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Variation in Carbon Storage Across Biomass Components and Soil Depths by Stand Age in *Acacia* Hybrid Plantations, Song Hinh District, Phu Yen Province, Vietnam

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ABSTRACT

To better understand the carbon (C) sequestration process of Acacia hybrid plantations in Song Hinh District, Phu Yen Province, Vietnam; this study was carried out to explore the distribution of C stocks among various biomass components and soil depths in three A. hybrid stands of different ages (3, 6, and 9 years old). The results showed that: (1) The amount of C stored in the biomass of trees, understory vegetation (shrubs and herbs), and litter on the forest floor increased with stand age. (2) The amount of C stored in tree biomass accounted for 79.20% of the total C stored in biomass. Other biomass C components (understory plants and litter on the forest floor) accounted for < 12% of the total C stored in biomass. (3) The soil organic C storage of 3-, 6-, and 9-year-old A. hybrid forests were 81.95, 103.60, and 140.78 Mg· ha^{-1}, respectively. Soil organic C stocks decreased continuously with increasing soil depth. (4) Total ecosystem C storage increased with plantation age, 119.35, 169.92, and 272.11 Mg· ha^{-1}, respectively, of which more than 60% was stored in the soil. Our results suggest that the large-scale planting A. hybrid plantations has a high potential for C sequestration during forest development in the study site.

Keywords: *Acacia* hybrid forests, biomass carbon stocks, soil carbon stocks, forest age

INTRODUCTION

Biomass and carbon (C) stocks in forest ecosystems are crucial for the global C cycle (Zhang *et al*., 2019). Among the components of these ecosystems, trees and soils have the highest potential for carbon storage (Cuong *et al*., 2020). To mitigate atmospheric CO_2 concentrations, increasing global C sequestration by expanding forest areas has been proposed as an effective strategy (Ming *et al*., 2014). However, as the area of natural forests has declined in recent decades, plantations have become increasingly important to the planet's forest resources. Commercial plantations are essential for promoting sustainable forest management worldwide. Well-designed, multi-purpose plantations can alleviate pressure on natural forests, restore certain ecosystem services, and contribute to climate change mitigation through direct C sequestration (Them & Toai, 2024).

Acacia hybrid is among the most widely used timber species for afforestation in the South Central Coast of Vietnam. This species is highly adaptable, grows rapidly, boasts good wood quality, and offers stable productivity. As a result, *A*. hybrid plantations play a significant role in promoting the national economy and enhancing ecological efficiency across various economic regions (Cuong *et al*., 2024a,b,c). Additionally, these plantations are crucial for providing environmental services (Chau *et al*., 2024). Therefore, accurate information regarding carbon (C) stocks in *A*. hybrid plantations is essential. Previous studies have explored growth characteristics, forest volume, biomass and carbon uptake, soil carbon storage, soil nitrogen storage, and the physical and chemical properties of soil at the local level (Hai *et al*., 2008; Bao & Hue, 2016; Thanh & Cuong, 2017; Cuong *et al*., 2024a,b,c).

Nonetheless, there is still a scarcity of information on biomass accumulation and carbon storage in plantation forests. In Song Hinh district, Phu Yen province, the total area of plantation forests reaches 7,613.54 hectares, with 89.9% (6,841.88 hectares) designated as *A*. hybrid forests (SHDPC, 2021). To contribute valuable information regarding forest carbon storage in Phu Yen province, Vietnam, which will help us better predict the response of regional and global C balances to future climate change; this study aims to assess the biomass and carbon stocks of *A*. hybrid plantations in Song Hinh district. The primary objective of the current study is to investigate how biomass and carbon storage change with the age of *A*. hybrid plantations in Song Hinh District of Phu Yen Province.

