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Increased shading in integrated agricultural systems in Southern Amazon reduces potential to store carbon and nitrogen in the soil

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ABSTRACT

Integrated crop-livestock-forestry (ICLF) systems have been used for food production in tropical environments, offering significant benefits to their components. However, shading can alter the exchange of energy and matter within the systems, directly affecting plant development and animal behavior and, ultimately, interfering with soil C and N dynamics. The aim of this study was to evaluate soil C and N pools in nine-year old integrated systems under the conditions of southern Amazon. The experimental systems comprised crop-livestock under full sunlight (ICL), moderately shaded ICLF ($ICLF_{MS}$) and strongly shaded ICLF ($ICLF_{SS}$), implemented in Sinop, Mato Grosso state, Brazil. Incidence of photosynthetically active radiation in $ICLF_{MS}$ and $ICLF_{SS}$ was suppressed by 18 % and 50 %, respectively, compared with ICL. The ICL presented the highest stocks of soil organic carbon (SOC; 84.9 Mg ha^{-1}), total nitrogen (TN; 5.3 Mg ha^{-1}), dissolved organic C (160 mg kg^{-1}), and C and N stocks in the mineral-associated organic matter fraction, as well as the lowest C lability index. In contrast, $ICLF_{SS}$ exhibited the lowest values for these parameters (SOC; 71.6 Mg ha^{-1}; TN 4.1 Mg ha^{-1}), indicating that shading alters soil C and N dynamics, resulting in reductions more than 15 % of SOC and 22 % of TN compared with ICL. Furthermore, $ICLF_{SS}$ exhibited the highest levels of labile forms of C in the particulate organic fraction and the highest lability index. Hence, shading decreased the addition and stabilization processes of C and N in soil organic matter, resulting approximately 1.5 Mg ha^{-1} year^{-1} less C and 0.14 Mg ha^{-1} year^{-1} less N accrual. The results presented herein will support decision-making processes related to soil management strategies and the implementation of systems aimed at low-emission livestock-based protein production.

1. Introduction

Integrated agricultural systems such as crop-livestock (ICL), crop-forestry (ICF), livestock-forestry (ILF) and crop-livestock-forestry (ICLF) (Embrapa, 2016) have been evaluated comprehensively under tropical climate conditions by virtue of their potential to promote significant benefits across all of the components, including soil, plants, animals and atmosphere (Marchão et al., 2024). In order to make pasture-based dairy farming viable in the southern of Amazon Biome, the forest component is essential since it improves the microclimate for lactating cows (Morenz et al., 2024), helping to face the high temperature and humidity found daily in this tropical climate (Alvares et al., 2013).

Although a solid scientific basis has been constructed regarding the feasibility of ICF, the practical adoption of ILF and ICLF systems still depends on the clarification of many aspects, one of which concerns the impact of shading on the dynamics of soil carbon (C) and nitrogen (N). A substantial body of evidence indicates that the rotation of crops grown between tree rows leads to improved soil health and increased productivity (Marchão et al., 2024; Matos et al., 2025). Moreover, the presence

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of trees improves the infiltration of water into the soil, reduces soil erosion (Sone et al., 2019; Matos et al., 2025), lowers greenhouse gas (GHG) emissions (Nascimento et al., 2020; Marchão et al., 2024) and enhances the thermal comfort of animals, especially of lactating dairy cows living under the tropical conditions (Morenz et al., 2024). On the other hand, tree canopies restrict the photosynthetically active radiation *(PAR)* received by the inter-row crops, thereby reducing the production of primary organic compounds (Pezzopane et al., 2024).

Although the photosynthetic activities of eucalyptus trees in a monoculture and those in an agroforestry system are highly comparable (Morales et al., 2023), shading in the latter reduces the biomass production by crops/grasses planted between the tree rows in a linear fashion ($R^2 = 0.70$) (Silva, 2021; Pezzopane et al., 2024). Such decreases lead to reductions in the amount of organic material incorporated into the soil surface and the rhizosphere, thereby changing the dynamics of decomposition processes, the soil microclimate and microbial communities (Berber et al., 2020; Sarto et al., 2020; Santos et al., 2024). According to Damian et al. (2023) and Tenelli et al. (2025), such alterations were responsible for the differences observed in soil C and N stocks in tropical areas under conventional management and ICLF conditions, indicating that the amount of organic material added to the soil was lower in the agroforestry system. In contrast, Matos et al. (2025) reported an increase in soil C stocks in areas cultivated under shaded systems. Additionally, it is reported that shading not only alters the production of primary organic compounds by crops, but also influences the behavior of grazing cows and, consequently, the distribution of their excreta (Carnevalli et al., 2019; Carpinelli et al., 2020), thereby influencing C and N dynamics and cycling in shaded environs.

The aim of the present study was to evaluate soil C and N pools after nine years of management in integrated agricultural systems in the southern Amazon. The levels of PAR incidence and transmittance, the total soil C and N content and their distribution between particulate organic matter (POM) and mineral-associated organic matter (MAOM) fractions, the dissolved organic C and N (DOC and DON, respectively), and soil organic C (SOC) and total N (TN) stocks were evaluated in three different integrated agricultural systems with analogous fertilization treatments and crop succession management but under dissimilar degrees of sunlight/shading. This study hypothesized that the shading provided by the tree components in integrated systems, recognized as a key element for enhancing animal welfare in pasture-based dairy systems within the humid tropic, directly influences the dynamics of soil C and N in the southern Amazon.

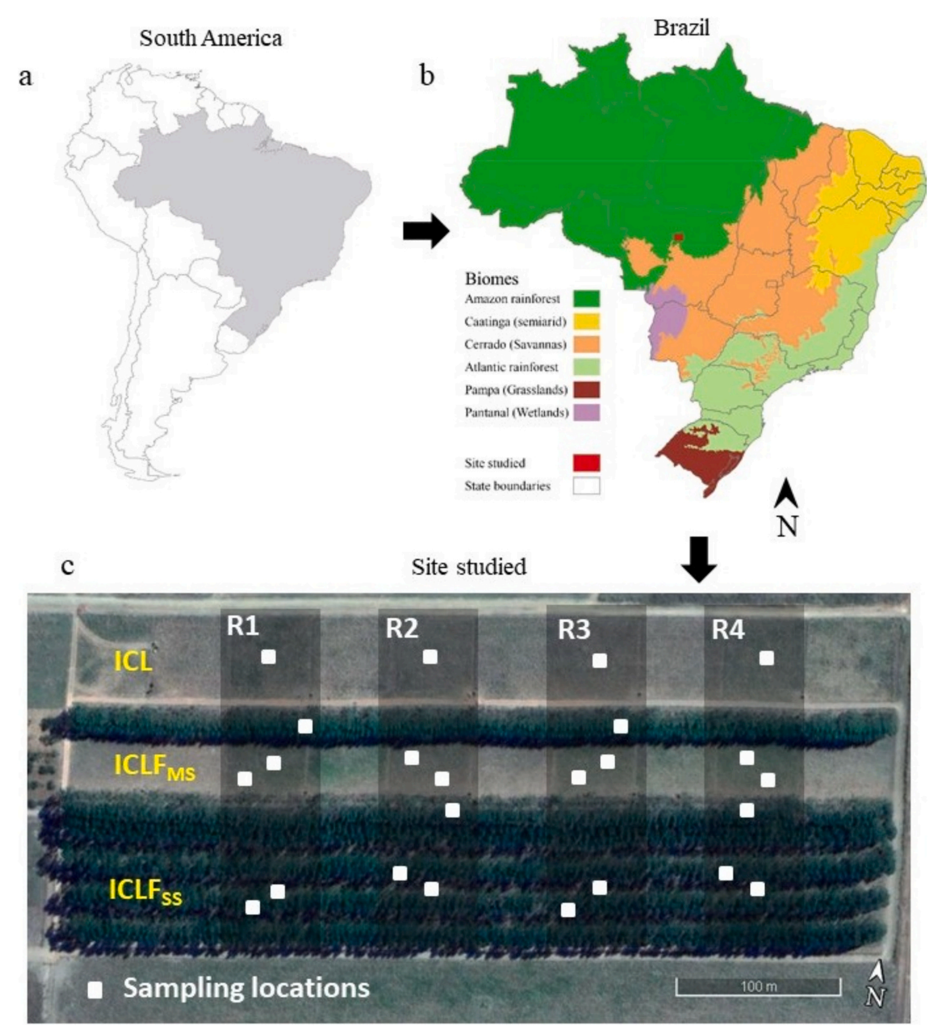

Fig. 1. Location of the study area (outlined in red) in South America (**a**) showing the distribution of the Brazilian biomes (**b**). The experimental systems consisted of integrated crop-livestock under full sunlight (ICL), moderately shaded integrated crop-livestock-forestry ($ICLF_{MS}$) and strongly shaded ICLF ($ICLF_{SS}$). Soil samples were collected from four trenches (one at each of the repeat locations R1 to R4) in the ICL, from 12 trenches (two in the crop/pasture component and one in the forest component at each of the repeat locations), and eight trenches in the $ICLF_{SS}$ (one in the crop/pasture component and one in the forest component at each of the repeat locations) (**c**).

