



Cocoa based agroforestry systems enhance carbon storage in deep horizons of Amazonian soils

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Abstract Cocoa-based agroforestry systems have the capacity to increase carbon storage in areas degraded by pasture in the Amazon. Therefore, we evaluated the soil carbon storage capacity down to 150 cm depth in cocoa-based agroforestry systems in a deforested Amazonian landscape of Colombia. Each shade tree species (Abarco *Cariniana pyriformis*, Capirón *Calycophyllum spruceanum*, Caracolí *Anacardium excelsum*, and Huito *Genipa americana*) were independently combined with cocoa. The performance of these systems was compared with mature forests and degraded pastures taken as references. Soil carbon content was evaluated at five depths (0–30, 30–60, 60–90, 90–120, and 120–150 cm). Carbon fractions were extracted with acid

solutions of increasing concentrations and classified accordingly into very labile (VL), labile (L), less labile (LL), and non-labile (NL). The C stock down to 150 cm was maximum in forest (209.6 ± 3.5 Mg C ha⁻¹) and decreased from Caracolí (190.2 ± 6.2 Mg C ha⁻¹) to Abarco (162.9 ± 2.9 Mg C ha⁻¹), Huito (150.5 ± 4.6 Mg C ha⁻¹), Capirón (137.1 ± 4.5 Mg C ha⁻¹), Full-sun cocoa (135.6 ± 3.5 Mg C ha⁻¹), and pasture (110.3 ± 3.9 Mg C ha⁻¹). In the 15-year experimental period, cocoa based agroforestry systems enhanced soil carbon contents to values up to 2.73 times the content in the initial degraded pasture, close to the performance of the forest ($\times 2.96$). Huito and Caracolí shade trees were the most efficient at storing carbon in deep soil layers.

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Introduction

Globally, the carbon cycle has been altered, specifically during the period 2011–2019, when an irrecoverable loss of 4.0 Gt of carbon was recorded, with an annual average of 0.45 Gt, equivalent to 1.65 Gt CO₂e (carbon dioxide equivalents). This loss represents approximately one-fifth of the 8.1 Gt CO₂e in annual emissions from deforestation, where total carbon storage amounts to 139.1 ± 443.6 Gt, distributed 57%

(79.0 Gt) in biomass and 43% (60.0 Gt) in soil (Noon et al. 2022). Within this global context, Continental Amazonia is home to the world largest tropical forest, providing ecosystem services of global significance (Wang et al. 2020). This biome is one of the largest planetary carbons (C) reservoirs, with approximately 123 ± 23 Pg C stored in aboveground and below-ground biomass and soil (Hubau et al. 2020). Soil carbon accounts for another 10–32% of the carbon accumulated in plant biomass (Pan et al. 2011).

However, specifically the Colombian Amazon has experienced an annual deforestation rate of approximately 119,800 hectares during 2020–2021, representing a loss of carbon stocks estimated at 45–60 Mg C ha⁻¹ in aboveground biomass (Teixeira et al. 2025; Murcia et al. 2011; Beuchle et al. 2021). This significant deforestation, coupled with inadequate land management practices, has led to a substantial decline in carbon accumulation across all compartments, with the Amazon's carbon sink capacity decreasing by approximately 33% in the past decade (Hubau et al. 2020; Gatti et al. 2021). The conversion of forest to grassland has locally increased soil-based CO₂ emissions to ranges of 16–80 Mg C ha⁻¹ (Hubau et al. 2020), resulting in highly degraded grasslands with low agronomic capacity, altered soil properties (Polanía-Hincapié et al. 2021), and diminished ecosystem services (Grimaldi et al. 2014; Rodríguez et al. 2021b). Some regions have even transitioned from carbon sinks to carbon sources, particularly during drought years, exacerbating the overall environmental degradation in the region.

Agroforestry systems represent a management option to reduce atmospheric CO₂ concentration through their potential to enhance carbon accumulation in stable soil C fractions and plant biomass (Rumpel 2022; Silver et al. 2000). Agroforestry systems established in highly fragmented landscapes of the Amazon are likely a suitable option for their capacity to sustain the provision of soil-based ecosystem services (Rodríguez et al. 2021a; Suárez et al. 2021) since the inclusion of trees increases C storage in both plant and soil compartments (Kumar and Kunhamu 2021). Cocoa-based agroforestry systems have the potential to improve fertility in degraded soils (Suárez et al. 2021), increase C stocks in plant biomass (Monroe et al. 2016), promote soil macrofauna communities (Duran-Bautista et al. 2023), and enhance the occlusion of C in soil aggregates (Rodríguez et al. 2021a). These systems

can host soil organic carbon stocks of up to 220 Mg C ha⁻¹, higher total carbon accumulation down to 1 m depth compared to adjacent degraded soils (Gama-Rodrigues et al. 2010; Monroe et al. 2016).

Total soil organic carbon (SOC) serves as a general indicator of soil quality (Andrade et al. 2022). In agroforestry systems, SOC dynamics are understood through three pools: Active (microbial biomass and metabolites), slow (physically protected organic debris), and passive (Lavelle and Spain 2001; Lehmann and Kleber 2015). While active and slow pools are highly sensitive to soil management practices, the passive pool is primarily protected through physical mechanisms within microaggregates and in deep soil layers where biological activity is limited (Datta et al. 2010; Jiang et al. 2014). The response of labile pools (active and slow) to land use changes (Quanying et al. 2014) provides crucial information about soil quality, health (Vieira et al. 2007), and SOC storage potential (Chen et al. 2017).

The storage potential of SOC and its fractions in agroforestry systems has been demonstrated across numerous sites and designs (Maia et al. 2007; Barreto et al. 2011; Kumar et al. 2020; Singh et al. 2021, 2023; Kaur et al. 2023; Melkani et al. 2024; Suárez et al. 2024). However, information on the composition and distribution of carbon stocks below 30 cm depth remains limited. A review of 34 papers (listed in Annex 1) revealed that only six provided data below 60 cm depth, and none examined soil depths beyond 1 m. This study aimed to address this knowledge gap by measuring carbon concentration and its distribution among fractions of decreasing lability at various soil depths. The research focused on soil profiles from cocoa plantations associated with five different shade tree species and adjacent degraded pastures. We hypothesized that cocoa-based agroforestry systems would increase carbon content across all fractions following their establishment in degraded pastures, and that shade trees would significantly influence deep soil carbon storage through their extensive root systems, with variations among species due to distinct chemical qualities of their leaf and root litter inputs.