MATERIALS AND METHODS

Study area

The study site is located in Song Hinh district of Phu Yen province, Vietnam, with coordinates ranging from 12°05′00″ to 13°19′00″ N latitude and 108°37′00″ to 109°10′00″ E longitude (Figure 1). This area experiences a tropical monsoon climate, characterized by distinct dry and rainy seasons. The dry season typically occurs from December to July of the following year, while the rainy season lasts from August to November. The average annual rainfall at the site ranges from 1485 to 2154 mm. The relative humidity is consistently between 80% and 84%. Additionally, the average annual temperature falls between 25°C and 27°C. The soil in this region belongs to the red-yellow soil group (Cuong *et al*., 2024b).

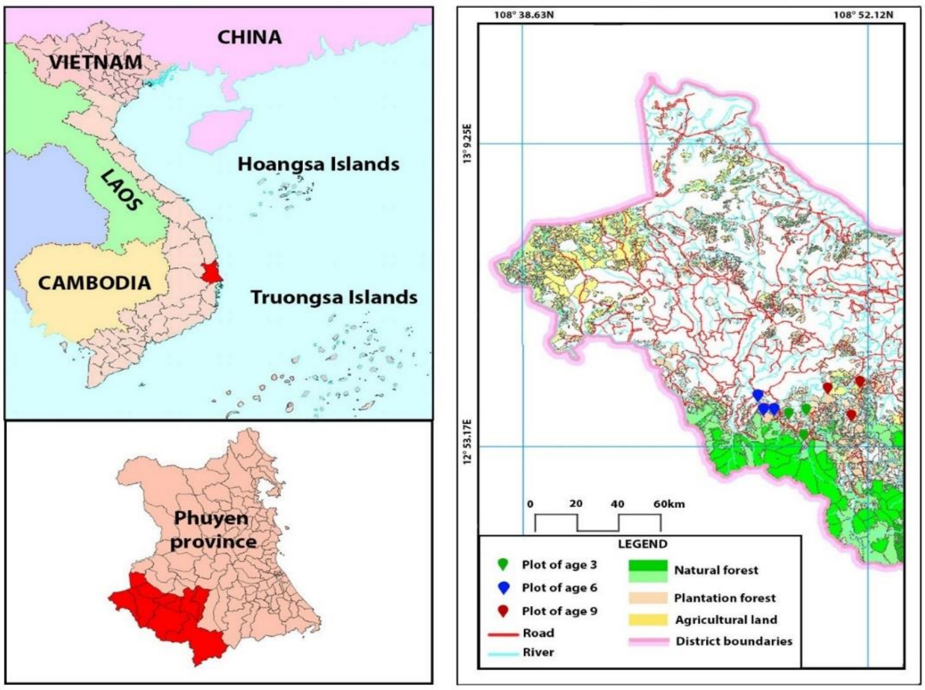

Figure 1: Map illustrating the locations of sampling points in the Song Hinh district of Phu Yen province, Vietnam

Experimental design

In 2023, three *A.* hybrid plantations with varying stand ages were selected based on their topographic similarities, including an elevation range of 140–152 m and a slope range of 3–6°. The plantations represented chronological sequences of *A.* hybrid stands with ages of 3, 6, and 9 years. All stands were in their first growth rotation and had previously replaced *Cassava* (Khoaimi in Vietnamese) before planting. Notably, no fertilizers were applied after planting. The initial planting density was set at 2,500 trees per hectare (2 m × 2 m). Thinning, a crucial and common silvicultural practice for *A.* hybrid stands, was conducted to enhance tree growth, vitality, and the quality of stems and wood. Thinning took place after three years, with an intensity that did not exceed 30% of the total stand volume.

From May to June 2023, three standard plots (each measuring 25 m × 20 m) were established for each age group of plantations. Within each plot, we measured the diameter at breast height (DBH) of each tree using a diameter measuring tape and recorded tree height, and height under branch using a Haglöf Vertex IV altimeter. Additionally, five quadrates containing understorey vegetation (shrubs and herbs) (each 2 m × 2 m) and five sub-plots with plant litter (each 1 m × 1 m) were established at the corners and the center of the standard plots to investigate indicators of understorey vegetation and plant litter. We recorded various characteristics of the plant species, including number, height, and cover. Environmental factors such as elevation and slope were also documented. Basic information regarding the stand features was provided in our previous study (Cuong *et al*., 2024b) and was represented in **Table 1**.

