N stocks

C and N stocks suffer losses after the conversion of native areas into production areas (Medeiros et al., 2022). Data from the Atlantic Forest region, between the states of Alagoas and Rio de Janeiro, show that C stocks were estimated between 61.3 and 91.7 Mg ha^{-1} at depths up to 42 cm, highlighting the loss of carbon after several years of conversion to production areas (Gomes et al., 2018). In other biomes, such as the Cerrado, reductions in C stocks have also been observed, with losses of 29.7% and 16.4% in pasture and silvopastoral systems, respectively (Ribeiro et al., 2023), and in the conversion to pasture in the Caatinga, with losses of 15.1% and 12.4% for C and N, respectively (Medeiros et al., 2023).

The accumulation of organic matter in the soil occurs through the constant input of residues (Cordeiro et al., 2022; Wang et al., 2023). In well drained soils and under temperatures favorable to microbial activity, the high rates of C oxidation to CO_2 require greater input to increase soil stocks. Therefore, under the edaphoclimatic conditions of the experiment, continuous and long-term input is essential for improving C and N stocks in the soil (Souza et al., 2023).

The short time of adoption of silvopastoral systems could be a reason for the small differences observed between the treatments, comparing the contents before the installation and after 36 months of implementing the production systems. DAMIAN et al. (2023), evaluating silvopastoral systems 3 years after the planting of African Mahogany (Khaya ivorensis), and BIELUCZYK et al. (2020) in agro silvopastoral systems (corn x pasture x eucalyptus) did not find significant differences compared to monoculture pastures. Simulations of C behavior in the soil conducted by RIBEIRO et al. (2023) show that the use of silvopastoral systems can increase stocks, however, significant results are typically obtained after several years of system implementation. On the other hand, ALMEIDA et al. (2021) found that after 4 years of implementation, integrated production systems promoted increases in C and N stocks. The PA treatment and leucaena in the PEL treatment have slower growth compared to eucalyptus, which may lead to a delay in expressing the benefits of grass-legume tree integration.

The monoculture pasture system presented C and N stock values equal to or higher than the adopted treatments. Improved genetics pastures have a high biomass production capacity, both above and below the soil, primarily associated with good water distribution and nutrient supply compared to degraded pastures (Campos et al., 2022; Damian et al., 2023). In association with leguminous plants capable of BNF or recent studies that have selected strains of bacteria able to associate with pastures themselves, this system creates better conditions for pasture production, as N is correlated with greater root development of plants (Marques et al., 2017; Pinheiro et al., 2021). The differences found in N stocks at different depths may be attributed to the release of nitrogen through root exudates in deeper soil layers, while the carbon input is greater via leaf deposition on the surface.

In this study, we evaluated soil stocks without considering the contribution to atmospheric carbon sequestration, which becomes part of the aboveground biomass of tree species and contributes to the emission balance of gases from the activity (Parra et al., 2022). Studies conducted within the same experiment estimated wood production at 7 years after planting as 291.68, 106.30, and 125.47 m^3 ha^{-1} for the Eucal, PE, and PEL treatments, respectively (Silva et al., 2021). Considering that wood typically contains 50% carbon and has a basic density of 500 kg m^{-3}, this results in C sequestration of 72.92, 26.58, and 31.25 Mg ha^{-1} for these treatments, respectively. The incorporation of soil nutrients into the biomass of trees may be one of the reasons for the reduction in N stocks at 36 months compared to the initial sampling (0 months) in the Past (P3) and PEL (P4) treatments.

In addition to wood production, the use of mixed plantations proves to be beneficial for the environment as a whole (Kruchelski et al., 2023; Roese et al., 2020). The activity of microorganisms in agricultural systems plays a crucial role in the physical and chemical fragmentation and rearrangement of the molecules in the residues added to the soil (Brewer et al., 2023). These microorganisms are capable of interacting with the mineral fraction of the soil through chemical bonds, thereby increasing its stability within the system. This process, known as humification, results in substances responsible for improving soil aggregation, generating negative and positive charges, metal complexation, and pH buffering in the soil solution (Brewer et al., 2023; Coelho et al., 2013; Damian et al., 2023).