Material and methods

Study area and experimental design

The study was carried out at the Centro de Investigaciones Amazónicas CIMAZ—Macagual, owned by

the Universidad de la Amazonia located southwest of the department of Caquetá, Colombia (1° 30' 4.87" N and 75° 39' 47.16" W, 300 m.a.s.l.), in a biome of type tropical rain forest (Holdridge 1978) in north-western Amazonia. The climate is tropical rainy Afm type (humid and hot tropical forest) with an average annual temperature of 25.5 °C, average sunshine of 1707 h yr⁻¹, average relative humidity of 84.3% and an average annual precipitation of 3793 mm with monomodal regime, subdivided into a rainy season (March to June) and a dry season (November to February) (IGAC 2014). Soils are Typic Paleudults and Oxic Dystrudepts (Soil Survey Staff 2022), associated with a high content of kaolinite clay and quartz, high acidity levels (pH 4.3–4.5), saturation of iron (Fe) and aluminum (Al) sesquioxides, and low concentrations of available phosphorus and exchangeable bases (Ca²⁺, Mg²⁺ and K⁺) in the mineral horizons (IGAC 2014).

A completely randomized block design (n=9) with factorial arrangement (i. land use and ii. depth) was used in the field. The total experimental area, including all seven land-use systems within the nine blocks, comprised 8.5 hectares. The study compared four cocoa agroforestry systems, each established with a different shade tree species (*Abarco Cariniana pyriformis*, Capirón *Calycophyllum spruceanum*, Caracolí *Anacardium excelsum*, and Huito *Genipa americana*), against forest, Full-sun cocoa, and pasture systems. Cocoa-based agroforestry systems were implemented in 2012, with cocoa trees aligned north–south at a distance of 3.5×3.5 m, and shade trees when used, planted as a monospecific canopy at 8×8 m intervals. Each plot measured 30×45 m and included a net plot of 18×32 m (576 m²), with 44 cocoa trees and 8 shade trees, as appropriate. These experimental plots were selected from larger areas of Forest (55 ha, naturally regenerated since 1996 on abandoned pastureland) and managed pasture (45 ha, established in the 1950s). The Forest hosts diverse tree species including *Cedrela odorata*, *Laurus nobilis*, *Inga* sp., *Miconia appendiculata*, *M. elata*, *Cecropia peltata*, and *Cedrelinga cateniformis* (Rodríguez et al. 2021a). The managed pasture, established in the 1950s, maintains approximately 20 cattle per hectare under a rotational grazing system with 30-day alternating periods of grazing and recovery. The pasture vegetation consists primarily of *Urochloa decumbens*

and *U. humidicola*, which are periodically overseeded to maintain productivity.

Soil carbon fractions and stability

At equal distance of four shade trees, a test pit of 1×1 m and 1.5 m depth was dug. A 1 kg composite sample was taken from the different cardinal points from each depth layer (0–30, 30–60, 60–90, 90–120 and 120–150 cm) for a total of 315 samples (7 land-use systems, 9 plot repetitions, 1 pit per plot, 5 depths), sampling was carried out in the year 2023. Each soil sample was air-dried at room temperature and sieved at 2 mm for subsequent organic matter fractionation analysis. At the northern position of each test pit, an undisturbed soil sample was extracted with a fixed volume cylinder for each depth and dried in an oven at 105 °C until a constant weight was reached, for calculation of soil bulk density (Blake and Hartge 1986).

The SOC fractions were separated using a sequential extraction by a suite of acidic solutions of increasing concentrations, following Chan et al. (2001). Acid-aqueous solutions with respective ratios of 0.5:1, 1:1, and 2:1 were used to obtain H₂SO₄ concentrations of 6, 9, and 12 mol L⁻¹, respectively. This chemical extraction process, based on different degrees of oxidation relative to total SOC, yielded four distinct fractions of decreasing oxidizability: Very labile (VL), labile (L), less labile (LL), and non-labile (NL). Carbon accumulation was further evaluated using the Carbon Pool Index (CPI) as described by Blair et al. (1995), with pasture serving as the reference land use. Additionally, the recalcitrant index (RI), defined as the ratio of labile (VL+L) to non-labile (LL+NL) fractions, was calculated following the methodology proposed by Datta et al. (2018). The C stocks (Mg C ha⁻¹) were calculated considering soil bulk density in all soil depth layers.

Statistical analysis

A linear mixed model (LMM) was used to detect significant differences among carbon fractions, carbon stocks in different land uses and depths and carbon stability indexes. Fractions and depth were considered as a fixed factor, and plots as a random factor. The analysis of model assumptions included the assessment of normality and homoscedasticity

using graphical diagnostics (fitted-plot and QQ-plot). Deviations from homogeneity of variances were corrected by applying the *varIdent* function of the *Nlme* statistical package (Pinheiro et al. 2018). Differences in the means of the fixed factors were verified using the DGC test (Di Rienzo et al. 2002; $P < 0.05$). In addition, principal component analyses (PCA) were performed with the variables related to carbon fractions and stocks and effects of land uses were tested with Monte-Carlo tests. The LMM was performed using the *lme* function of the *nlme* package. PCA was performed using the *fviz_dend* and *fviz_pca_ind* functions, respectively, of the *factoextra* package. Statistical analyses were performed in the statistical software *InfoStat* version 2020 (Di Rienzo et al. 2020) and the R language version 4.3.3 (R Development Core Team 2025).