Table 1: The general status of three age classes of *A.* hybrid forests

Characteristics parameters	Forest age (years)		
Altitude (m)	140	145	152
Slope (°)	3	4	6
Mean diameter at breast height (cm)	7.56 ± 0.02^{a}	11.94 ± 0.15^{b}	17.61 ± 0.06^{c}
Mean height (m)	7.66 ± 0.05^{a}	13.02 ± 0.03^{b}	20,12 ± 0.21^{c}
Mean height under branch (m)	4.03 ± 0.04^{a}	6.53 ± 0.03^{b}	7.45 ± 0.04^{c}
Mean canopy density	0.67 ± 0.06^{a}	0.70 ± 0.00^{a}	0.83 ± 0.05^{b}
Mean plan density (Plant· ha^{-1})	2400 ± 16^{a}	1800 ± 16^{b}	1633 ± 25^{c}
Mean total tree biomass (Mg· ha^{-1})	51.63 ± 0.27^{a}	107.25 ± 4.49^{b}	230.41 ± 5.47^{c}
Mean total understory vegetation biomass (Mg· ha^{-1})	12.68 ± 0.34^{a}	14.89 ± 0.22^{b}	16.41 ± 0.54^{c}
Mean plant litter biomass (Mg· ha^{-1})	10.49 ± 0.48^{a}	10.49 ± 0.48^{a}	15.83 ± 0.30^{c}
Major understory shrubs and herbaceous plants	*Chromolaena odorata* (L.) R.M. King & H. Rob., *Tetracera scandens* (L.) Merr., *Lophatherum gracile* Brongn., *Chrysopogon aciculatus* (Retz.) Trin., *Lygodium microphyllum* (Cav.) R. Br., *Mallotus apelta* (Lour.) Müll. Arg.		

Note: Data show the mean ± SD. Lowercase letters denote significant differences at $p < 0.05$, as determined by the one-way ANOVA and LSD test.

Determination of biomass

To avoid affecting the productivity of *A.* hybrid forests due to cutting down sample trees, the relative growth equation of *A.* hybrid trees established by Hai *et al*. (2008) was applied to estimate the biomass (including above-ground and below-ground dry biomass) for each tree and the total biomass storage for each age of the stand. The biomass of each tree (kg· $tree^{-1}$) was calculated using the following equation:

$$W_t = 0.225 \times DBH^{2.244} \qquad \text{(Equation 1)}$$

Where W_t is the individual tree biomass; DBH is the diameter at breast height of each tree.

We employed the whole-cut method to measure the biomass of the aboveground and belowground parts of the understorey and litter layers (Cuong *et al*., 2024a). The fresh weights of these samples were measured directly using weighing. We then selected approximately 500 grams from each sample and transported them to the laboratory, where they were dried in an oven at 65°C until reaching a constant weight. After drying, we weighed the samples to determine the dry weights of the shrub and litter biomass. This allowed us to estimate the biomass of understorey and litter per unit area ($Mg \cdot ha^{-1}$).

Soil sample collection

In each standard plot, five soil profiles were randomly collected, forming an S-shaped curve across three depth layers: 0–20 cm, 20–40 cm, and 40–60 cm. Soil samples from each depth layer within the same standard plot were mixed in equal proportions. These mixed samples were then air-dried at room temperature (25°C). After drying, the samples were ground and passed through a 0.15 mm mesh sieve to remove roots, gravel, and other debris before analyzing the physical and chemical properties of the soil. To measure bulk density, a stainless steel soil sampling ring (with a volume of 100 cm^3) was used to collect samples from different soil layers. These samples were transported to the laboratory for bulk density measurement using the ring method.

