# **TERRA LATINOAMERICANA**



## **Carbon Reservoirs in the Biomass and Soil in Livestock Systems with Scattered Trees in Pastures in the Humid Tropics of Mexico Reservorios de Carbono en la Biomasa y el Suelo en Sistemas Ganaderos con Árboles Dispersos en Pasturas del Trópico Húmedo de México**

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### **SUMMARY**



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Maintaining trees in grassland areas is an option for enhancing carbon stocks, yet the contribution of dispersed trees in pastures has been little studied in livestock systems in the humid tropics of Mexico, despite being one of the region's most widely employed silvopastoral modalities. We conducted a study to assess biomass and soil carbon storage in a silvopastoral system, consisting of *Brachiaria brizantha* grass with native trees dispersed in paddocks (STP) and compared the findings with a pastureland consisting of *Brachiaria decumbens* grass without trees (WT). Five sampling plots of 1000  $m^2$  size, were randomly selected for each livestock system (STP and WT). At each site, tree biomass was estimated by allometric equations, and herbaceous, litter biomass, and soil organic carbon at four different depths were quantified by direct sampling method. Of the total biomass in the STP, above-ground biomass contributed 57.3%, below-ground biomass 20%, and ground litter 22.7%. In the case of WT, above-ground biomass represented 40.9% of the total biomass, below-ground biomass 6.4%, and litter 52.7%. The STP system stored a total of 162.3 Mg C ha<sup>-1</sup>, of which 149.7 Mg C ha<sup>-1</sup> in the soil to 70 cm depth and 12.7 Mg C ha<sup>-1</sup> in the biomass. WT system stored a total of 116.6 Mg C ha<sup>-1</sup>, of which 110.7 Mg C ha<sup>-1</sup> in the soil and  $5.5$  Mg C ha<sup>-1</sup> in the biomass. It is concluded that the STP livestock system increased 28% of the total carbon storage compared to the WT. However, more studies with greater species diversity and at different densities are needed to understand better the contribution of trees to carbon accumulation in livestock systems.

*Index words: environmental services, grasslands, native tree species, silvopastoral system, tabasco.*

### **RESUMEN**

La presencia de árboles en pastizales es una opción para aumentar las reservas de carbono, aun el aporte de árboles dispersos en pastos ha sido poco estudiado en sistemas ganaderos del trópico húmedo de México, a pesar de ser una de las modalidades silvopastoriles más utilizadas en la región. Aquí, estudiamos la biomasa y el almacenamiento de carbono en el suelo en un sistema silvopastoril compuesto por pasto *Brachiaria brizantha* asociado con árboles nativos de *Cedrela odorata*, *Cordia alliodora*, *Spondias mombin* y *Zanthoxylum kellermanii* dispersos en potreros

(ADP) y lo comparamos con un sistema ganadero compuesto por *Brachiaria decumbens* sin árboles (SA). Se seleccionaron al azar cinco parcelas de muestreo de 1000 m<sup>2</sup> para cada sistema ganadero. En cada sitio, la biomasa arbórea se estimó mediante ecuaciones alométricas, la biomasa herbácea y de hojarasca, así como el carbono orgánico del suelo a diferentes profundidades se cuantificaron mediante métodos de muestreo directo. De la biomasa total en los sistemas ganaderos con ADP, la biomasa aérea contribuyó con el 57.3%, la biomasa subterránea con el 20% y la hojarasca con el 22.7%. En el caso del sistema ganadero SA, la biomasa aérea representó el 40.9% de la biomasa total, la subterránea el 6.4% y la hojarasca el 52.7%. El sistema ADP almacenó un total de 162.3 Mg C ha<sup>-1</sup>, de los cuales 149.7 Mg C ha<sup>-1</sup> en el suelo a 70 cm de profundidad y 12.7 Mg C ha<sup>-1</sup> en la biomasa. Mientras que el sistema SA almacenó un total de 116.6 Mg C ha-1, de los cuales 110.7 Mg C ha-1 en el suelo y 5.5 Mg C ha-1 en la biomasa. Se concluye que el sistema ganadero con ADP incrementó un 28% de las reservas totales de carbono en comparación con SA. Sin embargo, se necesitan más estudios con mayor diversidad de especies y a diferentes densidades para comprender mejor la contribución de los árboles en la acumulación de carbono en sistemas ganaderos.