Results

The SOC exhibited considerable variations in the relative concentration of fractions and total accumulated carbon, according to soil depth and land use types. Based on the results of principal component analysis (PCA), applied to the complete set of concentrations of four carbon fractions at 5 depths, the seven land use types were significantly separated ($P < 0.01$; 25% of explained variance) (Fig. 1). The first factor (30.6% of explained variance) was defined by the abundance of the total set of fractions, which were projected towards the same side of the axis. Land use types were arranged following a clear gradient along axis 1, from forests with the highest concentrations in all carbon classes, to cocoa based agroforestry systems, full-sun cocoa, and pastures. Non-labile fractions, at all depths, were the most determinant of this effect, with correlations of 0.66–0.77 with F1 coordinates, and labile fractions being the least sensitive. Cocoa

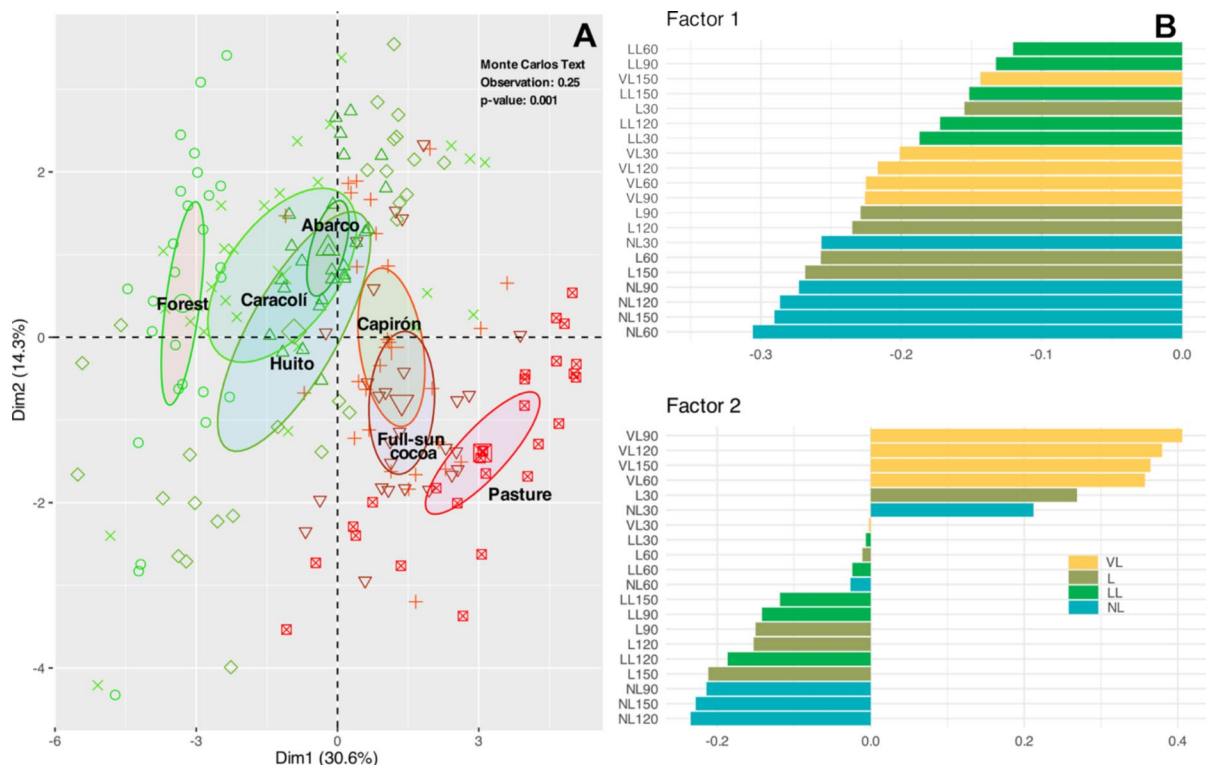


Fig. 1 The PCA analysis of C concentrations at different depths in the 7 different land uses. A. Projection of sampling points according to land use in factorial F1/F2 space; B: Correlations among variables and factor values. NL: Non labile,

LL: Less labile; L Labile; VL: Very labile. Numbers from 30 to 150 indicate the range of depths: 30, from 0 to 30 cm; 60, from 30 to 60 cm etc.

plantations with shade trees of Caracolí, Huito, and Abarco were projected close to the forest, associated with similar carbon concentrations across all fractions and depths. The cocoa plantation with Capirón or Full-sun cocoa showed lower concentrations, although still higher than those in the pasture system. Factor 2 (14.3%) was mainly determined by the non-labile fraction in the deeper soil layers, from 90 to 150 cm. This factor tended to show higher values along the gradient from forest to pastures, with the highest relative values of non-labile carbon in deep soils of pastures and full-sun cocoa, and the lowest values in agroforestry systems and forest.

Very labile (VL) and non-labile (NL) fractions were the most concentrated fractions with maximum average values in the first 30 cm of all soils, from 9.97 to 15.59 g C kg⁻¹ for VL and 7.14 to 13.36 g C kg⁻¹

for NL (Fig. 2). In the same soil layer, the intermediate L and LL fractions were much less concentrated, with values in a 5.07–9.35 range for L and 2.06–4.4 for LL. Concentrations decreased very sharply with depth, below the 30 cm in all categories. However, the relative importance of the NL fraction increased with depth, until it became the fraction best represented in the last layer of 120–150 cm where it ranged from 0.96 to 1.94 g C kg⁻¹ while the very labile fraction stayed behind with a 0.26–1.55 g C kg⁻¹ range.