Laboratory analysis

Soil bulk density was calculated by drying core samples at 105°C until constant weight (Blake & Hartge, 1986). Soil organic C content was analyzed by the H_2SO_4 - $K_2Cr_2O_7$ oxidation method (Six *et al*., 2002).

Calculation of biomass carbon storage in trees, understorey, and litter layers

The biomass C storage of trees, understorey, and litter layers was determined by multiplying their biomass values by a default factor of 0.5 adopted by the Intergovernmental Panel on Climate Change (IPCC, 2003). Biomass carbon storage in trees, understorey, and litter layers was calculated using the following equation:

$$C_v = B \times C_f \qquad \text{(Equation 2)}$$

Where C_v is the biomass carbon storage in trees, understorey, and litter layers (Mg/ha); B is the biomass of the trees, understorey, and litter layers (Mg/ha); and C_f is the default carbon factor.

Calculation of soil organic C storage

The stocks of soil organic C were calculated based on the organic C content of the soil layer, the soil bulk density, and the sampling depth. The following equation was used to calculate the soil organic C storage (Wang *et al*., 2019).

$$Cs_i = Sc_i \times BD_i \times K_i \times 0.1 \qquad \text{(Equation 3)}$$

Where Cs_i is the soil C storage ($Mg \cdot ha^{-1}$) in soil layer i; BD is the soil bulk density in soil layer i ($g \cdot cm^{-3}$); Sc_i is the C content of soil layer i ($g \cdot kg^{-1}$); K_i is the depth of soil layer i (cm); i represents the soil layers 0 – 20 cm, 20 – 40 cm, and 40 – 60 cm.

Statistical analysis

One-way analysis of variance (ANOVA) followed by the Least Significant Difference (LSD) test ($p < 0.05$) was used to assess the differences in C content and C storage among *A*. hybrid forests of different ages. All data were tested for normal distribution (Kolmogorov-Smirnov test) and homogeneity of variance (Levene test) before statistical analysis. All statistical analyses were performed using R software, version R 3.5.2 (Team, 2022). Statistically significant differences were detected at $p < 0.05$.

RESULTS AND DISCUSSION

Biomass C storage

Estimation of C storage at different stages of forest development is essential to assess the role of forest ecosystems in managing C pools at regional and global levels (Du *et al*., 2015). The C storage in the biomass of 3-, 6-, and 9-year-old *A*. hybrid trees was 25.81, 53.63, and 115.21 t/ha, respectively **(Figure 2a)**. The 9-year-old forests achieved as high as 115.21 Mg$^{\cdot}$ ha^{-1}, which was 4.46 and 2.15 times those of the 3- and 6-year-old forests respectively. This demonstrates that the forest ecosystems of current 3- and 6-year-old forests can deposit C when they reach the 9-year-old forests. The C storage in tree biomass was the largest contributor to the total biomass C pool across all stand ages, representing 69.02%, 80.86%, and 87.73% of the total biomass C pool for the 3-, 6-, and 9-year-old stands, respectively **(Figure 2b)**. These results showed that the amount of C stored in the biomass of *A*. hybrid trees increased significantly with forest age (p<0.05). Similar results have been reported in previous studies (Hai *et al*., 2008; Zhang *et al*., 2019; Cuong *et al*., 2020).