*Palabras clave: servicios ambientales, pastizales, especies de árboles nativos, sistema silvopastoril, tabasco.* 

### **INTRODUCTION**

Globally, the expansion of extensive livestock production has imposed destructive pressure on forests and woodlands, resulting in the disappearance or displacement of numerous species of flora and fauna (Grande, de León, Nahed, and Pérez, 2010; Martínez-Encino, Villanueva, and Casanova, 2013). In the state of Tabasco, Mexico, over the past 60 years, deforestation and degradation of tropical jungles and forests, primarily attributed to the expansion of agriculture and livestock production, has resulted in the loss of approximately 90% of the vegetation, mainly due to the for establishment of extensively managed native and improved pastures (Rullán-Silva, Gama, Galindo, and Olthoff, 2011; Villanueva-Lopez *et al*., 2019). This process has yielded diverse consequences and negative impacts on the environment and natural resources within the region (De Jong *et al*., 2010). These land use changes also contribute to the increase in atmospheric  $CO<sub>2</sub>$  concentrations since the arboreal component plays an essential role in absorbing and storing atmospheric CO<sub>2</sub> through photosynthesis, and its removal results in the release of significant amounts of CO<sub>2</sub> into the atmosphere (Villanueva-López, Martínez, Casanova, Ramírez, and Montañez, 2015; López-Santiago, Villanueva, Casanova, Aryal, and Pozo, 2023). A decrease in soil organic matter and nutrient content has been documented as a consequence of these land use changes, this leads to fertility problems that pose a threat to the stability of various production systems and result in an imbalance between the input and output of carbon, reflected in a higher concentration of atmospheric CO<sub>2</sub> (Rosenzweig, Carson, Baer, and Blair, 2016; Hu *et al*., 2019).

In this context, there is an urgent need to identify and evaluate farming alternatives that are more sustainable than current business-as-usual practices, permitting the improved use and management of local natural resources while simultaneously contributing to a reversal of the deleterious environmental impacts of livestock activities. Silvopastoral systems (SPS) emerge as a technological alternative for sustainable livestock production (Jose and Bardhan, 2012). These systems involve the presence of woody perennials (trees or shrubs) that interact with the grasses and animals under an integrated management system (Casanova-Lugo *et al*., 2022; Villanueva-López *et al*., 2015). In addition to providing higher plant and animal biodiversity, these systems reduce the pressure on temperate and tropical forests in tropical regions and contribute to the reduction of greenhouse gas emissions to the atmosphere, in comparison to livestock systems without trees (Harvey and González, 2007; Nair, Kumar, and Nair, 2021). They also enhance forage yield and quality, which contributes to improved animal productivity (Casanova-Lugo *et al*., 2022), and provide other environmental benefits such as the fixing and recycling of atmospheric nitrogen, soil protection from erosion with the addition of organic matter, enhancement of the microclimate in grazing pastures by providing larger shaded areas, and increased incorporation of atmospheric carbon (C) into the biological cycle through photosynthesis (Harvey and González, 2007; Nair *et al*., 2021; Poulton, Johnston, MacDonald, White, and Powlson, 2018).

Despite the valuable environmental services offered by forested vegetation areas such as the SPS in southeastern Mexico, there is a tendency for their conversion to other land use types that exclude arboreal vegetation, potentially causing significant impacts on the quantity of stored C (Valenzuela-Que *et al*., 2022). While various types of SPS have been documented in this region, particularly livestock systems with scattered trees in grazing pastures, there is limited information on their potential for carbon storage. Among the notable studies is the one conducted by Valenzuela-Que *et al*. (2022), in Tabasco Mexico which highlights improved C storage through litter production and woody biomass growth; Aryal, Gómez, Hernández, and Morales (2019) conducted a study in Chiapas, Mexico, reporting higher quantities of C stored in SPS with scattered trees in grazing pastures, compared to livestock systems with monoculture pastures (104.82 vs 58.63 Mg C ha<sup>-1</sup>); in Yucatan, Mexico; Casanova-Lugo *et al*. (2018), found, in forage banks, that woody biomass can represent up to 20% of the total C stored in this SPS variant system (175 Mg C ha-1); in Tabasco, México Villanueva-López *et al*. (2015) similarly reported higher quantities of stored C in livestock systems with live fences, compared to livestock systems with monoculture pastures (119.82 vs 113.34 Mg C ha-1). López-Santiago *et al*. (2019) in their study of SPS involving *Leucaena leucocephala* and grasses in Michoacán, Mexico, demonstrate that woody biomass constitutes 31% of the total C stored in the system (120.7 Mg C ha<sup>-1</sup>), suggesting that SPS can rival the C storage potential of regenerating tropical forests (120.9 Mg C ha<sup>-1</sup>). The quantity of stored C varies based on factors such as system type, management practices, tree density, incorporated species, land-use history, degree of degradation, and the time of establishment of the SPS. Therefore, this study aimed to assess the role of SPS with scattered native trees in pastures (STP) of *Brachiaria brizantha* for carbon storage in biomass and soils, comparing them to livestock systems based on *Brachiaria decumbens* pastures without trees (WT) under humid tropical conditions in Tabasco, Mexico.