The second most important source of variation in concentrations, after soil depth, was the type of land cover (Fig. 2). Soil bulk density varied from a minimum value of 1.23 ± 0.02 g cm⁻³ in the Capirón and Huito treatments in the 0–30 cm layer, to a maximum of 1.55 ± 0.03 g cm⁻³ in the 120–150 cm depth layer of the pasture site (Table 1). There was a general

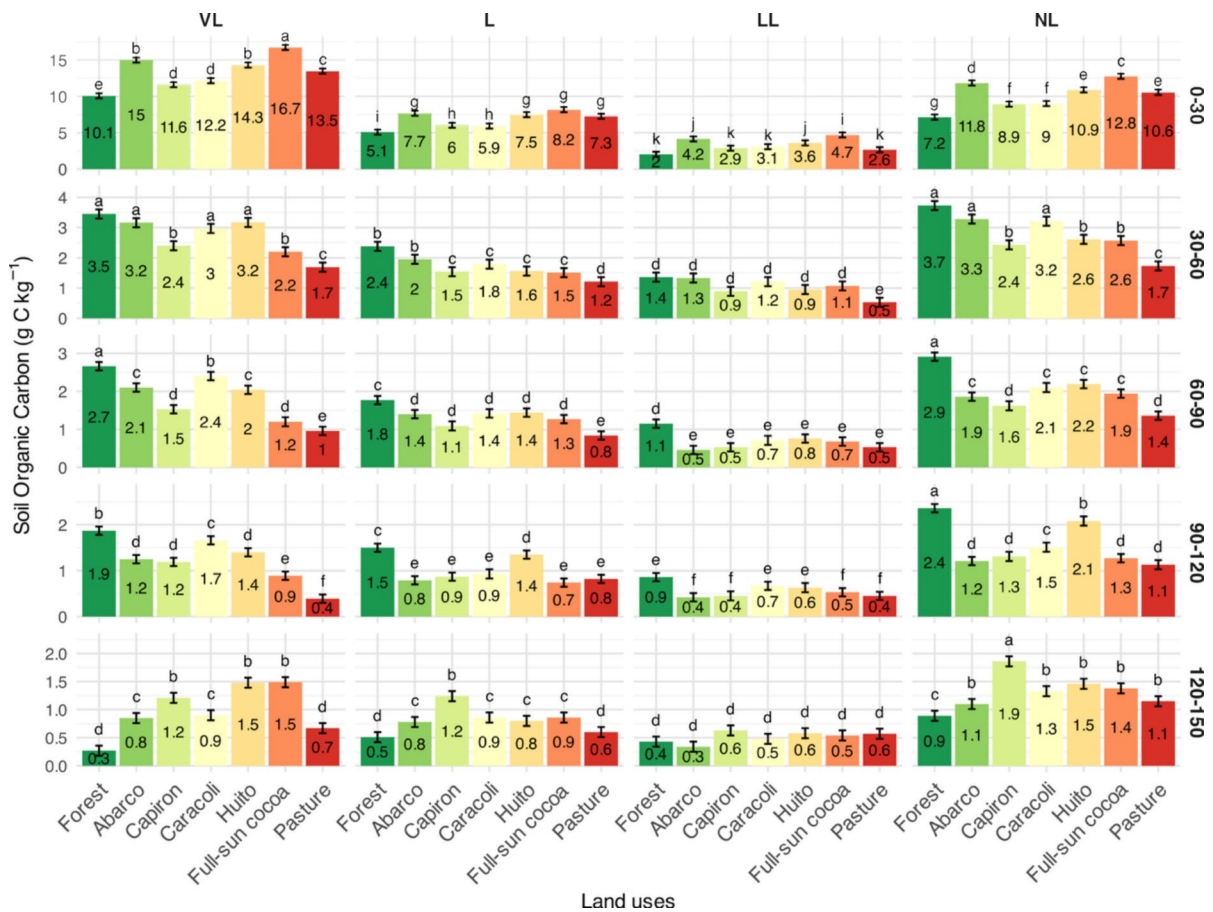


Fig. 2 Variation of soil C concentrations (g C kg⁻¹) in four different pools (VL: Very labile; L: Labile; LL: Less labile and NL: Non labile) according to soil depth (in cm) and the type of

land uses. ^a, ^b, ^c: Indicate statistically significant differences by the DGC means test ($P < 0.05$) between land uses at each depth for each carbon fraction

trend for bulk density to increase with depth. Pastures always had high to maximum values at all depths, slightly higher than Full-sun cocoa. Forest and agroforestry systems exhibited significantly lower values at all depths.

The C stocks significantly differed among land use systems (Figs. 3 and 4). The C storage was minimum in pastures (110.3 ± 3.9 Mg C ha⁻¹) and maximum in forests (206.9 ± 3.5 Mg C ha⁻¹), Full-sun cocoa (135.6 ± 3.5 Mg C ha⁻¹) being slightly better than pasture. Shade trees significantly improved C

storage with 190.2 ± 6.2 Mg C ha⁻¹ in Caracolí stands, 162.9 ± 2.9 Mg C ha⁻¹ in Abarco and 150.5 ± 4.6 Mg C ha⁻¹ in Huito. Capirón (137.1 ± 4.5 Mg C ha⁻¹) was the only treatment that did not improve C storage as compared with pure cocoa stands.

Although the increase in C storage as compared with the initial pasture occurred at all depths, accumulation below 120 cm is much more limited in Full-sun cocoa ($+4.56 \pm 0.32$ Mg C ha⁻¹), Capirón and Abarco ($+4.97 \pm 0.45$ Mg C ha⁻¹) than in Caracolí ($+36.24 \pm 1.1$ Mg C ha⁻¹) and Huito treatments

Table 1 Bulk density (g cm⁻³) in each column corresponds to the land uses to the row corresponds to the different depths

Depth (cm)	Land use						
	Forest	Abarco	Capirón	Caracolí	Huito	Full-sun cocoa	Pasture
0–30	1.24 ± 0.03^c	1.28 ± 0.02^c	1.23 ± 0.02^c	1.25 ± 0.03^c	1.23 ± 0.03^c	1.35 ± 0.03^b	1.33 ± 0.02^b
30–60	1.34 ± 0.04^b	1.35 ± 0.03^b	1.33 ± 0.03^b	1.3 ± 0.03^c	1.28 ± 0.04^c	1.41 ± 0.03^b	1.48 ± 0.03^a
60–90	1.42 ± 0.03^b	1.35 ± 0.02^b	1.36 ± 0.02^b	1.34 ± 0.03^b	1.33 ± 0.03^b	1.43 ± 0.03^b	1.51 ± 0.02^a
90–120	1.39 ± 0.04^b	1.36 ± 0.03^b	1.43 ± 0.03^b	1.36 ± 0.03^b	1.32 ± 0.04^b	1.42 ± 0.03^b	1.48 ± 0.03^a
120–150	1.41 ± 0.03^b	1.38 ± 0.03^b	1.39 ± 0.03^b	1.34 ± 0.03^b	1.33 ± 0.03^b	1.45 ± 0.03^a	1.55 ± 0.03^a

a, b, c indicate significant statistical differences by DGC means test ($P < 0.05$) of the interaction between depth and land use