A. hybrid is a fast-growing species; The mean total tree biomass C in this study was 64.88 Mg$^{\cdot}$ ha^{-1}, which was significantly higher than the values reported for 2- to 12-year-old *A. auriculiformis* plantations (21.5 Mg$^{\cdot}$ ha^{-1}) and 1- to 7-year-old *A*. hybrid forests (21.73 Mg$^{\cdot}$ ha^{-1}) in Vietnam, as noted by Hai *et al*. (2008). However, it was slightly lower than the 68.4 Mg$^{\cdot}$ ha^{-1} observed in 4- to 11-year-old *A. mangium* forests in Tay Ninh province of Vietnam (Cuong *et al*., 2020) and the 71.5 Mg$^{\cdot}$ ha^{-1} found in 4- to 11-year-old *A. mangium* forests in Guang Xi province of China (He *et al*., 2009). Despite this, the measured biomass falls within the range of 34.4 to 85.6 Mg$^{\cdot}$ ha^{-1} for Asian forests and 44.8 to 118.2 Mg$^{\cdot}$ ha^{-1} for global forests, as reported by the FAO (2010). The variations in results may be attributed to differences in tree species, age, climatic conditions, and forest planting density (Hai *et al*., 2008; Noh *et al*., 2010).

Understory vegetation plays an important role in stabilizing ecosystems and absorbing CO_2 (Xu *et al*., 2000). The biomass of understory vegetation had a clear relationship with the age of the stand (p<0.05), with the lowest (6.34 Mg$^{\cdot}$ ha^{-1}) and highest (8.21 Mg$^{\cdot}$ ha^{-1}) values recorded in 3- and 9-year-old *A*. hybrid forests **(Figure 2a)**. The C value of understory vegetation biomass accounted for 6.25%–16.95% of the total C stored in biomass, with the largest contribution in 3-year-old *A*. hybrid forests **(Figure 2b)**. In most previous studies, scientists found that there was a clear trend of change in understory vegetation biomass with age, similar to this study (He *et al*., 2009; Cuong *et al*., 2020). Some studies showed that as the age of the stand increased, understory vegetation biomass decreased (Cao *et al*., 2012), first increased and then decreased (Li *et al*., 2011), or only increased in the late stage (Chen *et al*., 2013). This difference in understory biomass may be due to differences in age, structure, climate, and soil conditions among these reports (Du *et al*., 2015).

The amount of C stored in the biomass of the litter on the forest floor was significantly lower in 3-year-old plantations than in other stages (**Figure 2a**). The contribution of forest floor litter to biomass C storage decreased from 3-year-old *A*. hybrid forest (14.02%) to 6-year-old *A*. hybrid forest (7.91%) and 9-year-old *A*. hybrid forest (6.03%) (**Figure 2b**). The lower C storage of forest floor litter in the first three years of forest development was due to the reduced biomass of leaves, branches, and understory vegetation in young forest stands.