### **MATERIALS AND METHODS**

#### **Study Area**

This study was conducted in the municipality of Tacotalpa, located in the Sierra Sur of Tabasco, Mexico 17° 30' 31" N and 92° 41' 12" W (Figure 1b) at an elevation of 55 meters of altitude. The regional climate is classified as warm humid Af (m) w' (i') g, with rainfall throughout the year (García, 1988). During the study period, the annual minimum and maximum temperatures were 22.2 and 32.2 °C, respectively, with an annual rainfall of 2013.9 mm (Valenzuela-Que *et al*., 2022) Figure 2. The soils dominant are Vertisols (Palma-López *et al*., 2017), and their physicochemical characteristics are presented in Table 1. The soils in the grass monoculture plots were slightly more acidic than silvopastoral plots but the clay contents were higher in silvopasture. In general, soil nitrogen and organic carbon contents were also better in silvopasture than in pasture without trees (Table 1).



**Figure 1. (a) Location of Tabasco in Mexico; (b) location of the study area: Municipality of Tacotalpa, Tabasco in the Southwest of Mexico; (c) livestock systems without trees (WT); (d) livestock systems with scattered trees in pastures (STP).**



**Figure 2. Monthly variation of temperature and rainfall pattern from January to December 2018 in the Municipality of Tacotalpa, Tabasco, Mexico. Source: (http://smn.conagua.gob.mx).**

#### **Description of the Systems**

Two different traditional livestock systems were selected in the region: one consisting of *B. brizantha* with scattered native trees in grazing pastures (STP) Figure 1d, which was characterized by the deliberate association of native trees such as *Cedrela odorata*, *Cordia alliodora*, *Spondias mombin* and *Zanthoxylum kellermanii,* with an irregular arrangement and spacing between trees, and the other system consisting of a monoculture of *B. decumbens* without trees (WT) Figure 1c. Table 2 shows the main characteristics of both systems.

#### **Experimental Plots**

In each livestock system, we randomly selected five circular plots of 1000  $m^2$ , resulting in a total of 10 sampling plots. Each plot was further divided into four quadrants, where a floristic inventory was conducted. Additionally, a randomly assigned sub-plot of 250 m<sup>2</sup> was used for sampling herbaceous vegetation. Soil samples were taken from the center of each plot. The design for herbaceous vegetation and soil sampling in the WT livestock system was consistent with that of the STP livestock systems.

#### **Table 1. Physical and chemical properties of the soil (0–70 cm depth) for livestock systems with scattered trees in pastures and without trees, in the Municipality of Tacotalpa, Tabasco, Mexico.**



STP = scattered trees in pastures; WT = without trees.



#### **Table 2. Biophysical characteristics of the livestock systems with scattered trees in pastures (STP) and without trees (WT), in the Municipality of Tacotalpa, Tabasco, Mexico.**

\* Best herd health management (i.e., vaccination and deworming), artificial insemination for cattle, use of machinery and technologies. NA = does not apply; STP = scattered trees in pastures; WT = without trees.

### **Estimation of Tree Biomass**

A dasometric inventory of the trees was conducted in each of the experimental plots to determine the aboveand below-ground biomass. The normal diameter of each tree was measured with a diametric tape at a height of 1.3 m above ground level, and the total height was measured with a Criterion® RD 1000 (Laser Technology, Inc., Colorado, USA). Tree biomass was estimated indirectly, initially for each tree using equation (1), developed by Chave *et al*. (2014) for humid tropical zones.

$$
AGB = exp[-2.977 + ln(\rho \cdot Dn^2 \cdot ht)] \tag{1}
$$

AGB is the above-ground biomass (kg tree<sup>-1</sup>);  $\rho$ , is the basic wood density of the species; Dn, is the normal diameter of the tree (cm); and ht is the total height of the tree (m). Subsequently, we calculated AGB per hectare using the expansion factor (ratio between one ha and the sample plot size) and the sum of the individual tree biomass (equation 2).

$$
AGB = \frac{\sum AGBtree (kg)}{S} * 10000
$$
\n<sup>(2)</sup>

Where AGB<sub>stock</sub> (Mg ha<sup>-1</sup>) is the total amount of above-ground biomass per hectare; AGB<sub>tree</sub> (kg tree<sup>-1</sup>) is the amount of biomass from individual trees within the sampling area (S,  $m<sup>2</sup>$ ).

Root biomass was estimated using equation (3), developed by Cairns, Brown, Helmer, and Baumgardner (1997).