2. Materials and methods

2.1. Site of study and background history

Sampling was carried out in an experimental area located at Embrapa Agrossilvipastoril, Sinop, State of Mato Grosso, Brazil (11°51′43" S, 55°35′27" W, altitude 384 m; Figs. 1a and b). According to the Köppen classification system, the climate of the region is Am (tropical monsoon) with rainfall concentrated mainly in the spring/summer period (Alvares et al., 2013). The soil in the area described by Viana et al. (2015) is classified as Hapludox by the U.S. Department of Agriculture (USDA) Soil Classification System (USDA, 1999) and as dystrophic red yellow latosol by the Brazilian Classification System (Santos et al., 2018), with a clayey texture and flat relief.

The entire area was originally covered by native ombrophilous forest (Araujo et al., 2009), but from 1980s onwards the native vegetation was progressively cleared to allow the cultivation of crops (maize, cotton, rice, soybean, etc). The history of the removal of native vegetation from 1975 up to the preparation of the experimental area was recorded by Landsat satellite images (Figs. S1 – S9).

The three experimental systems employed in the study comprised ICL under full sunlight, ICLF with moderate shade ($ICLF_{MS}$) and ICLF with strong shade ($ICLF_{SS}$). Implementation of the systems commenced in 2010 with subsoiling, correction of soil acidity and corrective fertilization (Coletti, 2016; Geremia et al., 2018). In January 2011, *Eucalyptus urograndis* hybrid (*Eucalyptus urophylla* x *Eucalyptus grandis*, clone H-13; Myrtaceae) was planted in rows in the east-west direction, with spacings of 2 m between trees and 3 m between rows, following two configurations involving either double-row bands of trees placed 50 m apart (initial density 338 trees ha^{-1}; $ICLF_{MS}$) or triple-row bands of trees 15 m apart (initial density 714 trees ha^{-1}; $ICLF_{SS}$) (Fig. 2). In 2015, the trees were submitted to selective thinning, reducing the densities to 240 and 340 trees ha^{-1}, respectively.

During the cultivation season 2011/2012 and 2012/2013, the ICL and the two ICLF systems were cultivated with maize (*Zea mays* L.; Poaceae) intercropped with Piatã grass [*Urochloa brizantha* (Hochst. ex A. Rich.) R.D. Webster. cv BRS Piatã; Poaceae]. Following harvesting of the maize, Piatã grass was maintained under rotational grazing by Girolando (Holstein X Gir) heifers. At the end of 2016, Piatã grass was desiccated using herbicide and subsequently replaced by Massai grass [*Megathyrsus maximum (Jacq.*) B.K.Simon & S.W.L.Jacobs cv Massai; Poaceae) intercropped with maize sown at the beginning of 2017. Following harvesting of the maize, Massai grass was maintained under rotational grazing by lactating Girolando crossbred cows. Pasture management was 95/50, defined as a pre-grazing sward height corresponding to 95 % light interception and post-grazing height of 0.50 m (Silva, 2021). Soil management procedures, fertilization and crop rotation are summarized in Figs. S10 - S12.

2.2. Soil sampling, preparation and analysis

In all of the production systems, the useful cultivation area (crop and/or pasture) comprised 2.4 ha of the total area (Figs. 1c and 2). Soil samples were collected in December 2019 from four trenches (one at each of the repeat locations R1 to R4; Fig. 1c) in the ICL, from 12 trenches in the $ICLF_{MS}$ (two in the crop/pasture component and one in the forest component at each of the repeat locations), and eight trenches in the $ICLF_{SS}$ (one in the crop/pasture component and one in the forest

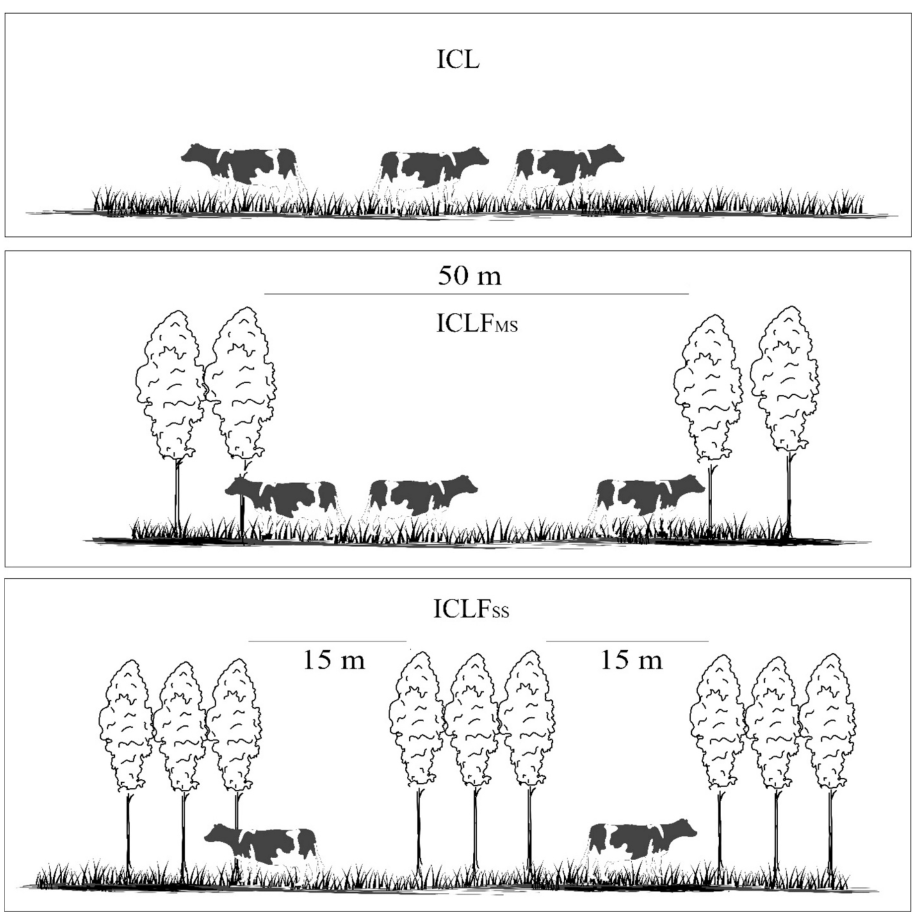

Fig. 2. Cross-sectional representation of the experimental systems showing: integrated crop-livestock under full sunlight (ICL), moderately shaded integrated crop-livestock-forestry ($ICLF_{MS}$) and strongly shaded ICLF ($ICLF_{SS}$).

component at each of the repeat locations). The opening of two trenches in the crop/pasture component of $ICLF_{MS}$ was performed with the purpose of forming composite samples per layer, since this system was configured with 50 m space between tree rows and had distinct environments, whereas the space in $ICLF_{SS}$ was just 15 m.

Soil samples were collected from secondary native forest (reference area) and from the repeat locations in the experimental systems at five different layers (0.00–0.05, 0.05–0.10, 0.10–0.20, 0.20–0.30 and 0.30–0.50 m). Disturbed samples, collected using a shovel and a knife without preserving the original field volume, were air-dried and passed through 2 mm mesh sieves to obtain air-dried fine soil (ADFS). Aliquots of the ADFS were then ground to fine powder using an agate mortar and pestle. Undisturbed samples, collected carefully to preserve the original soil volume in the field, were taken from each layer using a 100 cm^3 cylinder to determine the bulk soil density (Grossman and Reinsch, 2002).

The total elemental C and N concentrations present in disturbed soil samples were determined by weighing 50 mg of ADFS using a digital scale with a precision of four decimal places and analyzed according to the dry combustion method (Nelson and Sommers, 1982). ADFS samples were also subjected to physical fractionation to separate the SOM fractions following the procedure described by Cambardella and Elliott (1992). Briefly, a 5 g aliquot of ADFS was transferred to a 50 mL Falcon tube followed by the addition of 30 mL of deionized water (Milli-Q) and sodium hexametaphosphate (5 g L^{-1}) solution. The mixture was subjected to constant agitation on an orbital shaker for 16 h and subsequently sieved through a 53 μm mesh sieve. The material retained on the sieve was classified as POM, whereas the material that passed through the sieve was classified as MAOM. The elemental C and N content of the separated fractions were analyzed by the dry combustion method (Nelson and Sommers, 1982).