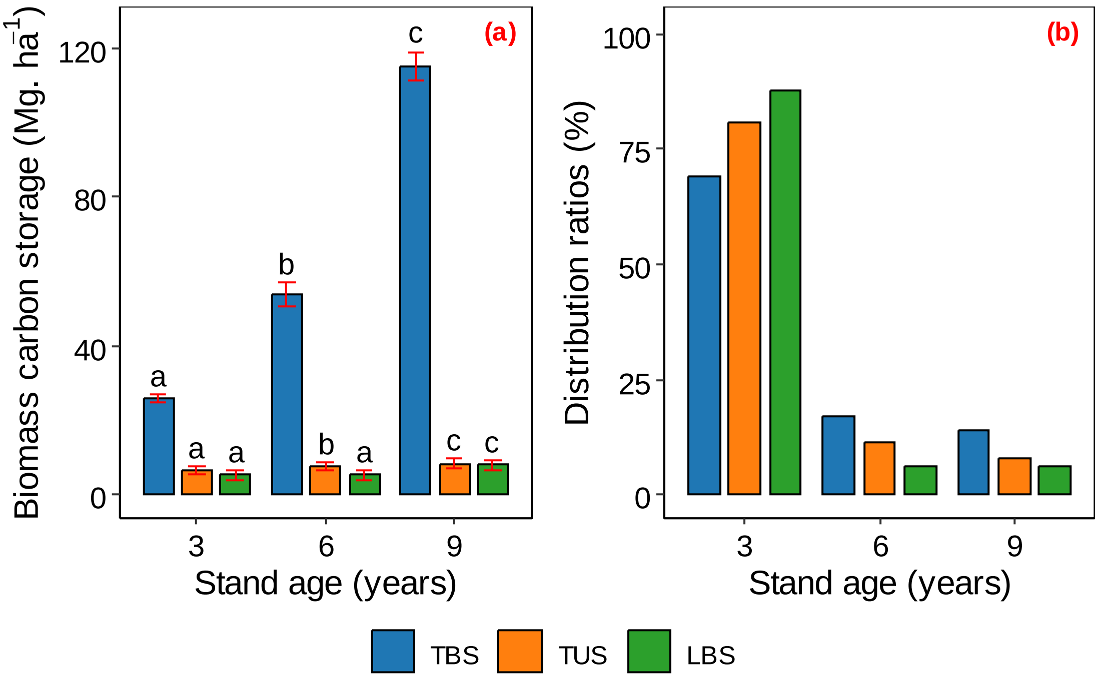

Figure 2. Biomass carbon stocks (a) and their distribution ratios (b) in different biomass components of *Acacia* hybrid forests

Note: TBS, Total tree biomass carbon stocks (Mg· ha^{-1}); TUS, Total understory vegetation carbon stocks (Mg· ha^{-1}); LBS, Litter biomass carbon stocks (Mg· ha^{-1}). Different lower-case letters indicate significant differences between forest ages at $p < 0.05$ (one-way ANOVA and LSD test). Values represent mean ± standard deviation (SD).

Soil carbon storage

Data from **Figure 3** shows that based on soil bulk density (**Figure 3a**) and organic C content (**Figure 3b**), soil organic C stocks in *A.* hybrid forests significantly increased with stand age ($p < 0.05$; **Figure 3c**). In the 3- to 9-years-old forests, C storage in the 0–20 cm layer rose from 34.33 Mg· ha^{-1} to 52.34 Mg· ha^{-1}, in the 20–40 cm layer from 29.12 Mg· ha^{-1} to 50.53 Mg· ha^{-1}, and in the 40–60 cm layer from 18.50 Mg· ha^{-1} to 37.91 Mg· ha^{-1}, leading to an increase in the total 0–60 cm layer from 81.95 Mg· ha^{-1} to 140.78 Mg· ha^{-1}. This clearly indicates a significant accumulation of organic C in the soil layers following afforestation. These results are consistent with previous studies (Thanh & Cuong, 2017; Ngoan *et al*., 2023; Cuong *et al*., 2024c). Gong *et al*. (2022) identified that sources of soil organic C include aboveground litter and root exudates, which accumulate as forests mature. Furthermore, older forests improve soil conditions, enhance microbial activity, and increase plant biomass (Cuong *et al*., 2020).