### $BGB = exp[-1.0587 + 0.8836 \text{ (ln AGB)}]$  (3)

Where BGB, is the underground biomass (Mg ha<sup>-1</sup>); AGB, is the above-ground biomass (Mg ha<sup>-1</sup>).

#### **Sampling of Herbaceous Plant Biomass, Roots, and Leaf Litter**

In both livestock systems, herbaceous biomass was quantified through direct measurement. Monthly sampling occurred three days before livestock grazing, employing a 1 m × 1 m PVC quadrat. All grass within the quadrat was harvested at ground level and its fresh weight was recorded *in situ* using a precision digital scale (±1.0 g). Simultaneously, all leaf litter (senescent material from the grass and tree canopy) within the square was weighed and 100 g sample was collected during each harvest and placed in brown paper bags labeled with the plot data, system, date, and herbaceous plant type. Subsequently, herbaceous biomass and leaf litter samples were taken to the Plant Ecophysiology and Agroforestry Systems laboratory (LEVSA) of El Colegio de la Frontera Sur (ECOSUR) in Villahermosa, Tabasco, and dried at 60 ºC until constant weight. For biomass calculations in both cases, the mean values (g DM  $m<sup>2</sup>$ ) from the six months of sampling were used and then extrapolated to megagrams of dry matter per hectare.

The root biomass of the herbaceous plants was determined by sampling within each square in February. A metal cylinder 30 cm in length and 8 cm in diameter was introduced at the following soil depths: 0-10 cm, 10- 20 cm, 20-30 cm, and 30-70 cm. The collected samples were transported to the LEVSA laboratory at ECOSUR in Villahermosa. In the laboratory, the root biomass was separated from the soil by washing with pressurized water. Subsequently dried in a forced air circulation oven at 60 °C until reaching a constant weight. The final weights were recorded and extrapolated to Mg ha<sup>-1</sup> using the appropriate expansion factor described above.

### **Soil Sampling**

A soil pit 1 m × 1 m × 0.7 m was manually excavated in each experimental plot and a representative soil sample was taken from each stratum (0-10, 10-20, 20-30, and 30-70 cm respectively), due to in most of the evaluated plots there were adult trees with heights greater than 17 m and diameters of up to 34 cm. Furthermore, we chose these differences in the depth intervals because previous studies in this region indicate that the depth-related changes in SOC content were greater in the upper soil layer up to 30 cm, while the soil was found to be more homogeneous below 30 cm (De la Cruz-López, Villanueva-López, Casanova-Lugo, Martínez-Zurimendi, and Aryal, 2024; López-Hernández *et al*., 2024). Carrying out profiles up to 70 cm deep allowed us to evaluate in greater detail the effect of trees on storing carbon at depths greater than 30 cm from the ground. Manually digging the pit was more practical than inserting probes to 70 cm because the soils were heavy and with stones in some cases. The samples were placed in polyethylene bags labeled with the data of the system, plot, depth, and date of sampling and transferred to the Biogeochemistry laboratory at ECOSUR in Villahermosa. These samples were placed in polyethylene bags labeled with the data of the system, plot, depth, and date of sampling and transferred to the Biogeochemistry laboratory at ECOSUR in Villahermosa. These soil samples were air-dried in the shade at room temperature, milled (Mill Model and Brand), and sieved through a 2 mm mesh before analyzing their physical and chemical characteristics. Soil pH, total nitrogen, sand, silt, and clay contents were analyzed following the procedure described in the Mexican norm NOM-021-RECNAT-2000 (2002). Soil organic carbon (SOC) content was analyzed by the dry combustion method using an elemental analyzer (Shimadzu TOC-V CSN) equipped with a non-dispersive infrared detector (Shimadzu Corporation). To determine the soil bulk density (BD), we parallelly collected soil samples using cylindrical metal cores for each depth category, and the samples were oven-dried at 105 °C until reaching the constant weight and weighed to determine the BD as the proportion of the dry soil mass and the volume of the cylinder (Blake and Hartge, 1986). Coarse fragments (rocks and roots) greater than 2 mm were separated before weighing.

#### **Quantification of Carbon in the Tree, Herbaceous Plant, and Leaf litter Biomass**

Carbon stored in the tree (above- and belowground) and herbaceous plant (above- and belowground) biomass and litter was determined by multiplying the estimated dry weight values of each of these components by a factor of 0.5, as suggested for tropical regions (Brown, 2002).