The first and second superficial soil layers, which contained the highest elemental C and N contents, were selected for analysis of DOC and DON. For this purpose, equal proportions of ADFS samples from the top layers (0.00–0.05 and 0.05–0.10 m) were combined and extracted with deionized water (soil:water ratio of 1:5) for 30 min, centrifuged for 5 min at 4500 rpm (rotor diameter 252 mm) and the supernatant filtered through 0.45 mm membrane filters (Gregorich et al., 2003). The concentrations of DOC and DON were determined using a C and N high-temperature combustion analyzer (Qiu et al., 2015).

2.3. Calculation of soil C and N stocks

C and N stocks in each layer were calculated according to eq. 1 (Batjes, 1996):

$$\text{C or N stocks } \left(\text{Mg ha}^{-1}\right) = \text{C or N x Ds x E x } (1\text{–P}) \text{ x } 10^{-1} \quad (1)$$

Where: C and N represent the total elemental concentrations present in ADFS; Ds is the bulk soil density; E is the layer thickness and P is the percentage of gravel and stones. Values for SOC and total N stocks per equivalent soil mass in each system were calculated as described by Sisti et al. (2004), while the levels of C and N stocks present in POM and MAOM fractions were determined as described by Cotrufo et al. (2019). In order to calculate the levels of C and N stocks in the two ICLF systems, the areas occupied by the crop/pasture and forest components were calculated from their lengths and widths determined using a 50 m measuring tape. In the $ICLF_{MS}$, the crop/pasture component occupied 70 % of the total area and the remaining 30 % was taken up by the forest, whereas in the $ICLF_{SS}$ each component occupied 50 % of the total area.

2.4. Calculation of carbon indices

The results from the fractionation of POM and MAOM described in section 2.2 were used to calculate carbon indices. Carbon lability (L) was calculated according to eq. 2, in which C_L is labile C (POM, Mg ha^{-1}) and C_{NL} is non-labile C (MAOM; Mg ha^{-1}). On this basis, the lability index (LI) was calculated using eq. 3. Carbon pool index (CPI) was established according to eq. 4, in which C_T represents SOC (Mg ha^{-1}), and carbon management index (CMI) was estimated according to eq. 5, which was adapted by Diekow et al. (2005) from the original formula of Blair et al. (1995).

$$\text{Carbon lability (L)} = C_L / C_{NL} \quad (2)$$

$$\text{Lability index (LI)} = \text{L of experimental system/L of reference area} \quad (3)$$

$$\text{Carbon pool index (CPI)} = C_T \text{ of experimental system}/C_T \text{ of reference area} \quad (4)$$

$$\text{Carbon management index (CMI)} = \text{CPI x LI x 100} \quad (5)$$

2.5. PAR measurements

PAR data were collected using sensors, installed at a height of 1.5 m, in meteorological stations equipped with automatic data acquisition systems (dataloggers) programmed to record readings at 5 s intervals and to provide average and total values every 15 min, hourly and daily. One meteorological station was installed in the center of the ICL system and one in the center of the crop/pasture component of the $ICLF_{SS}$. In consideration of the greater distance between rows in the $ICLF_{MS}$ system, two sensors were installed in the crop/pasture component and the data used to determine a mean value. The mean PAR for each agricultural system was calculated from the hourly data collected between 8 am and 4 pm during the period 2013 to 2019. The mean PAR values for ICL, $ICLF_{MS}$ and $ICLF_{SS}$ were used to calculate the percentage PAR transmission in shaded systems according to eq. 6 (Pezzopane et al., 2024):

$$\text{PAR transmission (\%)} = \text{PAR in } ICLF_{MS} \text{ or } ICLF_{SS}/\text{PAR in ICL} \quad (6)$$

2.6. Statistical analysis

Data relating to total elemental C and N content, SOC and TN stocks, C and N stocks in the POM and MAOM fractions, C:N ratio, DOC, DON, L, LI, CPI, and CMI of the experimental systems were first-tested for normality and homoscedasticity of the variance using the Shapiro-Wilk and Levene's tests, respectively. When both assumptions were satisfied, the data were submitted to analysis of variance (ANOVA) and the means compared using Tukey test at 5 % probability. When necessary, the results relating to ICL, $ICLF_{MS \text{ and}}$ $ICLF_{SS}$ were compared with those of the reference area (native secondary forest) using Student's *t*-test for pairwise comparisons. Linear regression models were constructed for the relationships of PAR (average annual value) with SOC and TN stocks for each of the experimental systems. Principal component analysis (PCA) was performed to better understand the distribution of the evaluated parameters of the integrated systems and the native forest, and correlation tests were applied between the principal components and soil variables to identify the most influential factors. All analyses were performed using RStudio software (R Development Core Team, 2021) and the following package were used: stats package for the Shapiro-Wilk normality test, ANOVA, Student's t-test, linear regression and Tukey's HSD post hoc test; corrplot to generate correlation matrices and plots for visualizing relationships among variables; and factoMineR, factoextra, and ggplot2 for PCA and generating biplots.

3. Results

3.1. PAR incidence and transmission in the experimental systems

PAR incidence in the ICL system remained constant at approximately 900 μmol m^{-2} s^{-1} in the period 2013 to 2019 (Fig. 3). In the $ICLF_{MS}$, PAR incidence decreased progressively from 900 μmol m^{-2} s^{-1} in 2013 to

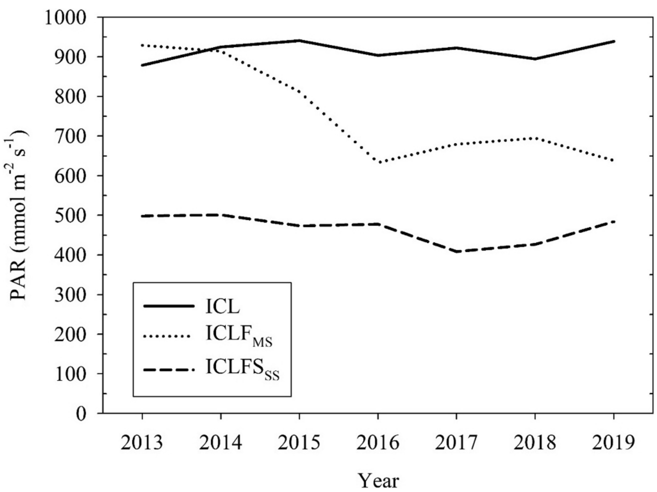

Fig. 3. Photosynthetically active radiation (PAR) assessed during the period 2013 to 2019 in the experimental systems showing: integrated crop-livestock under full sunlight (ICL), moderately shaded integrated crop-livestock-forestry ($ICLF_{MS}$) and strongly shaded ICLF ($ICLF_{SS}$).

633 μmol m^{-2} s^{-1} in 2016, following which it remained relatively stable up to 2019. In the $ICLF_{SS}$, PAR incidence during the period 2013 to 2019 was low and varied between 400 and 500 μmol m^{-2} s^{-1}. Over the seven-year-period, PAR incidences at the crop/pasture components measured daily from 08:00 to 16:00 h presented average values of 919, 749 and 450 μmol m^{-2} s^{-1} in the ICL, $ICLF_{MS}$ and $ICLF_{SS}$ systems, respectively (Fig. 4). PAR transmissions in the crop/pasture components of $ICLF_{MS}$ and $ICLF_{SS}$ were 82 and 49 %, respectively, indicating 18 and 51 % suppression of light under the respective conditions.

3.2. C and N pools

The physical and chemical characteristics of the top soil layer (0–0.20 m depth) in the evaluated integrated systems are presented in Table S1. As shown in Fig. 4, the C and N content of the soils of each system decreased from the 0 to the 0.50 m layers. The effects of system were observed mainly up to 0.30 m layer, and most especially up to 0.10 m. In the ICL (Fig. 4a and k) and the crop/pasture component of $ICLF_{MS}$ (Fig. 4c and m), the highest levels of C and N content were observed in the 0 to 0.05 m layer, and such values were significantly higher ($p < 0.05$) than those of the forest component of $ICLF_{MS}$ (Fig. 4b and l) and both components of $ICLF_{SS}$ (Fig. 4d, e, n and t). The high C content in the 0.05 m layer in ICL (Fig. 4f) and in the crop/pasture component of $ICLF_{MS}$ (Fig. 4h) was reflected in the SOC stocks, with both systems showing an approximate value of 16 Mg ha^{-1}. In contrast, the SOC stocks present in the 0.05 m layer of the crop/pasture component of $ICLF_{SS}$, 10.3 Mg ha^{-1} (Fig. 4i), were significantly lower than those in the same component of $ICLF_{MS}$, 16.2 Mg ha^{-1} (Fig. 4h). Furthermore, TN stocks present in the 0 to 0.05 m layers were also significantly higher in the systems with high C contents, around 1 Mg ha^{-1}.