Stable isotopes

The isotopic ratio found in the plant materials is consistent with values reported in the literature (Caxito and Silva, 2015), and the production systems did not cause differentiation in the isotopic ratio of the plant materials. This suggests that the assimilation of carbon isotopes is controlled by the specific photosynthetic cycle of each plant and is not influenced by the type of management system adopted.

However, when analyzing the isotopic ratio and the contribution of C from C_3 species in the soil, even after only 40 months of implementation, systems with the tree component Eucalyptus (Eucal and PE) showed incorporation of C from these trees into the soil's COT, with a more pronounced effect in the surface layer (P1) compared to the deeper layer (P2). Studies have shown that the incorporation of C into the soil occurs primarily through the deposition of leaves and branches on the soil surface, while in deeper layers, incorporation is mainly through roots. Given that C mobility in the soil profile is low, it is expected that the material input and differences between the systems would be more pronounced at the surface.

The PA treatment did not show any difference in the isotopic ratio in the soil, similar to the PE treatment, which has the same system design. This can be explained by the differential growth rates of the species. While Eucalyptus species exhibit significant growth, reaching up to 20 meters in height with a large canopy volume, Araribá has a slower growth rate, with smaller canopy formation and leaf litter input to the soil. As a result, the incorporation of C into the soil and the effects of nutrient cycling in this system may take longer to manifest (Miguel et al., 2013).

We observed that in the Past treatment, there was the presence of C derived from C_3 plants, even though these plants from the C_3 photosynthetic cycle were not cultivated in the plot. SOUZA et al. (2023) found that even after 24 years of pasture use, there is still C derived from C_3 plants, particularly in deeper layers. This phenomenon can be attributed to humified forms of C in the soil, which are highly recalcitrant and can remain in the soil for decades, even in tropical conditions with high mineralization of organic residues (Lustosa Filho et al., 2024). In fact, humic substances are the main source of C found in these tropical regions, particularly in the deeper layers of the soil profile, where they are stabilized and associated with the soil's mineral constituents and aggregates (Gama-Rodrigues et al., 2010; Gomes et al., 2018; Pessôa et al., 2022).

The N isotope ratios in the soil at both depths did not show any significant differences between treatments, which can be attributed to the time elapsed since the establishment of the systems and the limited contribution of nutrient cycling to N formation in the soil. Management techniques adopted may enhance atmospheric N fixation, such as inoculation with bacteria (Favero et al., 2022), reduced competition between plants (Bighi et al., 2021), or may hinder it, as seen in the availability of N in the soil (Aguirre et al., 2020; Paula et al., 2018).

The differences in nitrogen isotope ratios found in the leaves highlight that the Araribá species has the ability to associate with nitrogen-fixing bacteria, while the Eucalyptus species does not exhibit this capability. The N fixation capacity observed in leguminous species is an adaptive advantage that promotes their establishment, especially in tropical regions often associated with highly weathered and leached soils (Wang et al., 2023).

It was expected that the PEL treatment, which includes a leguminous species as part of the system, would show a decrease in δ^{15}N due to the translocation of N fixed by the legume and made available in the soil for the eucalyptus species. However, this was not observed when comparing this treatment to others that only included Eucalyptus in the system. PAULA et al. (2018) found in systems with 50% Acacia (*Acacia mangium*) and 50% Eucalyptus (*Eucalyptus grandis*) an increase in the N content in eucalyptus leaves, which was attributed to N fixation by the legume and its availability in the soil for assimilation by the eucalyptus.

At the same time, we observed that the plant material samples from the pasture showed a small contribution of atmospheric N in their tissues. Grasses do not form associations like legumes do, however free-living soil bacteria such as *Azotobacter*, *Azospirillum*, and *Herbaspirillum* have the ability to fix atmospheric N when associated with pasture (Aguirre et al., 2020; Marques et al., 2017).

Conclusion

The monoculture pasture and the Silvopastoral system with Pasture and Araribá were the ones that showed the highest nutrient levels in the soil, attributed to the lower absorption of nutrients for biomass composition compared to systems that include eucalyptus, a fast-growing biomass-producing tree. The levels of total organic carbon and total nitrogen tended to increase over the analysis period, while phosphorus, potassium, and sulfur levels showed a decrease. Calcium levels were not affected by the different systems implemented.