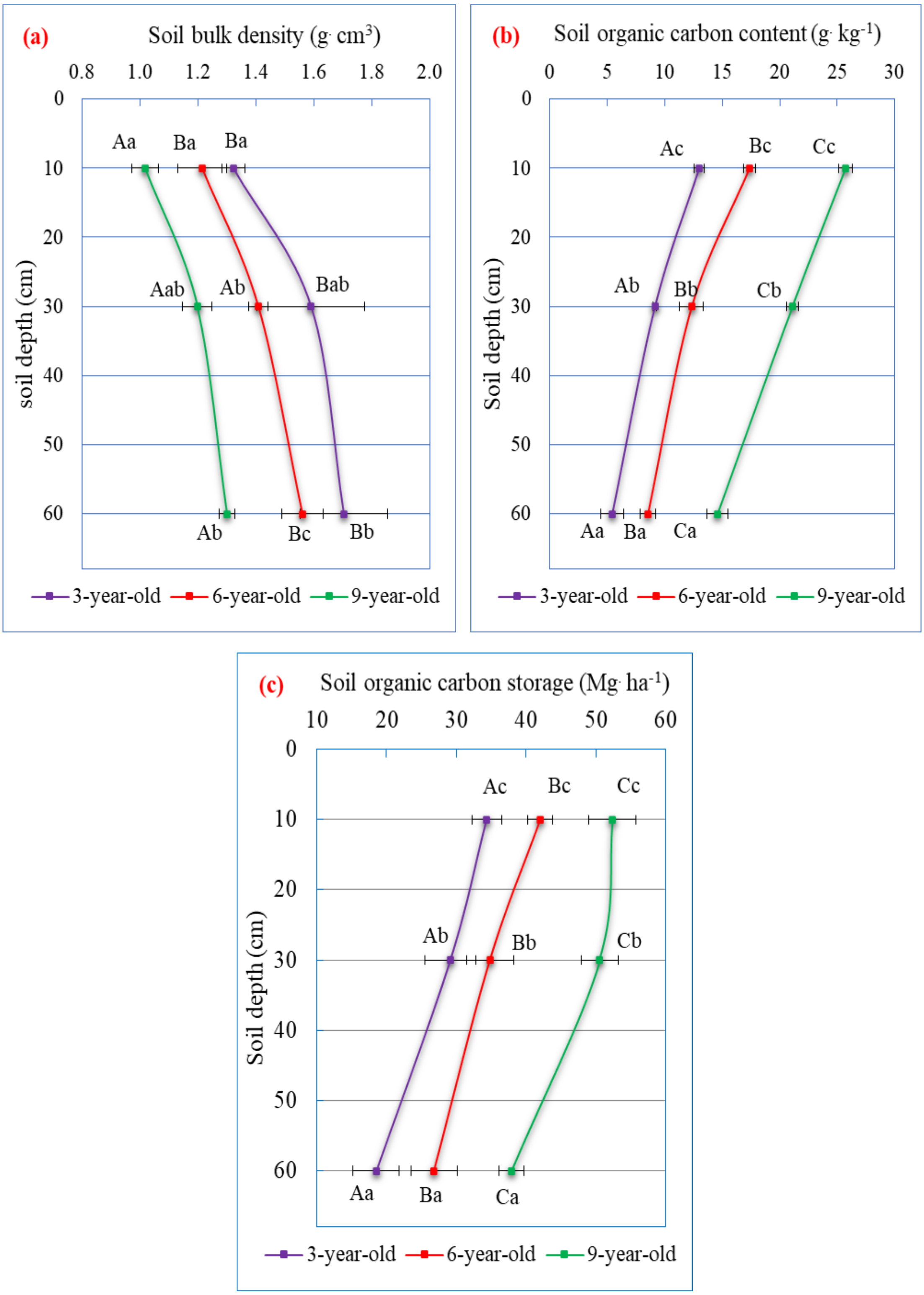

Figure 3. Soil bulk density (a), carbon content (b), carbon content storage of *Acacia* hybrid forest
Note: Error bars display standard deviation (SD). Divergent capital letters demontrate substantial variations among different stands on the same soil depth layer (p<0.05). Divergent lowercase letters demontrate substantial variations among distinct soil depth layers on the same stand (p<0.05).

Upon vertical examination (**Figure 4**), the respective contributions of soil layers to the stocks of soil organic C throughout the afforestation chronosequence were observed as follows: for the 0–20 cm layer, contributions ranged from 37.18% to 41.90%; for the 20–40 cm layer, contributions varied from 33.59% to 35.89%; and for the 40–60 cm layer, contributions ranged from 22.57% to 26.93%. Overall, as the stands aged, a larger proportion of soil organic C storage was found in the uppermost layer (0–20 cm) compared to the deeper soil layers (20–40 cm and 40–60 cm). This increase can be attributed to a higher amount of litter and more favorable environmental conditions. Poor aeration and lower nutrient content in deeper soils can restrict microbial activity (van Leeuwen *et al*., 2017), affecting C levels. Thus, maintaining C at the soil surface is crucial for effective carbon sequestration.