In the 0.05 to 0.10 m layer, dissimilarities in C and N content, when they occurred, were observed only between ICL and the crop/pasture component of $ICLF_{SS}$, while no differences between the experimental systems were observed in the 0.10 to 0.20 m and 0.30 to 0.50 layers. However, the C content and SOC stocks in the 0.20 to 0.30 m layer were higher in the forest component of $ICLF_{MS}$ (Fig. 4b and g) compared with those of the crop/pasture component of $ICLF_{SS}$ (Fig. 4e and j).

Analyses of the experimental systems and their components separately revealed that SOC stocks up to 0.5 m depth were higher in the ICL and the crop/pasture component of $ICLF_{MS}$ compared with those in the crop/pasture component of $ICLF_{SS}$, with mean values of 84.9, 81.7 and 67.1 Mg ha^{-1}, respectively. On the other hand, SOC stocks in the forest components of $ICLF_{MS}$ and $ICLF_{SS}$ were similar to those of ICL and $ICLF_{MS}$ crop/pasture components with values of 81.0 and 76.1 Mg ha^{-1}. Soil TN stocks in the ICL and the crop/pasture and forest components of $ICLF_{MS}$ were higher (5.3, 4.8 and 4.7 Mg ha^{-1}, respectively) than those in the crop/pasture and forest components of $ICLF_{SS}$ (3.8 and 4.3 Mg ha^{-1}, respectively).

Considering each system as a whole, and weighing the contribution of the two components in the agroforestry systems, revealed that the SOC stocks in ICL and $ICLF_{MS}$ were similar to that of the reference area and significantly higher ($p < 0.05$) than that of $ICLF_{SS}$ (Fig. 5a). Findings analogous to those relating to SOC stocks were also observed for TN stocks (Fig. 5b).

DOC content diminished in the order ICL > $ICLF_{MS}$ > $ICLF_{SS}$ and the differences between the systems were statistically significant ($p < 0.05$) (Fig. 6a). Furthermore, all of the experimental systems presented significantly lower contents of DOC compared with that of the reference area. In contrast, there were no significant differences between the experimental systems regarding the values of DON, but all were significantly higher than that of the reference area despite the high DOC associated with the latter (Fig. 6b).

The $ICLF_{MS}$ and $ICLF_{SS}$ systems presented the highest C-labilities with L values above 0.63, while the ICL system and the reference area showed the lowest L values of around 0.2 (Fig. 8a). Such large differences in C-labilities were reflected in the LI indices, which were between 3 and 4 for the agroforestry systems and around 1 for ICL and the reference area (Fig. 7b), and in the values of CMI, which were established to be in the range 250 to 300 for the agroforestry systems and between 90 and 100 for ICL and the reference area (Fig. 7d). The CPI values of the experimental systems were similar but significantly lower than that of the reference area (Fig. 7c). Thus, whilst the LI values indicated that the levels of C-lability in the shaded systems were three to four times greater than in the reference area, the CMI values, which take into account CPI weighting, suggested that C-labilities in the agroforestry systems were only two to three times higher than in reference area.

There were significant differences ($p < 0.05$) in the surface soil layer (0 to 0.05 m) between the experimental systems regarding C and N stocks present in the POM fraction and C stocks in the MAOM fraction (Table 1). The highest C and N stocks in POM fractions were recorded in the $ICLF_{MS}$ crop/pasture component, while the lowest were observed in the ICL. It is noteworthy that the crop/pasture components of $ICLF_{MS}$ and $ICLF_{SS}$ systems did not differ with respect to the C stocks in POM. In the MAOM fraction, the highest C stocks were registered in ICL whereas the lowest were found in $ICLF_{SS}$ crop/pasture component. Regarding the C:N ratios in the surface layer, significant differences ($p < 0.05$) between the experimental systems were observed in MAOM but not in POM. Interestingly, the statistically significant maximum C:N ratio was observed in the MAOM of $ICLF_{MS}$ forest whereas the minimum value was observed in the MAOM of ICL.

In the 0.05 to 0.10 m layer, significant differences between the experimental systems were detected only for C stocks in POM and MAOM fractions. In the 0.10 to 0.20 m layer, the C and N stocks of the POM were similar in all experimental systems although there were some significant differences regarding the C and N stocks of the MAOM, with ICL presenting the highest stocks of both elements and $ICLF_{SS}$ exhibiting the lowest.

In the 0.20 to 0.30 m layer, significant differences between the experimental systems were detected in the POM for C stocks, in the MAOM for C and N stocks, and in both fractions for C:N ratios. Concerning the POM fraction, the highest C stocks were recorded in $ICLF_{SS}$ forest component and the lowest in ICL. In relation to the MAOM fraction, the highest C stocks were found in ICL and the lowest in $ICLF_{SS}$ crop/pasture component, whereas the highest N stocks were detected in the $ICLF_{MS}$ forest component and ICL and the lowest in $ICLF_{MS}$ crop/pasture component. The POM fraction of $ICLF_{MS}$ forest component presented the highest C:N ratio, while the crop/pasture component of the same system presented the lowest ratio. Regarding the MAOM

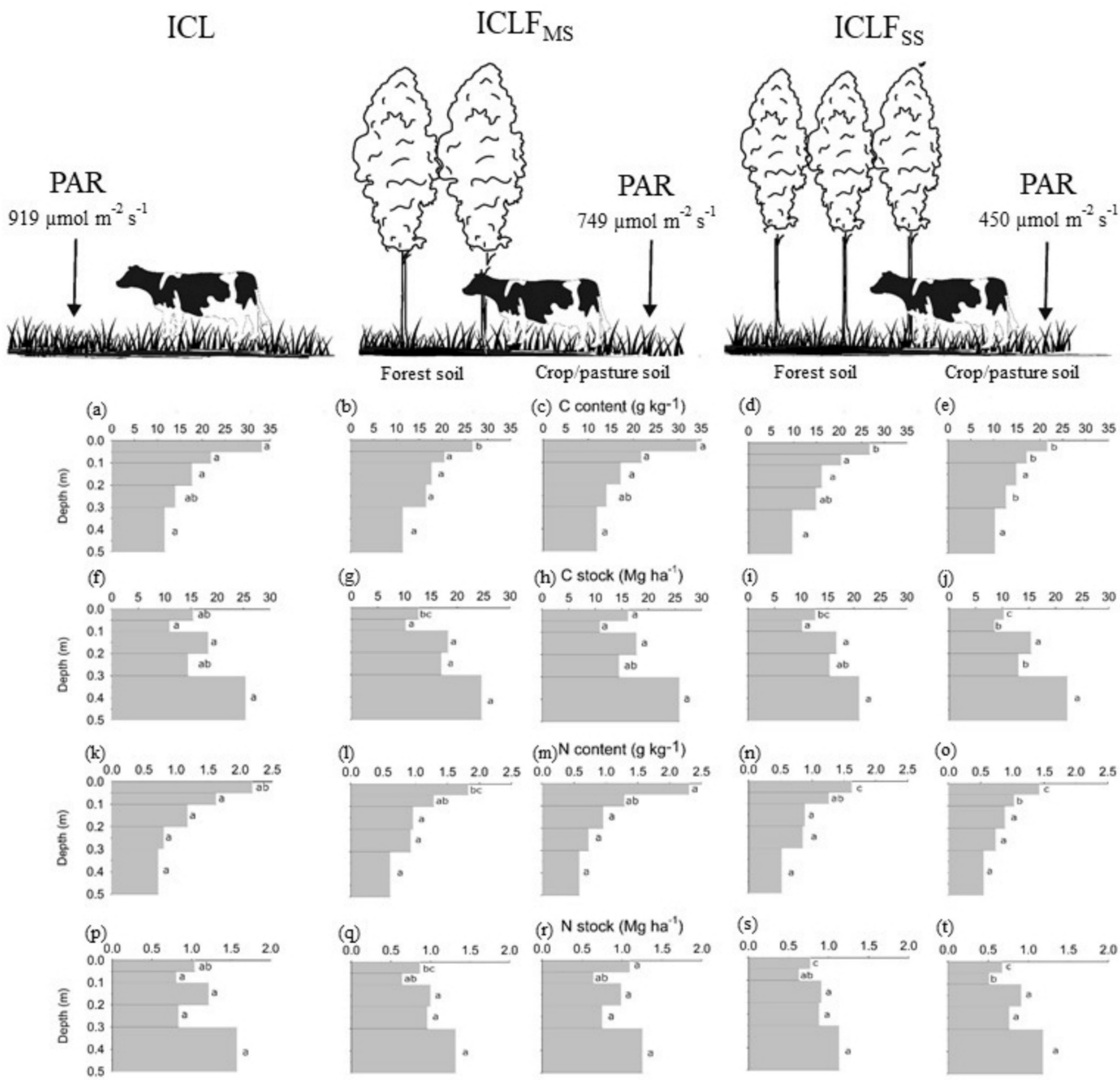

Fig. 4. Comparison of the experimental systems and their forest and crop/pasture regarding the C (**a, b, c, d,** and **e**) and N (**k, l, m, n,** and **o**) contents and SOC (**f, g, h, i,** and **j**) and TN (**p, q, r, s,** and **t**) stocks in soil up to a depth of 0.5 m showing: integrated crop-livestock under full sunlight (ICL), moderately shaded integrated crop-livestock-forestry ($ICLF_{MS}$) and strongly shaded ICLF ($ICLF_{SS}$). Bars bearing dissimilar lowercase letters are significantly different according to Tukeys test ($p < 0.05$).

fraction, the highest and the lowest C:N ratios were recorded in the $ICLF_{SS}$ crop/pasture component and ICL, respectively.