The carbon and nitrogen stocks in the soil did not show significant increases. Even considering that Eucalyptus and Araribá species have different growth rates, no differences were found in the accumulation of carbon and nitrogen in the soil after 36 months of system implementation. Only the monoculture pasture treatment showed higher nitrogen stocks when considering the depth up to 40 cm.

Although no differences were found in carbon and nitrogen stocks among the studied systems, we observed a shift in the surface soil (up to 5 cm) from a grass-derived carbon source to a tree-derived carbon source in the monoculture Eucalyptus and the Silvopastoral Eucalyptus-Pasture systems. However, this was not the case for nitrogen stocks, as the biological nitrogen fixation by the legumes did not promote an increase in this nutrient in the soil.

Further studies are needed regarding the estimation of nutrients present in the aboveground biomass and the movement of nutrients after 36 months, when the systems begin to rely less on soil fertility and more on nutrient cycling. Additionally, a complete life cycle analysis of the forest species and the return of biomass residues to the soil should be conducted.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the content of the submitted work.

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Tables and figures

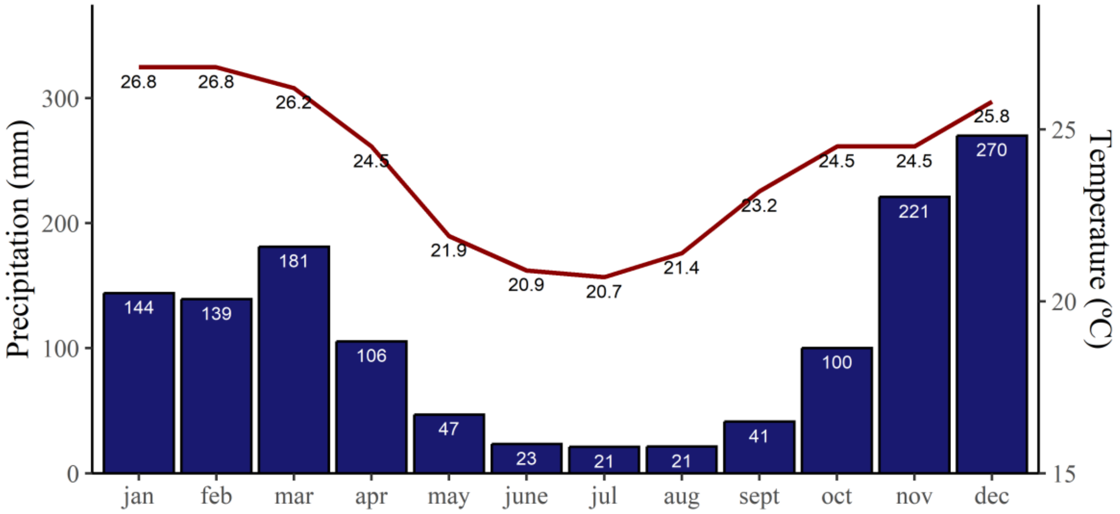

Figure 1: Average monthly temperature and rainfall from 2007 to 2022 in the experiment region. Source: INMET

Table 1: Data from chemical-physical analyzes of the experiment area.

Element	unit	depth (cm)	
		0-20	20-40
pH (1:2.5)	-	5.82	5.69
Ca	$cmol_c\ dm^{-3}$	2.36	2.00
Mg		0.99	0.86
P		12.13	7.05
K	$mg\ dm^{-3}$	94.41	45.16
MO	$g\ dm^{-3}$	12.18	10.82
Al	$cmol_c\ dm^{-3}$	0.05	0.09
H+Al		2.24	2.35
S	$mg\ dm^{-3}$	25.11	28.66
SB	$cmol_c\ dm^{-3}$	3.59	2.98
t		3.64	3.07
T		5.83	5.33
V	%	61.47	55.91
m		1.37	2.93

Sand		459	392
Silt	g kg^{-1}	258	293
Clay		283	315

SB: Sum of bases; t: effective CEC; T: CEC at pH 7; V: base saturation; m: aluminum saturation.