#### **Quantification of Soil Organic Carbon**

The SOC stored down to 70 cm in depth was calculated using the laboratory results of soil BD and SOC and the sampling depth, summing the SOC at each depth analyzed. The SOC at each depth range was then obtained using equation (4), proposed by Xu, Liu, Zhang, and Kiely (2011):

#### $SOC = CC \times BD \times SD \times A$  (4)

SOC = soil organic carbon stock (Mg C ha<sup>-1</sup>), CC = carbon content (%), BD = soil bulk density (Mg m<sup>-3</sup>),  $SD =$  sampling depth (m),  $A =$  sampling area (ha).

#### **Carbon Storage in the System**

The quantity of C stored in the system was calculated using equation (5), proposed by several authors for tropical systems (Soto-Pinto, Anzueto, Mendoza, Ferrer, and De Jong, 2010; Schmitt-Harsh, Evans, Castellanos, and Randolph, 2012).

### $Carbon \, Stokes \, (Mg \, C \, ha^{-1}) = AGB + BGB + SOC$  (5)

AGB is the above-ground biomass (Mg C ha<sup>-1</sup>); BGB is the below-ground biomass (Mg C ha<sup>-1</sup>); and SOC is the soil organic C (Mg C ha<sup>-1</sup>).

#### **Statistical Analyses**

Data on biomass and above-and below-ground C content were analyzed by mean comparisons using the Student's t-test, considering the influence of the STP and WT livestock systems. In the case of soil organic C content and BD, a two-way ANOVA was used to examine the effect of systems, soil depth, and the interaction between these two factors. Where significant differences were found, a Tukey test at 95% confidence was used. These analyses were performed with the statistical package StatSoft STATISTICA version 8.0.360 for Windows (StatSoft, 2011).

### **RESULTS AND DISCUSSION**

#### **Estimation of Biomass and Carbon Content**

Aboveground biomass was twice as high in the STP livestock system as in the WT livestock system (*F=* 91.96, *P* < 0.001). The same behavior was observed in belowground biomass, with the STP livestock system presenting greater biomass than the WT livestock system (*F=* 1428.38, *P* <0.001). However, litterfall was similar in both systems (*F=* 0.0007, *P=* 0.978; Table 3). Consequently, the STP livestock system had more than double the total biomass presented by the WT livestock system (*F=* 137.27, *P* <0.001; Table 3).

It is important to note that within the STP livestock system, aboveground biomass represented 57.3% of the total biomass, while root biomass accounted for 20%, and leaf litter for 22.7% (Figure 3). In the case of the WT livestock system, the aboveground biomass represented 40.9% of the total biomass, while belowground biomass accounted for 6.4% and leaf litter for 52.7% (Figure 3). These percentages indicate that in the STP livestock system, the C content of aboveground biomass represented 28.6% of the total C in the system, while the root biomass was 10%, and the leaf litter was 11.3%. On the other hand, in the WT livestock system, the C content of the aboveground biomass represented 20.5% of the total C in the system, while the belowground biomass was 3.2% and the leaf litter was 28.6 per cent.



**Table 3. Above and belowground biomass, herbaceous above and belowground biomass, litter, and total biomass (Mg DM ha-1) in livestock systems with scattered trees in pastures and without trees, in the Municipality of Tacotalpa, Tabasco, Mexico.**

Averages (± standard error) in the same row indicated by the same letter are not significantly different according to the Tukey test (*P* < 0.05). STP = scattered trees in pastures; WT = without trees. NA = does not apply.

The greater quantity of above- and belowground biomass in the STP livestock system, although surpassing values reported by Villanueva-López *et al.* (2015) and Morales-Ruiz *et al*. (2021) in livestock systems with live fences (11.3 Mg DM ha<sup>-1</sup> and 9.8 Mg DM ha<sup>-1</sup>, respectively); is lower than the values reported by López-Santiago *et al.* (2019) 41.8 and 32.7 Mg DM ha-1 in an SPS with *L. leucocephala* shrubs and in a tropical deciduous forest. Additionally, it is lower than the value reported by Valenzuela-Que *et al*. (2022) with 89.28 Mg DM ha-1 in an STP livestock system in this same region. This finding aligns with the results of other studies that also report higher productivity in SPS in the tropical region compared to other land uses (Nair *et al*., 2021; Feliciano, Ledo, Hillier y Nayak, 2018). These differences can be attributed to several factors, such as the size of the individual arboreal components, tree density, canopy cover, species diversity, and the spatial arrangement of the studied STP livestock system relative to the other systems mentioned above.