In the 0.30 to 0.50 m layer, significant differences between the experimental systems were detected only for C and N stocks of the MAOM fraction. ICL presented the highest C and N stocks while the $ICLF_{SS}$ forest component presented the lowest.

Overall examination of Table 1 shows that the highest C and N stocks in POM were found in the shaded systems ($ICLF_{MS}$ and $ICLF_{SS}$), but the highest levels of MAOM were detected in ICL. Furthermore, as soil depth increased, the proportion of C and N in MAOM fraction gradually increased relative to the POM fraction, indicating a shift toward more stable forms of organic matter with increasing depth.

3.3. Relationship between PAR incidence and SOC and TN stocks

The linear regression equations presented in Fig. 8 show that PAR incidence was positively and significantly correlated with SOC and TN stocks in all experimental systems, with shading negatively affecting these stocks most predominantly in $ICLF_{SS}$. The goodness of fit of the regression models was adequate, with r^2 values of 75 and 83 % for SOC and TN stocks, but would have been improved if the regressions had been adjusted using mean values of the parameters. Nevertheless, the linear regression models reveal that for every 100 µmol m^{-2} s^{-1} decrease in PAR caused by shading, SOC stocks diminished by 4 Mg ha^{-1} and TN stocks by 0.32 Mg ha^{-1}.

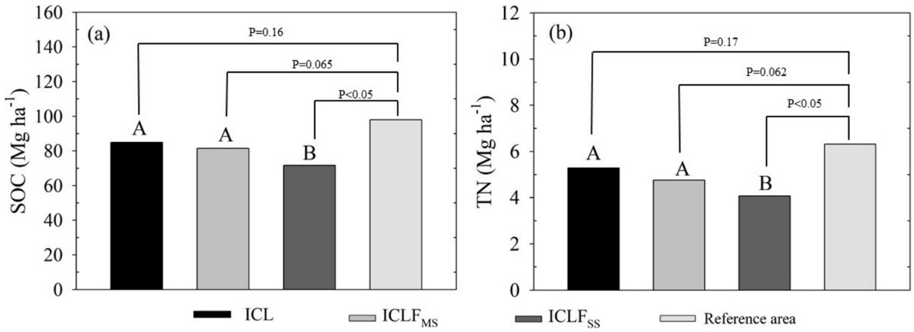

Fig. 5. Comparison of secondary native forest (reference area) and experimental systems regarding the soil organic carbon (SOC) and total nitrogen (TN) stocks up to a depth of 0.5 m layer showing: integrated crop-livestock under full sunlight (ICL), moderately shaded integrated crop-livestock-forestry ($ICLF_{MS}$) and strongly shaded ICLF ($ICLF_{SS}$). Bars bearing dissimilar capital letters are significantly different according to Student's *t*-test ($p < 0.05$).

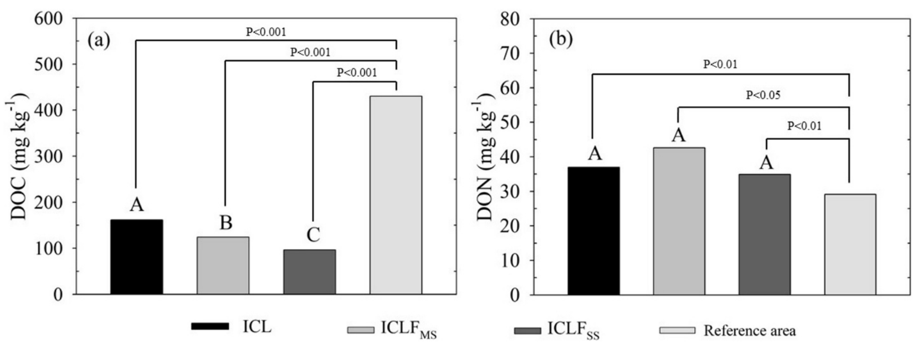

Fig. 6. Comparison of secondary native forest (reference area) and experimental systems regarding dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) showing: integrated crop-livestock under full sunlight (ICL), moderately shaded integrated crop-livestock-forestry ($ICLF_{MS}$) and strongly shaded ICLF ($ICLF_{SS}$). Bars bearing dissimilar capital letters are significantly different according to Student's *t*-test ($p < 0.05$).

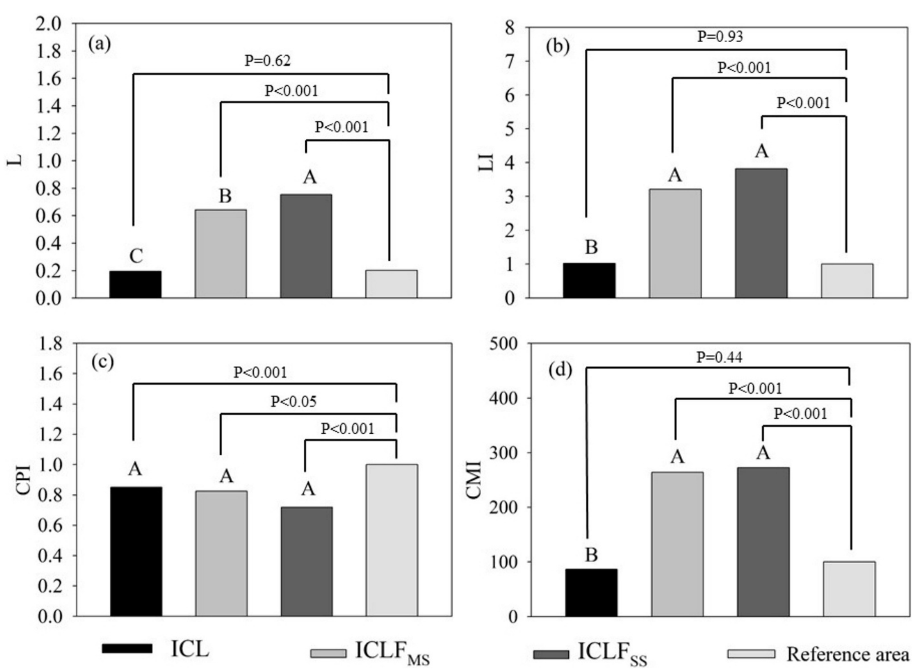

Fig. 7. Comparison of secondary native forest (reference area) and experimental systems regarding carbon lability (L,) lability index (LI), carbon pool index (CPI) and carbon management index (CMI) showing: integrated crop-livestock under full sunlight (ICL), moderately shaded integrated crop-livestock-forestry ($ICLF_{MS}$) and strongly shaded ICLF ($ICLF_{SS}$). Bars bearing dissimilar capital letters are significantly different according to Student's *t*-test ($p < 0.05$).

Table 1
Carbon (C) and nitrogen (N) stocks present in soil organic matter fractions of the experimental systems along with their percentage contributions and carbon C:N ratios.