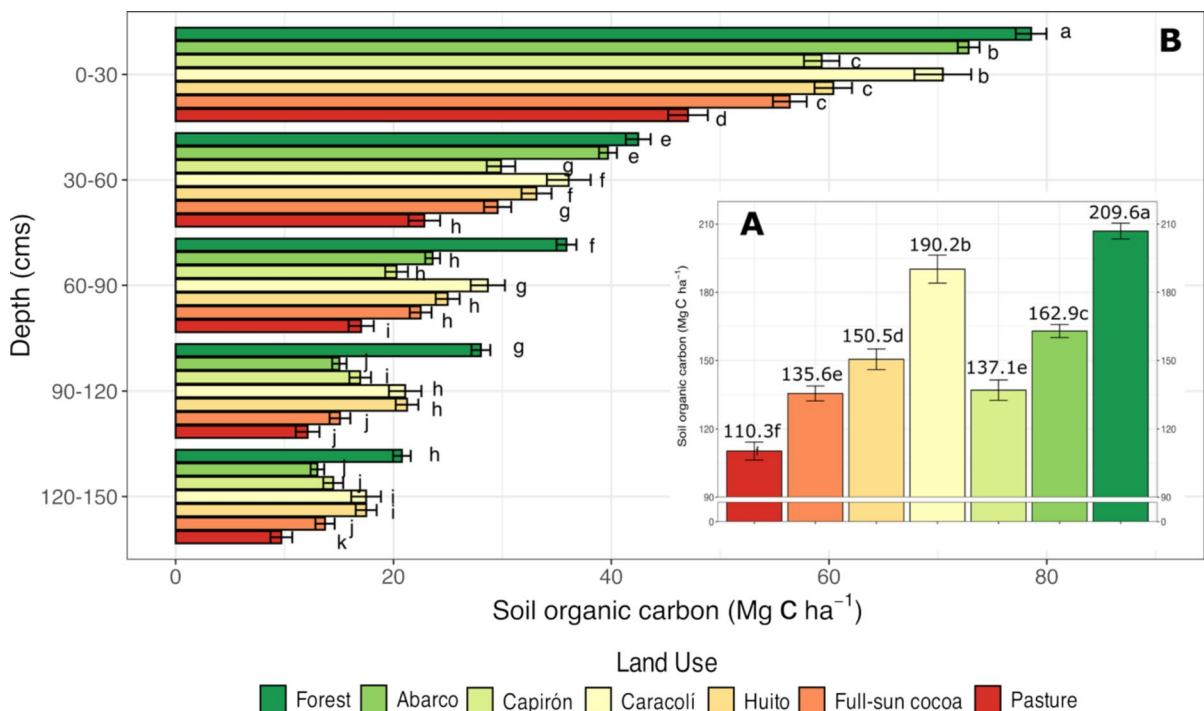


Fig. 3 Distribution of total C accumulated in different land uses (A), at different depths (B). a, b, c... to k letters indicate significant statistical differences by DGC means test ($P < 0.05$)