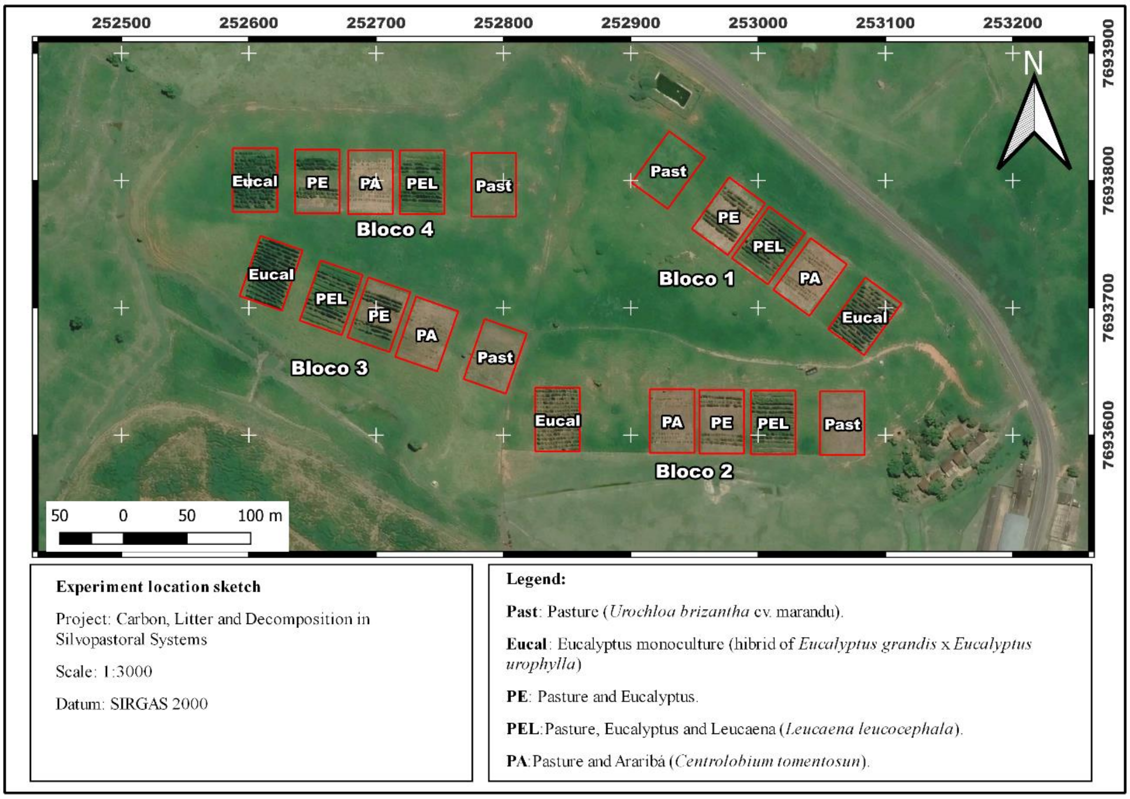

Figure 2: experiment location sketch

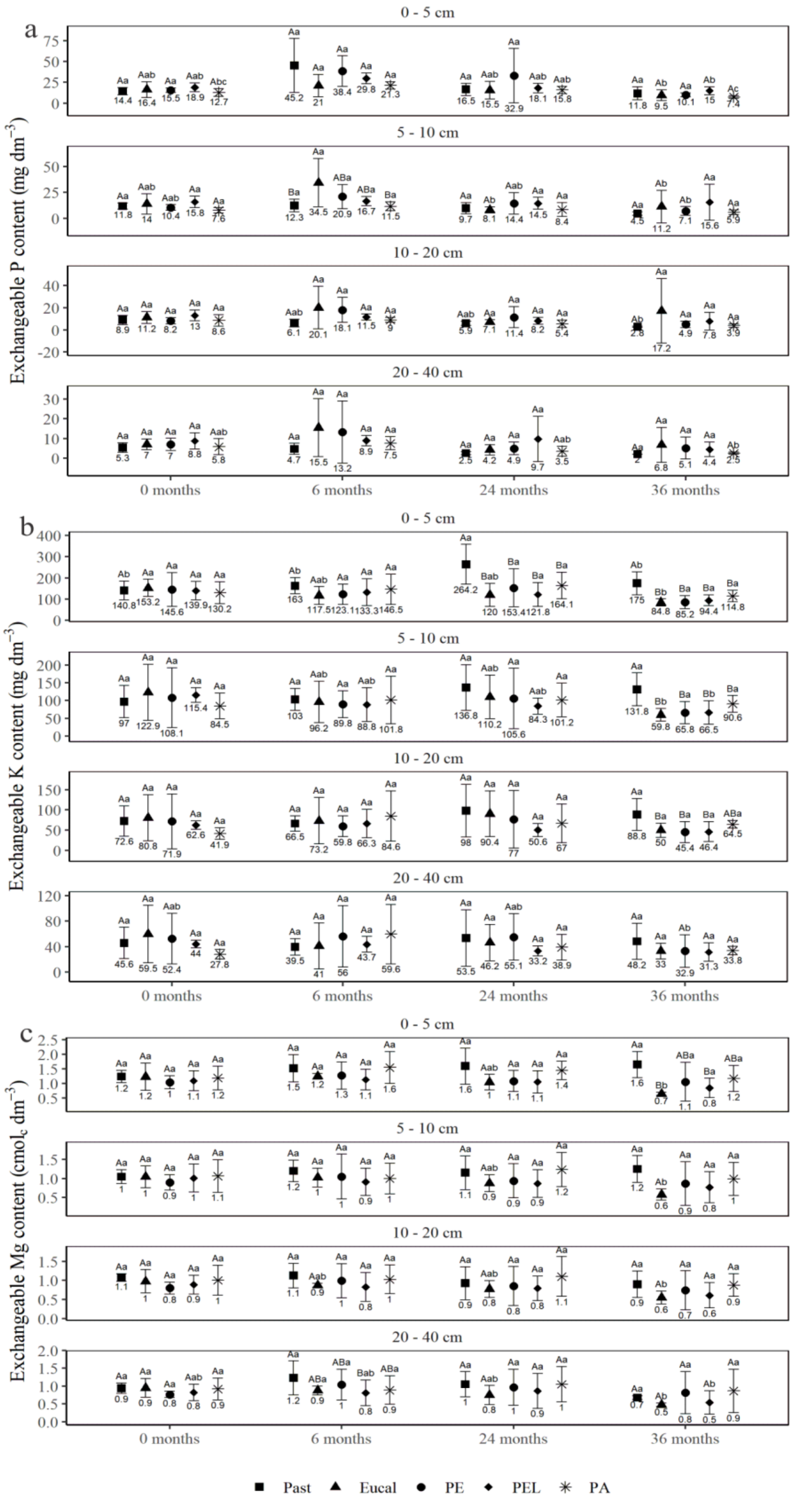

Figure 3: Soil content of: a) exchangeable P, b) exchangeable K, and c) exchangeable Mg in different Production Systems, at depths of 0 to 5, 5 to 10, 10 to 20, and 20 to 40 cm, and at times 0, 6, 24, and 36 months. Averages followed by the same uppercase letter between treatments and lowercase letter between times do not differ from each other by Tukey's test at 10% probability. Bars represent standard deviation. Past.: Pasture; Eucal.: Eucalyptus; PE: Pasture and Eucalyptus; PEL: Pasture, Eucalyptus and Leucaena; PA: Pasture and Araribá. n = 4.