Systems and their components	Soil organic matter fractions					
	POM			MAOM		
	C stocks Mg ha^{-1} (%)	N stocks Mg ha^{-1} (%)	C:N ratio	C stocks Mg ha^{-1} (%)	N stocks Mg ha^{-1} (%)	C:N ratio
0.00–0.05 m layer						
ICL	3.7 b (24)	0.22 b (27)	17.6	12.0 a (76)	0.62 (73)	12.5 c
ICLF$_{MS}$ forest	5.1 ab (38)	0.37 ab (43)	19.4	7.6 b (62)	0.50 (57)	20.3 a
ICLF$_{MS}$ crop/pasture	6.6 a (46)	0.48 a (47)	14.3	8.5 b (54)	0.54 (53)	16.3 b
ICLF$_{SS}$ forest	5.3 ab (42)	0.28 b (36)	18.7	7.4 b (58)	0.49 (64)	14.0 bc
ICLF$_{SS}$ crop/pasture	5.5 a (55)	0.37 ab (54)	18.1	4.4 c (45)	0.31 (46)	16.5 b
0.05–0.10 m layer						
ICL	2.9 b (26)	0.27 (34)	21.6	8.1 a (74)	0.54 (66)	14.6
ICLF$_{MS}$ forest	5.2 a (51)	0.30 (46)	18.8	5.1 b (49)	0.35 (54)	16.5
ICLF$_{MS}$ crop/pasture	4.6 ab (44)	0.26 (42)	17.6	5.8 ab (56)	0.35 (58)	15.1
ICLF$_{SS}$ forest	4.9 ab (48)	0.27 (42)	20.3	5.3 b (52)	0.37 (58)	15.6
ICLF$_{SS}$ crop/pasture	4.2 ab (48)	0.22 (43)	23.7	4.4 b (52)	0.29 (57)	18.6
0.10–0.20 m layer						
ICL	3.6 (20)	0.27 (22)	28.0	14.7 a (80)	0.95 a (78)	15.0
ICLF$_{MS}$ forest	6.7 (37)	0.25 (25)	27.4	11.6 ab (63)	0.75 ab (75)	14.6
ICLF$_{MS}$ crop/pasture	6.6 (38)	0.27 (28)	25.9	10.8 b (62)	0.68 ab (72)	15.7
ICLF$_{SS}$ forest	5.9 (35)	0.32 (35)	23.6	10.8 b (65)	0.59 b (65)	28.2
ICLF$_{SS}$ crop/pasture	5.7 (37)	0.31 (34)	24.8	9.7 b (63)	0.60 b (66)	18.1
0.20–0.30 m layer						
ICL	2.3 b (16)	0.16 (19)	47.5 ab	12.2 a (84)	0.68 a (81)	14.4b
ICLF$_{MS}$ forest	5.5 ab (32)	0.17 (18)	49.5 a	11.6 ab (68)	0.79 a (82)	17.6 ab
ICLF$_{MS}$ crop/pasture	5.4 ab (39)	0.23 (32)	27.9 b	8.5 bc (61)	0.49 b (68)	16.2 ab
ICLF$_{SS}$ forest	6.8 a (44)	0.31 (35)	36.0 ab	8.6 bc (56)	0.57 ab (65)	16.4 ab
ICLF$_{SS}$ crop/pasture	4.9 ab (38)	0.24 (31)	42.1 ab	8.2 c (62)	0.52 ab (69)	20.7 a
0.30–0.50 m layer						
ICL	4.9 (19)	0.41 (26)	32.4	20.5 a (81)	1.16 a (74)	16.0
ICLF$_{MS}$ forest	9.1 (37)	0.42 (32)	36.7	15.5 ab (63)	0.90 ab (68)	18.7
ICLF$_{MS}$ crop/pasture	9.1 (36)	0.32 (26)	34.9	15.9 ab (64)	0.90 ab (74)	19.4
ICLF$_{SS}$ forest	11.9 (57)	0.55 (48)	49.0	9.1 b (43)	0.59 b (52)	16.7
ICLF$_{SS}$ crop/pasture	7.3 (33)	0.39 (33)	37.6	14.9 ab (67)	0.79 ab (67)	20.7

Abbreviations: ICL, integrated crop-livestock under full sunlight; moderately shaded integrated crop-livestock-forestry (ICLF$_{MS}$); ICLF$_{SS}$, strongly shaded ICLF; MAOM, mineral-attached organic matter; POM, particulate organic matter.
Mean values followed by dissimilar lower case letters in the columns of each soil layer are significantly different according to Tukey test ($p < 0.05$).

3.4. Results from PCA

PCA analysis explained more than 82 % of the total variation in the dataset, with PC1 explaining 70.53 % and PC2 explaining 12.14 % (Fig. 9a). As shown in Fig. 9b, C and N stocks in MAOM, SOC stocks, TN stocks, CPI and DOC were all positively correlated with PC1 (correlation coefficient $R > 0.75$), whereas C and N stocks in POM, DON, L, LI and CMI were negatively correlated with PC1 ($R < -0.5$). All variables were positively correlated with PC2 ($R = 0.05$ to 0.59).

The C and N stocks of MAOM, along with SOC and TN stocks, CPI and DOC had positive weightings leading to grouping with samples from the reference area (native forest) on the upper right side of the PCA biplot (Fig. 9a). On the other hand, the C and N stocks of POM, as well as DON, L, LI and CMI were grouped on the left side of the biplot with samples from the ICLF$_{SS}$ system (Fig. 9a). The ICLF$_{MS}$ and ICL samples lie between the ICLF$_{SS}$ and the reference area clusters, with the former positioned nearer to the ICLF$_{SS}$ group and the second located in the center nearer to the reference area group. Consideration of all of the variables together facilitates our understanding of soil C and N dynamics/distribution and indicates that shade intensity promotes changes that segregate moderately and strongly shaded areas from native forest and sunlit ICL.

4. Discussion

4.1. Effects of shading on C and N stocks in the soil

The lowest C and N stocks found in ICLF$_{SS}$ had differences of more than 13 Mg ha^{-1} of SOC and 1.2 Mg ha^{-1} of TN in relation to ICL. Hence, after nine years of management of the experimental systems, C sequestration in ICL was almost 1.5 Mg ha^{-1} year^{-1} greater than in ICLF$_{SS}$. In marked contrast, the C and N stocks in ICL and ICLF$_{MS}$ differed little one from another. There is conflicting evidence in the literature about the effects of shading, with some articles claiming that the presence of trees does not increase C and N stocks in the soil (Damian et al., 2023; Tenelli et al., 2025) while others suggest the opposite (Matos et al., 2025).

The lower C and N stocks in ICLF$_{SS}$ crop/pasture can be explained by the lower input of organic material into the soil surface, which is due to the reduced production of dry plant material by the crop/grass and the smaller contribution by dead grass, all triggered by the tree shadow (Silva, 2021). The linear relationship between increased shading and decreased soil C and N stocks also confirm the relationship with the reduced primary organic compound production by crop//pasture components (Pezzopane et al., 2020, 2024). The fact that grasses had been the predominant during the entire history of the systems explains partially the low C and N stocks present in the crop/pasture component up to a depth of 0.5 m, because C_4 plants, such as grasses, are very sensitive to the restriction of light (Taiz and Zeiger, 2009)

On the other hand, forest components of ICLF$_{MS}$ and ICLF$_{SS}$ presented C and N stocks similar to those of the other components of the systems, indicating that the trees also contributed organic material to the soil. It is important to note that the forest components of ICLF$_{MS}$ and ICLF$_{SS}$ were not subjected to the same mechanized procedures as the crop/pasture components, which might have suffered soil disturbances caused by the sowing of maize and grass. It cannot be ruled out, however, that lactating cows could have played a significant role in this process by concentrating the deposition of excreta near to the trees (Carnevalli et al., 2019). Even though the C stocks in ICLF$_{SS}$ presented the lowest values of the studied systems, their mean of 71.6 Mg ha^{-1} is still considered high. Earlier studies asserted that the soil of ICLF systems contained lower C and N stocks in the 0 to 0.30 m layer in comparison with well-managed pastures (Rachwal et al., 2022; Tenelli et al., 2025).

Although the leaves of plants cultivated between tree bands in strongly shaded ICLF systems contained high levels of N (Silva, 2021), these were not sufficient to increase soil N stocks or reduce C:N ratios in POM or MAOM fractions (Table 1). Thus, while N content of plant

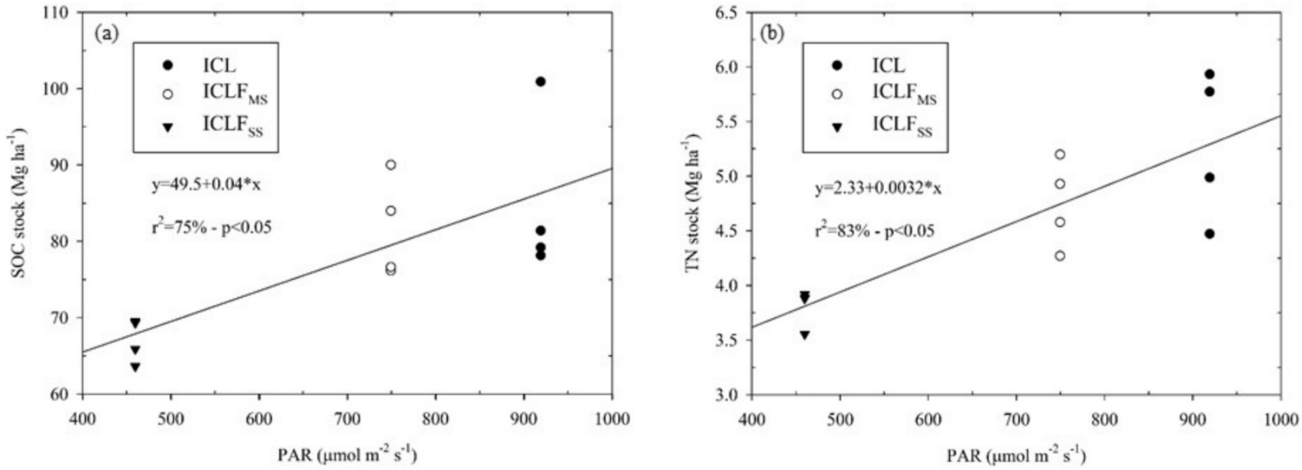

Fig. 8. Relationship of photosynthetically active radiation (PAR) incidence with soil organic carbon (SOC) and total nitrogen (TN) stocks up to a depth of 0.5 m depth in the experimental systems showing: integrated crop-livestock under full sunlight (ICL), moderately shaded integrated crop-livestock-forestry (ICLF$_{MS}$) and strongly shaded ICLF (ICLF$_{SS}$).