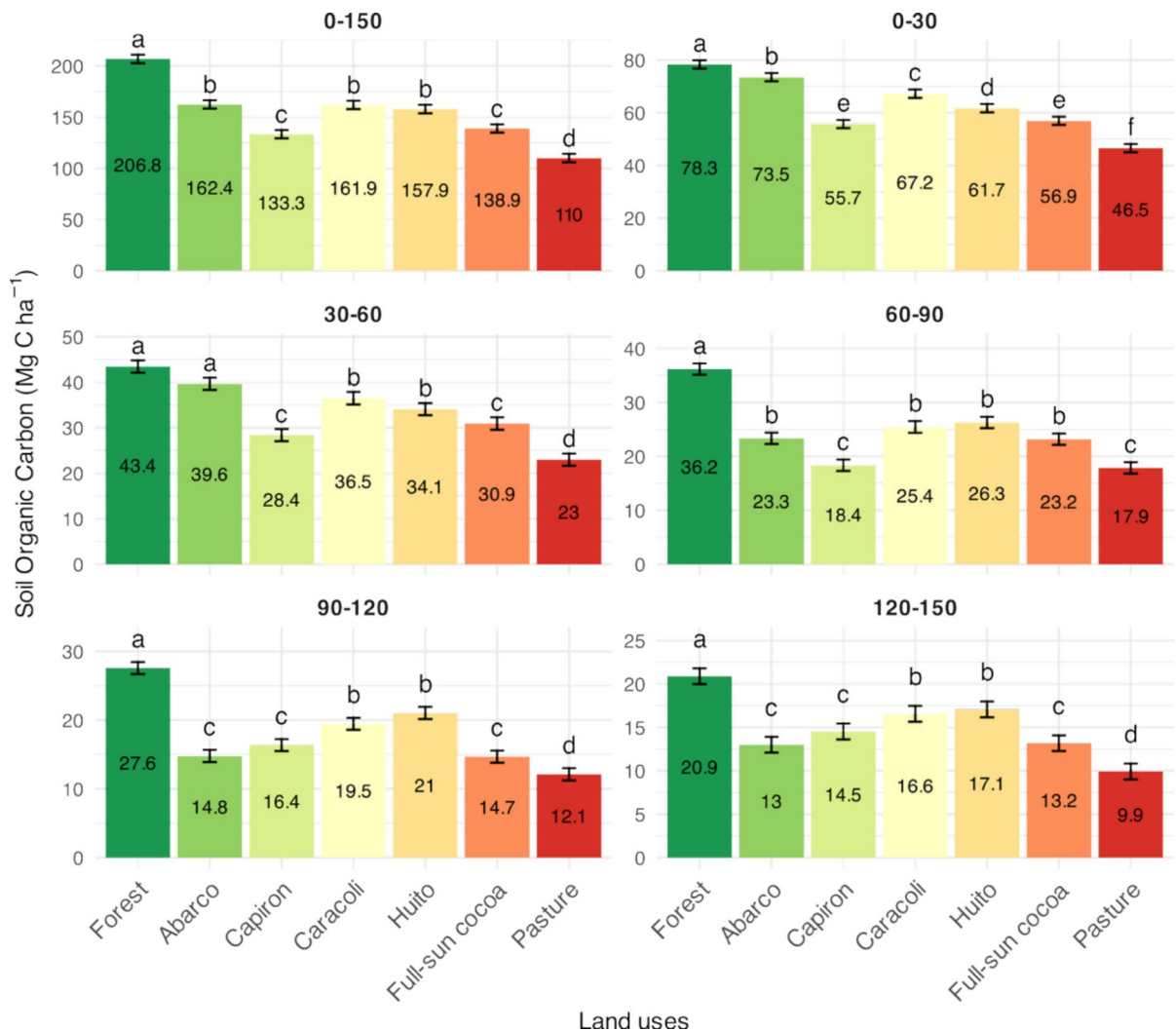


Fig. 4 Total C accumulated (Mg C ha^{-1}) at different depths (cm) according to land use. ^{a, b, c}: Indicate significant statistical differences by means of the DGC mean test ($P < 0.05$) of the interaction between land uses at each depth

($+40.80 \pm 1.7 \text{ Mg C ha}^{-1}$), that still lay behind the forest ($+63.72 \pm 2.4 \text{ Mg C ha}^{-1}$). The proportion of C stored in excess below 90 cm is remarkably higher in Huito (36.6%) than in Caracolí (26.1) and Capirón (23.1) and Cocoa (17.2) while Abarco increased much less C storage at these depths ($+9.6\%$).

The PCA analysis confirmed these observations, ranging systems along axis 1 (33.0% explained variance) from pasture to forest according to systems, in the order forest, Caracolí, Huito, Abarco, Full-sun cocoa, Capirón and pasture, according to values of C stored at all depths (Fig. 5). Axis 2 (11.9% explained variance) separated sites according to the relative

concentration of C stocks in the upper or deeper horizons, separating Abarco from the others (Monte Carlo test: 37% variance explained, $P < 0.01$).

The Carbon Pool Index (CPI) (ratio of the sum of C pools in the different treatments to the one measured in the original degraded pastures), increased in all treatments from the 0–30 cm surface layer down to the deepest soil in all treatments (Fig. 6). While forest always had the highest values, (2.0 ± 0.15 on average between 0 to 150 cm, progressively increasing from 2.00 ± 0.15 in the first layer to 4.86 ± 1.26 in the deepest one), plantations with shade trees always had high scores from Abarco (2.22 ± 0.60),

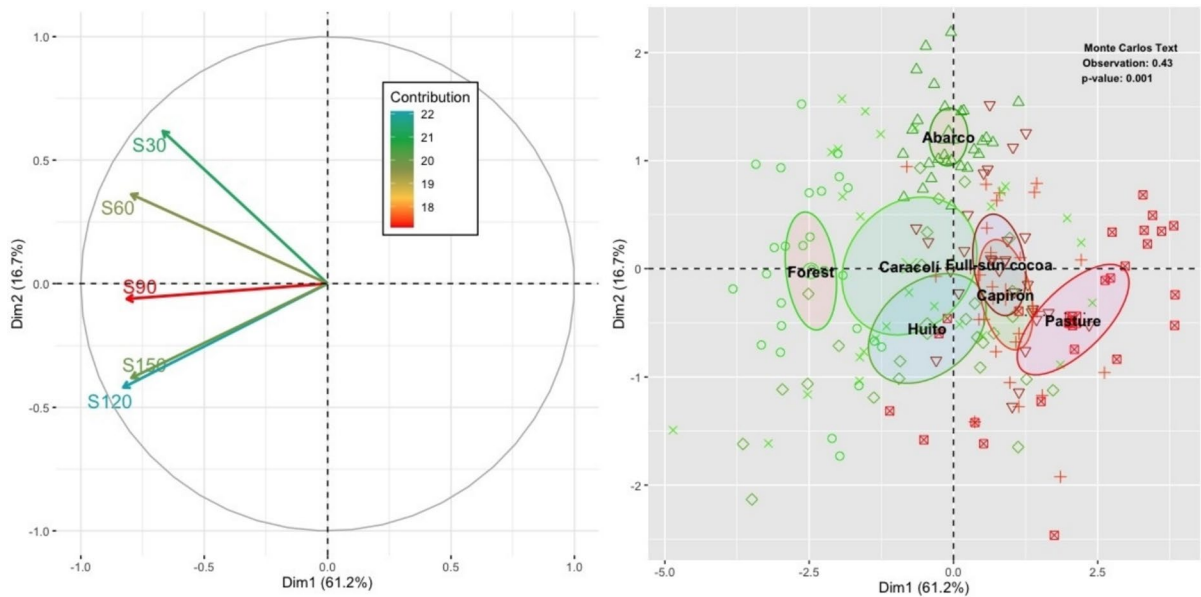


Fig. 5 The PCA analysis of C accumulated in sampling points of different production systems. S30–S150: Total C accumulated in soil strata 0–30 cm to 120–150 cm depth. $P < 0.01$; 36.5% variance explained

to Caracolí (2.40 ± 0.54) and Huito (2.73 ± 0.87), Capirón (1.91 ± 0.85) being the only one lower than Full-sun cocoa (1.98 ± 0.49). The recalcitrance index (RI) globally increased with depth in all treatments. While differences among treatments were not significant down to 90 cm (with one exception), pastures exhibited the highest values at the bottom of the soil profile.

Discussion

In the deforested Colombian Amazon, where significant carbon emissions result from land use conversion from forest to pasture, our study demonstrated that cocoa plantations and cocoa-based agroforestry systems can increase soil carbon accumulation within 15 years to levels 2.73 times higher than local degraded pastures, approaching the performance of Forests (2.96 times higher). Carbon accumulation under three of the four species used as monospecific shade trees was significantly higher than in cocoa monoculture and pasture systems, with differences primarily attributed to varying carbon distribution patterns with depth. Although these three shade tree species contributed substantially to building larger

carbon stocks compared to pure cocoa stands, their performance remained below that of forests, consistent with previous findings (Gama-Rodrigues et al. 2010). Fonkeng et al, (2024) found slightly higher performance of shade trees although not significant except under *Ceiba Pentandra* which showed significantly higher performance than forest in accumulation carbon in soil. However, note that their point of origin was degraded savannah, and their forest land use are highly degraded forest, reason why they observed slightly higher C values under shade tree species compared to forest.