**Figure 3. Proportions of different components to the total biomass for livestock systems with scattered trees in pastures (STP) and without trees (WT), in the Municipality of Tacotalpa, Tabasco, Mexico.**

On the other hand, it is worth noting that the greater biomass contained in the STP livestock system (Table 3), is evidence of the higher potential of this system, compared to the WT livestock system to store C and its importance in terms of fixing significant amounts of atmospheric  $CO<sub>2</sub>$ . In this context, the C content of biomass in the STP livestock system exceeded values reported by Aryal *et al*. (2019) who stated the storage values from 12.5 and 8.1 Mg C ha<sup>-1</sup> in trees with improved grasses and native grasses, respectively, and the 11.7 Mg C ha<sup>-1</sup> reported by Aryal, De Jong, Ochoa, Esparza, and Mendoza (2014) in a tropical secondary forest of five years of age in southeastern Mexico. These differences arise from the STP livestock system's high tree density (128 trees ha<sup>-1</sup>), with diameters reaching up to 34.2 cm and an average height of 17.3 m (Table 2). This high density contributes to substantial woody material, particularly in the form of aerial biomass (trunks and branches), which contributes to maintaining and increasing the reserves of C in the vegetation and soil.

Other studies have also reported increased C storage in SPS compared to WT livestock systems (Jose and Bardhan, 2012; Baumann *et al*., 2017; Feliciano *et al.,* 2018). According to these authors, these differences can be primarily attributed to the inclusion of the arboreal component. This component not only promotes higher C storage in their organs (roots, branches, leaves, and trunks) through photosynthesis (Zhu, Yan, Fan, Yang, and Hu, 2009; Djomo, Knohl, and Gravenhorst, 2011) but also contributes to improving the accumulation of organic material in the soil through its capacity to fix large quantities of atmospheric nitrogen. This is achieved through the fall of leaf litter, which constitutes another input of C due to its decomposition process and recycling of nutrients in the soil, in addition, due to the replacement of fine roots in the deeper horizons for to the presence of the tree component (Leblanc, McGraw, and Nygren, 2007; Munroe and Isaac, 2014).

### **Soil Organic Carbon and Bulk Density**

The results reveal significant differences in the SOC content between the STP and WT livestock systems (*F =* 26.56; *P =* < 0.001; Figure 4a), in which the C content was higher in the STP livestock system (Figure 4a). When comparing the SOC content of the livestock systems at different depths, it was found that they differed significantly (*F =* 115.48; *P =* < 0.001; Figure 4b), with the STP livestock system consistently presenting the highest values at each depth analyzed. The distribution of SOC in both systems followed a tendency to decrease with depth, being higher in the surface stratum (0-10 cm) with mean values of 35.5, 24.2, 16.9, 6.6 g kg<sup>-1</sup>, for soil layers of 0-10, 10-20, 20-30 and 30-70 cm for both livestock systems (Figure 4b).

In addition, soil bulk density differed significantly between the two livestock systems and among the depths evaluated (*F=* 6.25; *P=* 0.001). Apart from the 0-10 cm soil depth, in which BD was similar in both systems, the remaining sampled depths consistently presented their highest soil BD values in the WT system (Figure 5).



Figure 4. a) Soil organic carbon fractions (g kg<sup>-1</sup>) in livestock systems with scattered trees in pastures (STP) and without trees **(WT). b) Soil organic carbon fractions at various depths livestock systems with scattered trees in pastures (STP) and without trees (WT) in the Municipality of Tacotalpa, Tabasco, Mexico.**



**Figure 5. Soil bulk density (g cm-3) at various depths in livestock systems with scattered trees in pastures (STP) and without trees (WT), in the Municipality of Tacotalpa, Tabasco, Mexico.**

Moreover, significant differences were identified between the two evaluated systems in terms of total SOC stored (*F* = 36.10; *P* < 0.001; Table 4). The STP and WT livestock systems stored 149.7 (±4.4) Mg C ha<sup>-1</sup> and 110.8 ( $\pm$ 4.7) Mg C ha<sup>-1</sup>, respectively. When comparing C storage between the systems at different depths, it was observed that the SOC in the STP livestock system did not differ significantly between depth categories. However, the C distribution in the WT livestock system followed a tendency to decrease with depth, where the surface stratum (0-10 cm) presented values of 37.1 (±1.38) Mg C ha<sup>-1</sup>, while the 30-70 cm depth stratum presented values of 26.0 (±2.4) Mg C ha-1, a pattern that was less marked for the STP livestock system (Table 4). We noted that the SOC stocks begin to differ between STP and WT from 20 onward to deeper horizons. No differences were observed on the surface soil of 0-10 cm. This can be attributed to the role of trees in adding carbon to deeper soil horizons compared to grass monoculture.