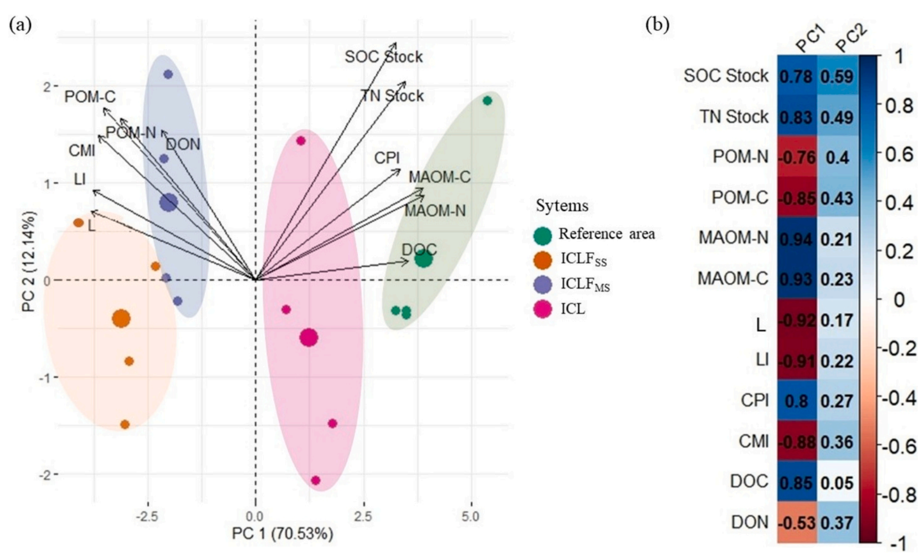

Fig. 9. Principal component analysis (PCA) biplot showing (**a**) the variance among the studied variables in different experimental systems and their environs and (**b**) the correlations between PC1 and PC2. Abbreviations: CPI, carbon pool index, CMI, carbon management index; DOC, dissolved organic carbon, DON, dissolved organic nitrogen; ICL, integrated crop-livestock under full sunlight; moderately shaded integrated crop-livestock-forestry (ICLF$_{MS}$); ICLF$_{SS}$, strongly shaded ICLF; L, carbon lability; LI, lability index; MAOM, mineral-attached organic matter; POM, particulate organic matter; SOC, soil organic carbon; TN, total nitrogen.

material and animal excreta made an important contribution to soil stocks, the added amount of organic material appears to have a greater influence in augmenting C and N stocks in the soil of the experimental systems evaluated herein.

4.2. Stabilization of C and N stocks in shaded systems

The strong relationships between the reference area and the variables C stocks in MAOM, SOC stock and CPI suggest that the native secondary forest favors the accumulation and stabilization of SOM resulting from thousands of years of C and N cycling. The ICL system, which lacks a forest component, most closely resembled the reference area regarding the C and N stocks in both bulk soil and MAOM fraction, as well as the CPI. Examining land use in the experimental systems in terms of the microbial efficiency-matrix stabilization framework (Cotrufo et al., 2013), the data support the hypothesis that the labile plant constituents are utilized more efficiently by microbes and are, therefore, the predominant source of microbial products (Cotrufo and Lavallee, 2022). These products would, therefore, serve as the primary precursors of stable SOM by facilitating aggregation and forming strong chemical bonds with the mineral soil matrix (Soong et al., 2021; Witzgall et al., 2021; Cotrufo et al., 2022). A corollary of this hypothesis is that low-quality residues favor the formation of POM whilst high-quality residues result in the formation of MAOM (Cotrufo and Lavallee, 2022). The results presented herein corroborate this hypothesis since the ICLF$_{MS}$ and ICLF$_{SS}$ systems added low-quality residues to the soil, including structural compounds from the forest component (eucalyptus) such as lignin, cellulose and hemicelluloses, which make up a large part of POM (Cotrufo et al., 2015). Crow et al. (2009) previously reported that the greater addition of low-quality woody materials led to increased POM in the soil.

The ICL system and the forest components of ICLF$_{MS}$ and/or ICLF$_{SS}$ showed similarities with respect to C and N stocks in the POM (0.00 to 0.05 m layer) and MAOM (0.10 to 0.20 and 0.20 to 0.30 m layers) fractions, whilst these variables differed between the ICL and the crop/pasture components of ICLF$_{MS}$ and ICLF$_{SS}$. Considering that soil management was analogous for all of these systems, the results indicate that the high C and N stocks present in the stable MAOM compartments may be associated with the intensity of PAR incidence in the inter rows. In this sense, it is likely that the microclimate provided by shading not only influenced the production of primary organic compounds by the crop/pasture component (Pezzopane et al., 2020, 2024), which generate DOC and POM, but also influenced the dynamics and population of the microbial community (fungi and bacteria and their ratios) (Berber et al., 2020; Sarto et al., 2020; Santos et al., 2024) responsible for the formation of MAOM from DOC and POM (Cotrufo and Lavallee, 2022). These processes of aggregation, which are promoted by the extracellular microbial polymers responsible for forming MAOM and binding the different soil constituents including minerals (Witzgall et al., 2021), serve to protect SOM. However, it is also possible that the higher PAR intensity over ICL and the crop/pasture component of $ICLF_{MS}$ may have increased soil temperatures, impacting directly the soil microbiology that enhanced MAOM formation (Soong et al., 2021).

A decline in the production of primary organic compounds not only reduces plant aerial biomass but also affects the roots, and this has remarkable consequences for the stabilization of C and N required for root growth (Ashworth et al., 2021; Yang et al., 2023). The alternation between root growth and death stimulated by the pasture grazing may have resulted in increased belowground C, including DOC, which is more efficient to form MAOM fraction (Ashworth et al., 2021; Cotrufo and Lavallee, 2022). Hence, in systems with high PAR, as in ICL, root growth is also enhanced and the subsequent decomposition of such tissues results in greater inputs of DOC and POM into the soil and, consequently, stabilization of C and N in the MAOM fraction (Ashworth et al., 2021; Cotrufo and Lavallee, 2022; Yang et al., 2023).

Although it is difficult to equate the contribution of animal feces and urine to the stabilization of C and N in the soil of grazing systems, it is of note that, according to Carnevalli et al. (2019), heifers grazing on Piatã grass in systems similar to those described herein deposited 72 % of their excreta under the trees. Such a distribution may explain why the C and N stocks in the forest components were similar to those of the crop/pasture components. In the present case, the organic material added to the soil of the forest component originated not only from eucalyptus but also from animal excreta, with the livestock acting as transport agent by removing C and N from the forage area and transferring them to the forest soil. Another important point is that the stocking rates (number of animals per unit area) in ICL, $ICLF_{MS}$ and $ICLF_{SS}$ were, respectively, high, intermediate and low (Silva, 2021), from which it may be presumed that the former system benefited from higher amounts and better distribution of C and N from animal excreta (Carnevalli et al., 2019), which have lower C/N ratio, resulting in improved stabilization (Samson et al., 2020). Furthermore, the contribution of animal excreta to SOM was substantiated by the higher DON content detected in the experimental systems compared to the reference area, a finding that has also been