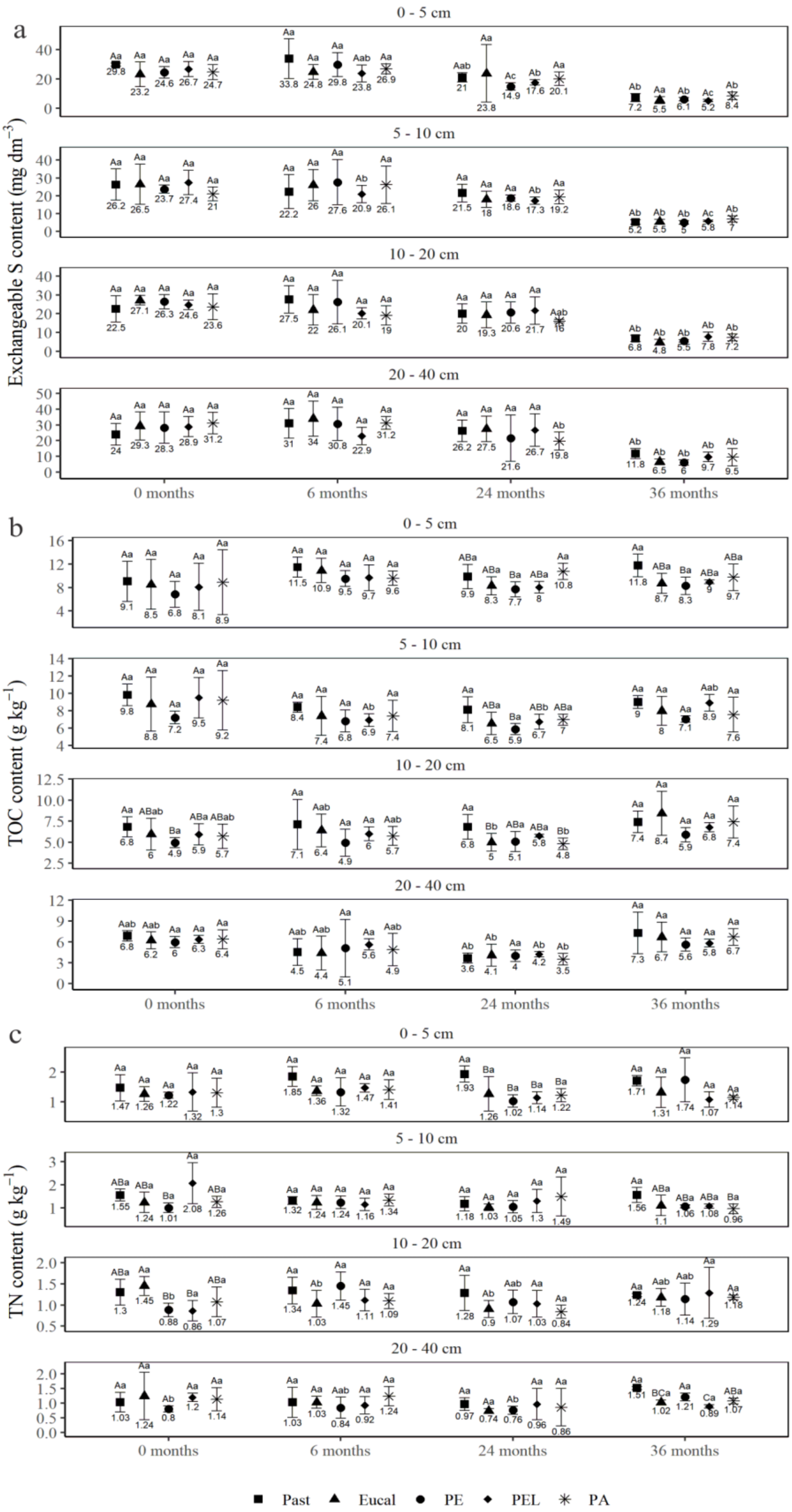

Figure 4: Soil content of: a) exchangeable S, b) total organic carbon (COT), and c) total nitrogen (NT) in different Production Systems, at depths of 0 to 5, 5 to 10, 10 to 20, and 20 to 40 cm, and at times 0, 6, 24, and 36 months. Averages followed by the same uppercase letter between treatments and lowercase letter between times do not differ from each other by Tukey's test at 10% probability. Bars represent standard deviation. Past.: Pasture; Eucal.: Eucalyptus; PE: Pasture and Eucalyptus; PEL: Pasture, Eucalyptus and Leucaena; PA: Pasture and Araribá. n = 4.