The higher C content in the soil of the STP livestock system (148.7 Mg C ha<sup>-1</sup>) compared to the WT livestock system (110.8 Mg C ha<sup>-1</sup>; Table 4), could be related to both SOC concentration and soil BD. The WT livestock system presented values of 1.34 Mg m<sup>-3</sup>, which were higher than those of the STP livestock system at 1.25 Mg m<sup>-3</sup> (Figure 5), similar to the soil BD reported by Villanueva-López *et al.* (2015) in livestock systems with live fences and grass monoculture in this same region (1.3 vs 1.5, respectively). According to these authors, the higher BD value

	Livestock systems	
Soil depth	<b>STP</b>	<b>WT</b>
$0-10$ cm	48.4 ( $\pm$ 3.9) a	$37.1 (\pm 1.3) b$
$10-20$ cm	$31.8 (\pm 1.7) a$	$29.5 (\pm 2.1) a$
20-30 cm	$27.1 (\pm 1.2) a$	$18.2 (\pm 1.8) b$
$30 - 70$ cm	42.4 $(\pm 2.4)$ a	$26.0 \ (\pm 2.4) b$
All profile (0-70 cm)	$149.7 (\pm 9.2)$ a	$110.8 (\pm 7.6) b$

**Table 4. Total carbon storage in the soil (Mg C ha-1) in livestock systems with scattered trees in pastures and without trees in the Municipality of Tacotalpa, Tabasco, Mexico.**

Averages (± standard error) in the same row indicated by the same letter are not significantly different according to the Tukey test (*P* < 0.05). STP = scattered trees in pastures; WT = pasture without trees.

is due to the presence of heavier and compaction soils produced by the action of trampling by grazing animals, and to higher silt contents (Table 1). Furthermore, vertosols are heavier due to the fine clay particles and get hard when dry. Such fine and heavy clay content could also have resulted in a slightly higher bulk density in this study. Another factor of carbon input in livestock grazing systems is the addition of animal manure and urine that increase C and N content in the soil. However, we assume the role of animals in carbon stock is indifferent between the two livestock systems. Other studies have also reported that the dynamics and longevity of fine roots are lower in systems without trees (Morales-Ruiz *et al*., 2021; Montejo-Martínez *et al*., 2020), can alter the physicochemical and biological properties of the soil, resulting in soil with greater compaction, as occurred in this study. However, in systems with trees, the root dynamics and longevity are higher and contribute to the maintenance of adequate levels of organic material, which improves soil structure, increases resistance to soil compaction, and reduces BD (Montejo-Martínez *et al*., 2020).

On the other hand, we observe that the greatest storage of C in the soil in both livestock systems was presented in the first 10 cm of depth, and although we found similar C contents in the 10-20 cm deep stratum in the two systems evaluated, they different at greater depth and was higher in the livestock system with STP (Table 4). This could be associated with the physiochemical properties of the soil, as well as the presence of the trees that, through the contribution of leaf litter and the recycling of nutrients, favored the incorporation of organic matter into the soil (Morales-Ruiz *et al*., 2021; Valenzuela-Que *et al*., 2022). For example, there is evidence that leaf litter acts as mulch and reduces evaporation, surface runoff, and erosion, hence protecting the topsoil, which contains more soil organic carbon and other soil nutrients than other soil layers. On the other hand, the livestock systems with STP were characterized by soils with higher quantities of OM and N and lower BD values (Table 1), due to the amount of litterfall (i.e., leaves, branches, and twigs) that enters the system and the accumulation of OM from the growth and decomposition of the finest tree and grass roots. In addition, below ground, the tree roots in livestock systems with STP penetrate to deeper soil layers than monoculture pasture roots facilitating the transport of nutrients such as C, providing a better nutrient balance in the soil compared to monoculture pasture systems (Villanueva-López *et al*., 2015; López-Santiago *et al*., 2019). In addition to the higher contents of organic matter in the surface layer of the soil in livestock systems with STP, the rainfall throughout the year and high temperatures characteristic of the humid tropics (Figure 2) contributed to the dynamics of soil microorganisms, which resulted in faster decomposition of the mulch by microorganisms and incorporating a greater amount of organic matter into the surface layer and even in the deeper layers (Tapia-Coral, Luizão, Wandelli y Fernandes, 2005; Dube *et al*., 2013; Kaempf, Hoelzel, Stoerrle, Broll y Kiehl, 2016).

### **Total Carbon Reserves in Livestock Systems**

The STP livestock system showed an increase of 40.37% (*F* = 18.86, *P* = 0.002) in the total carbon stored compared to the WT livestock system (155.4 *vs*. 110.7 Mg C ha-1). It should be noted that, in the STP livestock system, the soil contributed 96.3%, and the total biomass contributed 3.4% of the total C in the system, while the soil represented the only reservoir of C in the WT livestock system (Table 5).