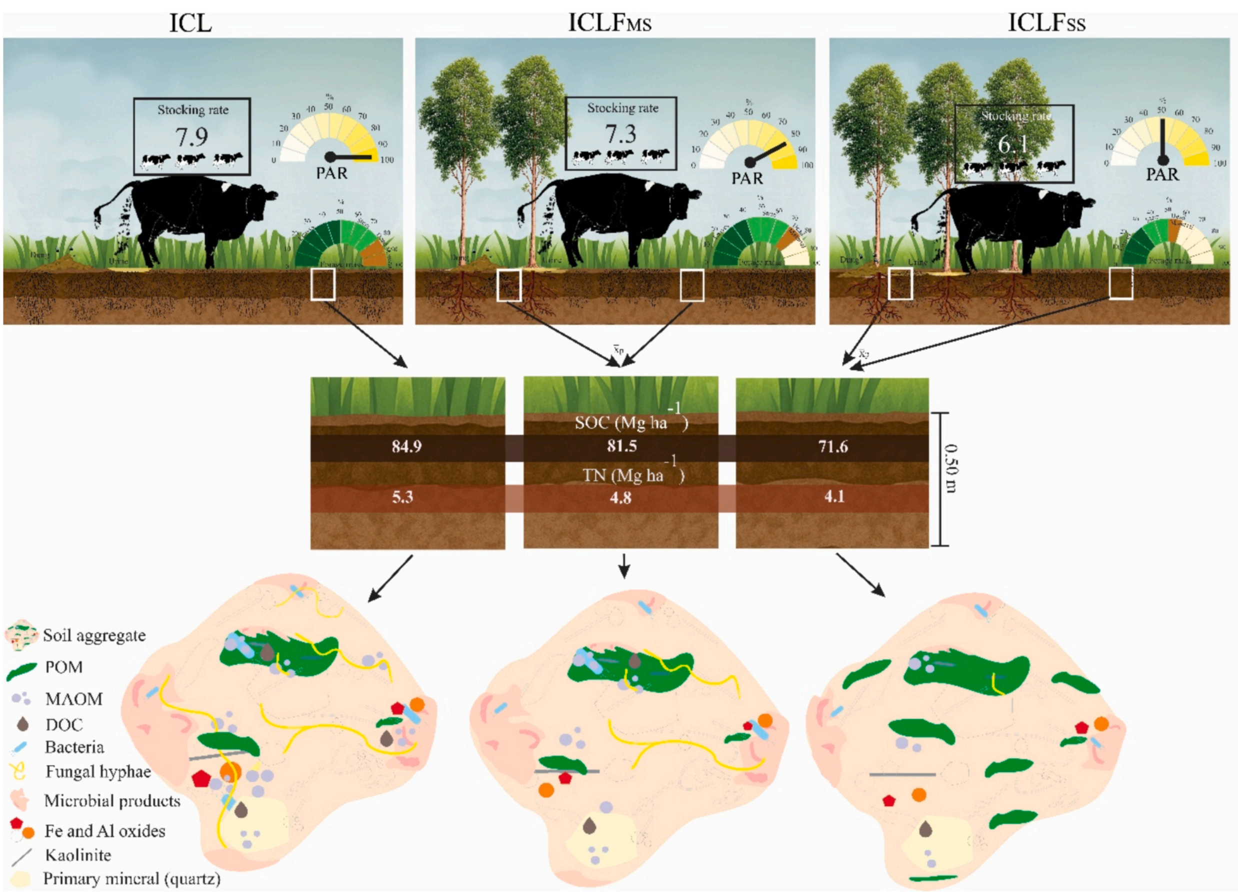

Fig. 10. Graphical summary of the main findings and aggregated data from other publications on the same experimental platform. The percentage of PAR and forage mass production were calculated considering the highest value system, in this case the ICL. Forage production and stocking rate data were extracted from Silva (2021). Soil mineral phase (Fe and Al oxides, kaolinite and quartz) was based on Viana et al. (2015). The components of soil aggregates were conceptualized based on Witzgall et al. (2021) and Cotrufo and Lavallee (2022). Data related to soil microorganisms (bacteria, fungal hyphae, and microbial products) were inferred based on the key results obtained. The greater or lesser presence of soil aggregate components is a simplified representation used to indicate their relative quantities in each system. Abbreviations: PAR, photosynthetically active radiation; ICL, integrated crop-livestock under full sunlight; $ICLF_{MS,}$moderately shaded integrated crop-livestock-forestry; $ICLF_{SS}$, strongly shaded ICLF; SOC, soil organic carbon; TN, total nitrogen; POM, particulate organic matter; MAOM, mineral-attached organic matter; DOC, dissolved organic carbon.

described by Stamati et al. (2011).

4.3. Summary and perspectives

In summary, the PAR reaching the crop/grass layer decreased with increasing shading, reducing PAR input by up to 50 % in the $ICLF_{SS}$ system (Fig. 10). This reduction directly impacted forage production, which declined in nearly the same proportion, as well as the stocking rate in each system. Consequently, the production of organic material, whether through forage accumulation or the distribution of dairy excreta, was higher in the ICL systems. It is also noteworthy that increased aboveground forage production was accompanied by enhanced root growth.

All this reflected in the lowest C stocks in the MAOM fraction of $ICLF_{SS}$ (strong shading), while the POM presented the highest, indicating that the C added, mainly, to the soil surface via plant material remained in the form of POM for an extended period. On the other hand, the incorporation of C into the soil of ICL was not only high but its amalgamation into MAOM was accelerated. Such differences in C stabilization within shaded systems was reflected in their higher C indices (L, LI and CMI), which confirmed that these systems retained C in the POM fraction unlike the system under full sunlight.

The results presented herein open up future research areas relating to the effects of microclimate (temperature and humidity) and quality of added organic material on the increase of soil microbial carbon use efficiency (CUE). While high temperatures favor microorganisms with high CUE (Ye et al., 2019), low quality organic materials select microorganisms with low CUE even when temperatures are high (Frey et al., 2013), and such changes in microclimate modify the dynamics of soil C and N (Cotrufo and Lavallee, 2022). Considering that in $ICLF_{MS}$ and $ICLF_{SS}$ most of the C is in labile forms and poorly protected in the POM fraction, it is possible that novel management strategies for these systems could lead to increased C oxidation and CO_2 production (Soong et al., 2021; Cotrufo and Lavallee, 2022). Hence, further research focused on soil management in ICLF systems is important in order to improve our understanding of the effects of pruning and thinning of the forest component to boost PAR transmission between tree rows, of the stability and loss of soil C and N, and of soil gas emissions.

Furthermore, future research should explore the potential of the tropical integrated systems as effective strategies for enhancing soil C sequestration and mitigating greenhouse gas emissions (including in the trees). Given that increased shading significantly reduces this soil potential, as observed in the $ICLF_{SS}$ system, further studies could quantify trade-offs between tree cover and C dynamics. An important methodological improvement would be to extend soil sampling to a depth of 1 m, as both tree roots and tropical grasses are capable of reaching deeper layers, potentially contributing to C and N accumulation at greater depths. Such investigations would support the development of climate-smart livestock systems aligned with the goals for low-emission protein production (Almeida and Alves, 2020; Oliveira et al., 2022).

5. Conclusion

Integrated agricultural systems employed for dairy production favored the augmentation of soil C and N stocks under the edaphoclimatic conditions of southern Amazon. The ICL system accumulated the highest C stocks in the stable fraction (MAOM), as indicated by the low L index (around 0.2), with amounts close to those of the reference area. The increased shading provided by $ICLF_{MS}$ and $ICLF_{SS}$ systems was linearly correlated with the reduction of soil C and N stocks in the soil, although such stocks were superior to those found in degraded pastures or in conventional systems for grain production. In addition to reducing C and N stocks, shading delayed the process of incorporation and stabilization of these elements into the more stable fraction of SOM. It is possible that such modification in soil C and N dynamics and storage may affect the resilience of shaded systems to changes in management practices. Apart from issues related to MOS stabilization, and considering only the C and N stocks of the systems and their benefits for the welfare of lactating cows, $ICLF_{MS}$ appears to be a suitable alternative for the edaphoclimatic conditions of the southern Amazon. The results presented herein will support decision-making regarding the selection of the most effective and context-appropriate sustainable land-use strategies for the southern Amazon including, for instance, the most suitable soil management approaches and/or the most adequate type of ICLF system to be implemented, aligned with the goals of low-emission livestock-based protein production.

CRediT authorship contribution statement

Alexandre Ferreira do Nascimento: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Jorge Lulu:** Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Funding acquisition, Formal analysis. **Admar Junior Coletti:** Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation. **Austeclínio Lopes de Farias Neto:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Anderson Ferreira:** Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation. **Sílvio Tulio Spera:** Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Formal analysis. **Roberta Aparecida Carnevalli:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

AFN reports equipment, drugs, or supplies was provided by Sustainable Rural. If there are other authors, they 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

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geodrs.2025.e00985.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials. Derived data supporting the findings of this study are available from the corresponding author [AFN] on request.

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