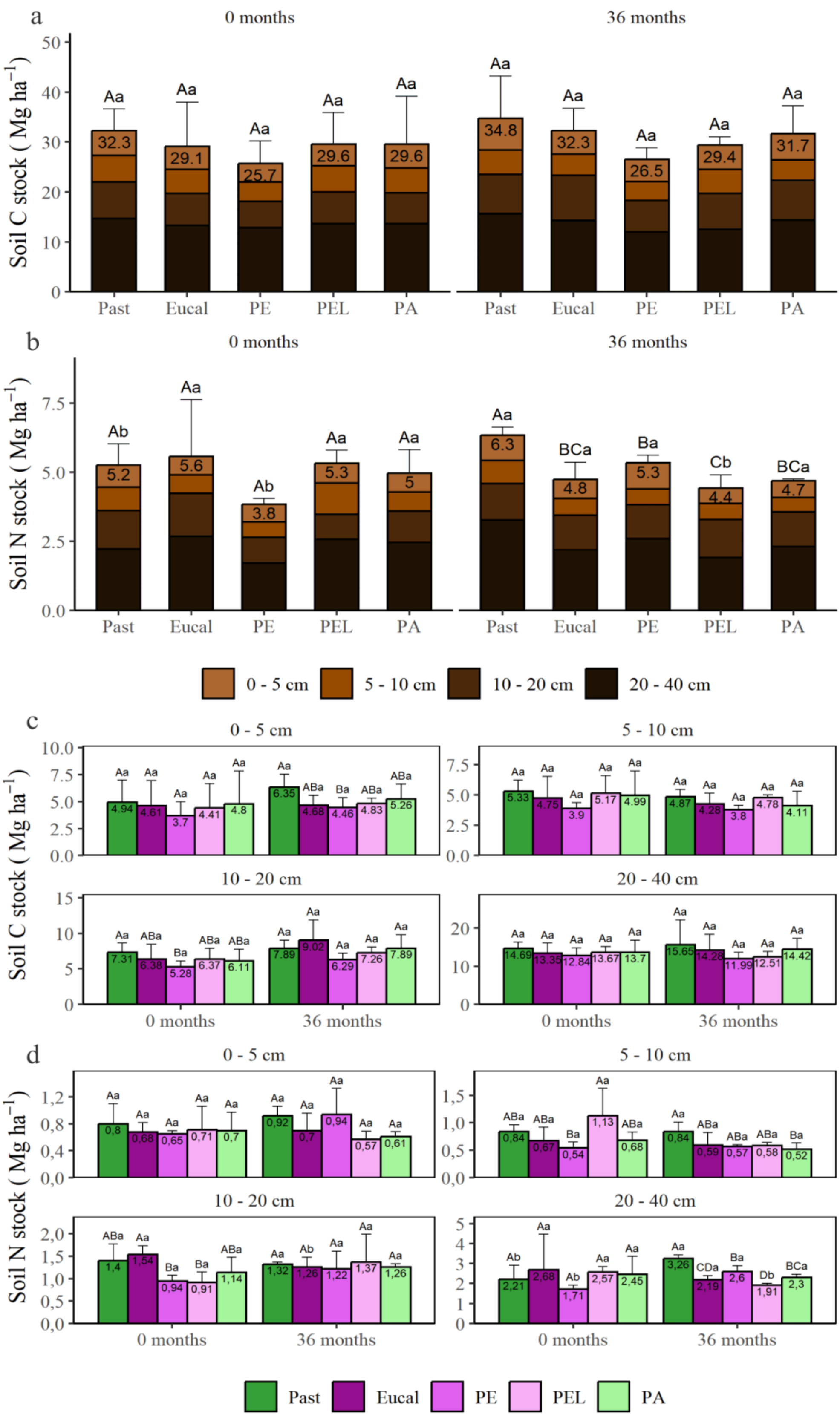

Figure 5: Stock at a depth of 0 to 40 cm of: a) C and b) N, and at depths of: c) C and d) N in different production systems. Averages followed by the same uppercase letter between treatments and lowercase letter between times did not show significant differences by Tukey's test at 10% and Student's t-test at 10%, respectively. Bars represent standard deviation. Past.: Pasture; Eucal.: Eucalyptus; PE: Pasture and Eucalyptus; PEL: Pasture, Eucalyptus, and Leucaena; PA: Pasture and Araribá. n = 4.