Our results align with numerous studies reporting lower carbon storage in pastures compared to forests and agroforestry systems, which typically achieve accumulations between 40 to 70 Mg C ha⁻¹ between 40 and 100 cm depth range (Kimaro et al. 2024; Lustosa Filho et al. 2024; Santos et al. 2024; Suárez et al. 2024). Cocoa-based agroforestry systems demonstrate high capacity for soil carbon accumulation, for example, Monroe et al. (2016) reported 60 Mg C ha⁻¹ in an 18-year-old system down to a depth of 60 cm, likewise, Goñas et al. (2022) in a 40-year-old crop at 30 cm depth a carbon content of 132 Mg C ha⁻¹. Similar findings have been reported for other agroforestry systems, which store between 60 to 140 Mg C ha⁻¹ of

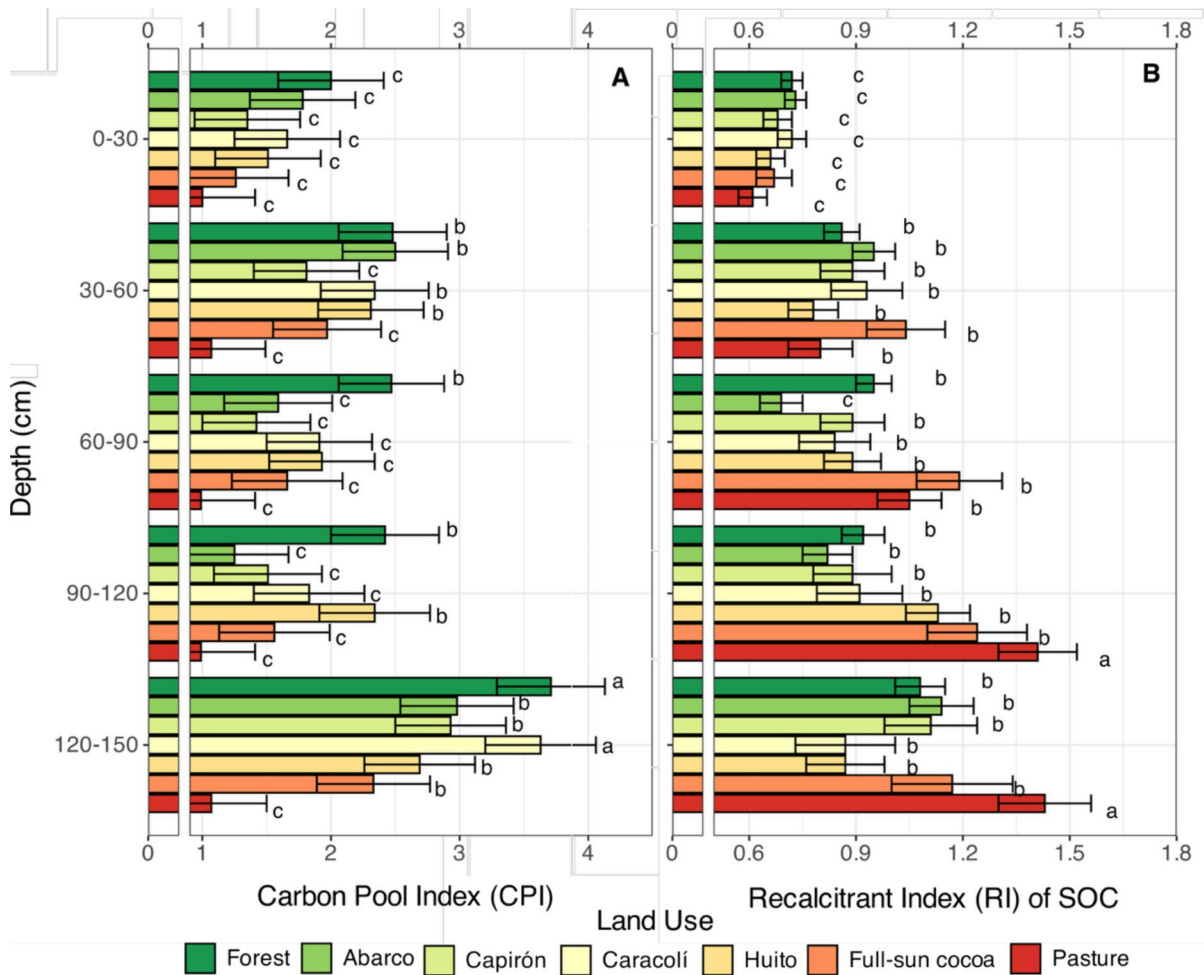


Fig. 6 Variations of the Carbon Pool Index (CPI) and Recalcitrance index (RI) according to depth and type of land use. a, b, c: Indicate significant statistical differences by DGC means test ($P < 0.05$) of the interaction between land use and depth

SOC, further evidencing how perennial land use systems can help mitigate soil carbon losses (Somarriba et al. 2013; De Stefano y Jacobson 2018; Shi et al. 2018; Salgado et al. 2019; Monroe et al. 2021; Suárez et al. 2024).