Table 5. Total carbon storage (Mg C ha<sup>-1</sup>) in the total biomass and soil and carbon fluxes **(Mg C ha-1 year-1) in livestock systems with scattered trees in pastures and without trees in the Municipality of Tacotalpa, Tabasco, Mexico.**

STP = scattered trees in pastures; WT = without trees; NA= does not applied.

The total stored C was higher in the STP livestock system at  $162.3$  Mg C ha<sup>-1</sup> year<sup>-1</sup> compared to the WT livestock system at 116.2 Mg C ha<sup>-1</sup> year<sup>-1</sup>, suggesting that the presence of trees in the SSP plays an important role in C storage. This is related to the vegetation structure, as observed in the STP livestock system, which features larger and thicker roots that penetrate deeper into the soil and facilitate an increased input of organic matter compared to the WT livestock system (Aryal *et al*., 2014; Mattsson, Ostwald, Nissanka, and Pushpakumara, 2015; Morales-Ruiz *et al*., 2021). An important factor in carbon storage in vertisol is the content of fine materials, which include clays and silts, and these particles play an important role because they can fix organic compounds and allow their storage in the soil. These soils have reduced pore spaces that limit the water flow velocity, which allows soil C to be distributed within the profile. On the other hand, there are studies such as those of Mikutta *et al*. (2007) and Barthès *et al*. (2008), which indicate that iron and aluminum sesquioxides are relevant in the stabilization of COS through the formation of organo-mineral complexes. It should be noted that, in the STP livestock system, aboveground biomass contributed 28.6% of the total system C, while belowground biomass contributed 10%, and litter contributed 11.3%, which was similar to the accumulated C in the WT livestock system. This is related to the vegetation structure, e.g., in the STP livestock system, which features larger and thicker roots that penetrate deeper into the soil and facilitate an increased input of organic matter compared to the WT livestock system (Mattsson *et al*., 2015). Other studies (Dornbush, Isenhart y Raich, 2002; Montejo-Martínez *et al*., 2020) also indicate that the decomposition of fine roots is the principal manner in which C and nutrients return to the soil from the plant tissues, reducing compaction and forming the main source of SOC. Sánchez-Silva *et al*. (2022) indicate that the C transported to the soil through fine root decomposition is probably 4 to 5 times higher than that derived from aerial litter decomposition. In addition, soil type influences the accumulation of C, due to the texture, which influences the speed of movement of the materials through the profile, allowing them to be distributed and accumulate in the profile, as shown in Table 4, where it is observed that the contents tend to decrease as depth increases. The management favors the increase in the contents for STP, since having a higher production of slow degradation compounds such as cellulose (Rabbi *et al*., 2024), allows the soil to fix more organic compounds, unlike WT in which mainly the biomass produced is easily decomposed, thus decreasing the possibility of being fixed through the profile. This is because the texture of soil contributes, among other things, to microbial activity due to the effect of porosity, which, as depth increases, promotes the accumulation and stabilization of organic compounds by adsorbing them on the surface of clays and silts. In addition, as depth increases, gas exchange decreases, microbial activity decreases and, due to the amount of clay, the formation of more stable organic compounds is promoted.

### **CONCLUSIONS**

We conclude that the STP livestock system has a higher potential to store C in its different pools compared to the WT livestock system. Our results also evidenced the increased potential of the STP livestock system to store C throughout the soil profile (compared to the WT livestock system as well as highlighting the importance of the latter in terms of fixing significant amounts of atmospheric CO<sub>2</sub>. Likewise, our results revealed that SOC is the main carbon reservoir, contributing 92.2% in the STP livestock system and 95.3% in the WT livestock system, relative to the total C of the system. These results are of considerable importance since the conversion of land from forest vegetation to monoculture pastures is an increasing phenomenon in the region. However, further research is required at different tree densities and species diversities in the system to further our understanding of the effects of the evaluated livestock systems on C accumulation.

### **ETHICS STATEMENT**

Not applicable.

### **CONSENT FOR PUBLICATION**

Not applicable.

### **AVAILABILITY OF SUPPORTING DATA**

All data generated or analysed during this study are included in this published article.

### **COMPETING INTEREST**

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

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### **AUTHOR ́S CONTRIBUTION**

Conceptualization and methodology: G.V.L., J.G.L.S., F.C.L., and P.M.Z. Investigation: J.G.L.S., and G.V.L. Data Curation and formal analysis: J.G.L.S., and F.C.L. Writing original draft: J.G.L.S., and G.V.L. Writing-reviewing and editing: F.C.L., F.B.O., and P.M.Z. Fund acquisition; G.V.L.

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