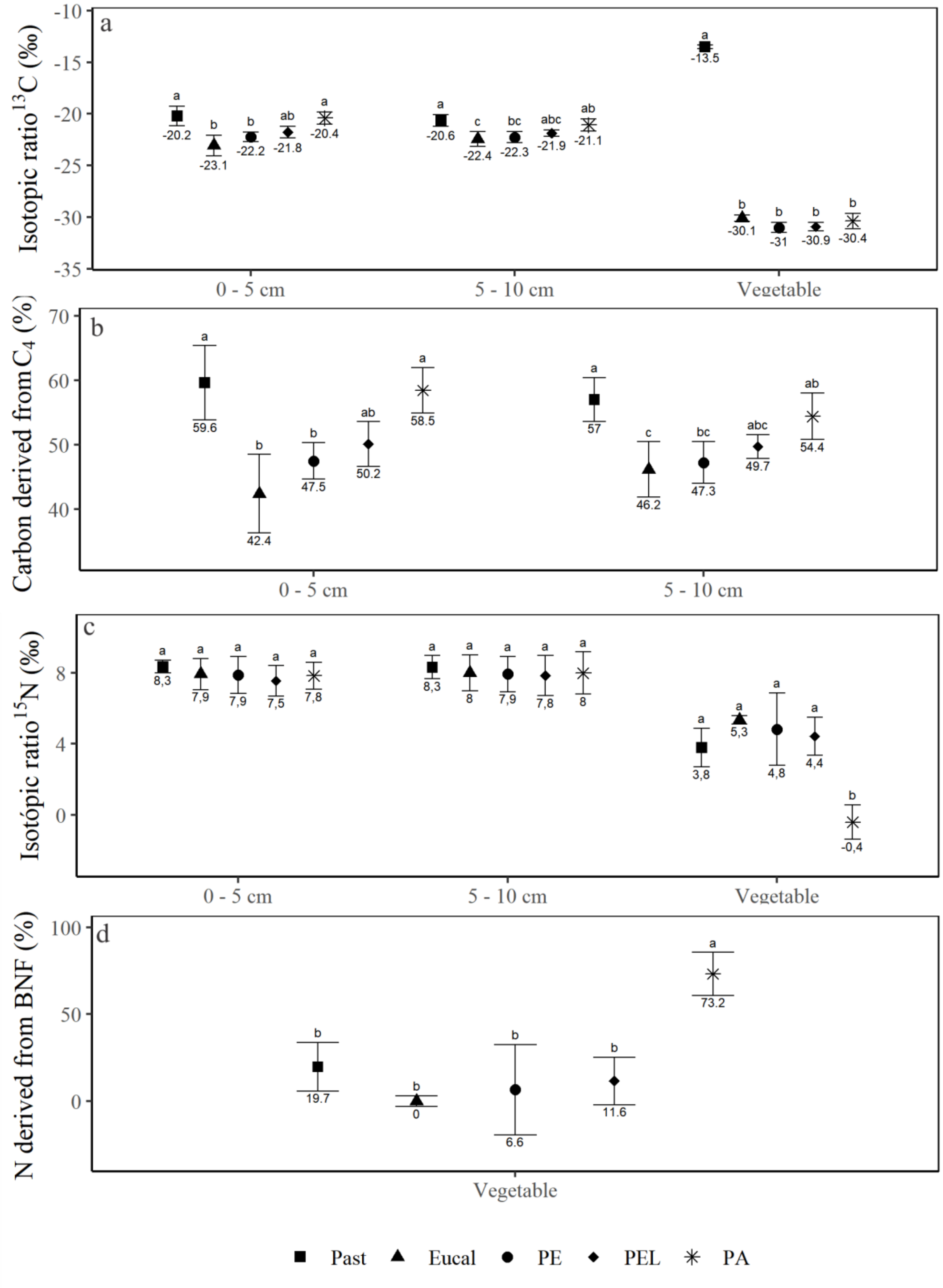

Figure 6: Isotopic ratio of: a) 13C, b) 15N, c) proportion of C derived from C4 plants, and d) contribution of Biological Nitrogen Fixation (BNF) in the soil at depths of 0 to 5 cm, 5 to 10 cm, and in the plant material in different production systems. Averages followed by the same letter did not show significant differences by Tukey's test at 10%. Bars represent standard deviation. Past.: Pasture; Eucal.: Eucalyptus; PE: Pasture and Eucalyptus; PEL: Pasture, Eucalyptus, and Leucaena; PA: Pasture and Araribá. n = 4.

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