The enhanced capacity for SOC fixation is attributed to the incorporation of trees as shade canopy in different land use systems. Studies have demonstrated that increasing tree components in pasture areas leads to higher SOC levels (De Oliveira et al. 2024; Salazar et al. 2024). When cocoa is associated with different tree species, carbon sequestration in soil increases through the contribution and decomposition of above and below-ground biomass, including leaf litter and roots (Gama-Rodrigues et al. 2010; Lima et al. 2023;

Rodriguez et al. 2023; Teramag et al. 2024). In our study the influence of shade tree species on SOC storage was significant, with cocoa agroforestry systems showing significantly higher SOC levels ($64.5 \pm 2.3 \text{ g C kg}^{-1}$) compared to pasture systems ($46.5 \pm 1.3 \text{ g C kg}^{-1}$) at 30 cm depth. This pattern is corroborated by Fonkeng et al. (2024), who reported higher SOC in cocoa agroforestry systems ($17.9 \pm 5.6 \text{ g C kg}^{-1}$) compared to degraded savanna ($10.6 \pm 3.1 \text{ g C kg}^{-1}$), with values comparable to nearby forests ($16.3 \pm 5.8 \text{ g C kg}^{-1}$). The SOC content varies notably among tree species: *Albizia adianthifolia* shows lower values ($13.9 \pm 2.8 \text{ g C kg}^{-1}$) compared to *Dacryodes edulis* ($16.8 \pm 6.5 \text{ g C kg}^{-1}$) and *Ceiba pentandra* ($23.5 \pm 5.5 \text{ g C kg}^{-1}$) (Fonkeng et al. 2024). Similar

species-specific variations have been documented with *Cariniana piriforme* ($11.3 \pm 0.5 \text{ g C kg}^{-1}$), *Tabebuia rosada* ($9.6 \pm 0.4 \text{ g C kg}^{-1}$), and *Terminalia superba* ($8.3 \pm 0.3 \text{ g C kg}^{-1}$) (Carvalho et al. 2023).

The SOC accumulation measured in our systems, in a range of $135\text{--}190 \text{ Mg C ha}^{-1}$, is much higher than equivalent figures from agroecological zones in Africa (Quaye et al. 2021) with very low SOC ($0.39\% \pm 0.23$), contents at 20 cm depth. This difference may be caused due to different soil types, especially soil ages and general composition of soil in Latin America and Africa (Monroe et al. 2016; Blaser et al. 2017). Likewise, it was found in the present study that variations in carbon concentrations are observed along the vertical soil profile, as $41.3 \pm 0.8\%$ ($63.5 \pm 4.1 \text{ Mg C ha}^{-1}$) and $21.4 \pm 0.4\%$ ($33.3 \pm 2.5 \text{ Mg C ha}^{-1}$) accumulated between 0 to 30 and 30–60 cm depth, respectively in cocoa-based agroforestry systems. This behaviour has been described by other studies (Duguma et al. 2001; Dawoe et al. 2014; Takoutsing et al. 2016), where a reduction of more than 25% in SOC content is evident at 30 cm depth relative to that found in the first 10 cm. In this sense, it was evidenced that SOC stocks decreased in the different land uses at greater depths, while carbon stability increased.

Our study clearly indicates that the highest increases in C stocks occur where shade trees develop large root systems, with best performances for Huito and Caracolí, and no additional effect of Abarco as compared with Cocoa. In addition, the level of litter contributed to the soil increases the amount of carbon in the soil (Teramage et al. 2024). Relative lability of C also needs to be taken into consideration (Fontaine et al. 2007; R  egg et al. 2019; Xu et al. 2020). In our study, the recalcitrant index (RI) had the highest values in deep horizons under pasture and cocoa monoculture. This higher stable C content in full-sun cocoa may be due to a comparatively shallow root system, a lower contribution of fine root biomass and leaf litter (Niether et al. 2019). At greater depth, SOC results from root turnover and exudates (Trumbore et al. 1995; Fontaine et al. 2007), and organic matter input could be limited by low soil permeability and microbial activity (Berger et al. 2002). Soils under cocoa-based agroforestry systems with bulk density in a $1.23\text{--}1.43 \text{ g cm}^{-3}$ range between surface and a 150 cm depth and forest ($1.24\text{--}1.41 \text{ g cm}^{-3}$) presented a lower bulk density than pasture and

cocoa monoculture ($1.33\text{--}1.55$ and $1.35\text{--}1.43 \text{ g cm}^{-3}$ respectively) that allows the free flow of water loaded with oxygen (Buritic   et al. 2023), contrary to what was presented in pasture and cocoa in full sun where the bulk density was higher.

This study represents a significant contribution to the knowledge of carbon storage in cocoa based agroforestry systems in the Colombian Amazon, standing out primarily for its unprecedented analysis of carbon distribution down to 150 cm depth. From a review of 34 related articles, only six provided data below 60 cm, and none examined depths beyond 1 m. The research is distinguished by its comprehensive analysis of different carbon fractions (very labile, labile, less labile, and non-labile) throughout the soil profile, evaluating how these fractions vary according to the type of agroforestry system and shade tree species used. The study systematically compares cocoa agroforestry systems with four different shade tree species (Abarco, Capir  n, Caracol  , and Huito) against Forests, Full-sun cocoa plantations, and degraded pastures, providing a comprehensive assessment of soil management options in the region. The findings are particularly relevant for the restoration of deforested landscapes, demonstrating that cocoa plantations and agroforestry systems can accumulate up to 2.73 times more soil carbon than local pastures over a 15-year period. The study reveals significant differences in carbon storage capacity according to shade tree species, with Huito and Caracol   showing better efficiency in storing carbon in deep soil layers. This research is especially relevant for the Colombian Amazon region, where deforestation and soil degradation are critical problems, providing solid evidence on how cocoa agroforestry systems can effectively contribute to soil carbon restoration in deforested landscapes.

Conclusion

Our study demonstrated the significant carbon storage potential of cocoa-based agroforestry systems. The incorporation of shade trees substantially enhanced carbon accumulation, with notable variations among tree species. While Forests stored 1.91 times more carbon than pastures, cocoa systems with shade trees-maintained ratios between 1.21 and 1.47, and Full-sun cocoa systems achieved a ratio of 1.26. This

enhanced carbon storage was primarily attributed to the extensive development of deep root systems rather than differences in the lability of accumulated organic matter. Under our experimental conditions, the observed carbon accumulation rates substantially exceeded those reported in previous literature.

Author contributions J.C.S. and E.A.C. planned the research project and sampling methodology. J.C.S. and P.L. carried out the statistical analyses and the preparation of the figures. J.C.S. wrote the first version of the manuscript. All authors reviewed and edited the manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflicts of interest The authors declare no competing interests.

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