Although previous studies have widely reported changes in soil organic C storage (Cs) following afforestation, there is still some controversy surrounding the effects of stand age on Cs levels (Sartori *et al.*, 2010; Chen *et al.*, 2017; Zhang *et al.*, 2019). The varying responses of Cs to stand age may partly result from differences in other influencing factors, such as climate, soil properties, forest type, and previous land use, all of which could overshadow the impact of stand age (Hai *et al*., 2008).

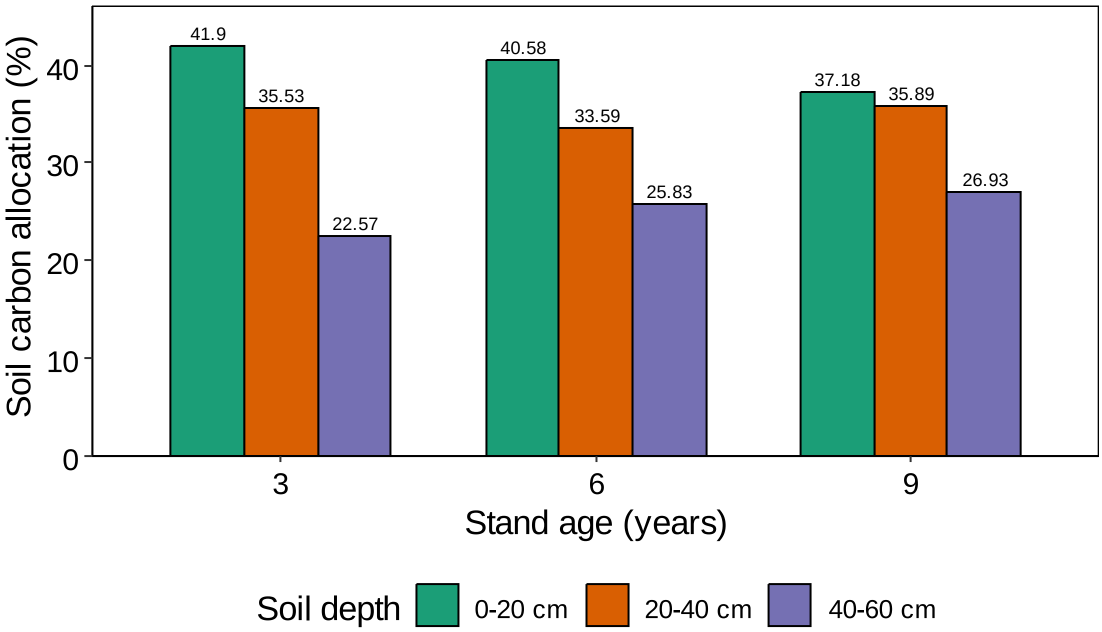

Figure 4. Percentage carbon allocation (%) at different soil depths of *Acacia* hybrid forests

Ecosystem C storage

The study's results indicated that the forest age significantly influences the carbon storage process within the entire ecosystem and offers detailed insights into C uptake process (Cuong *et al*., 2020). The total C storage in 3-, 6-, and 9-year-old *A.* hybrid forests showed significant differences ($p < 0.05$), with values increasing with forest age: 119.35, 169.92, and 272.11 Mg ha^{-1}, respectively (**Figure 5a**). In all age classes, the majority of C was stored in the soil rather than in biomass. Specifically, 68.66% of the total C in the 3-year-old *A.* hybrid forests was stored in the soil. This proportion decreased with forest age, dropping to 51.74% in the 9-year-old *A.* hybrid forests (**Figure 5b**). As the forests aged, C accumulation in biomass increased. The contribution of biomass C to the overall ecosystem C increased from 31.34% for 3-year-old *A.* hybrid forests to 48.26% for 9-year-old *A.* hybrid forests (**Figure 5b**). However, this trend was not consistent across all forests, as rates of aboveground and belowground C accumulation varied among different tree species throughout forest development (Li *et al*., 2011; Cao *et al*., 2012).

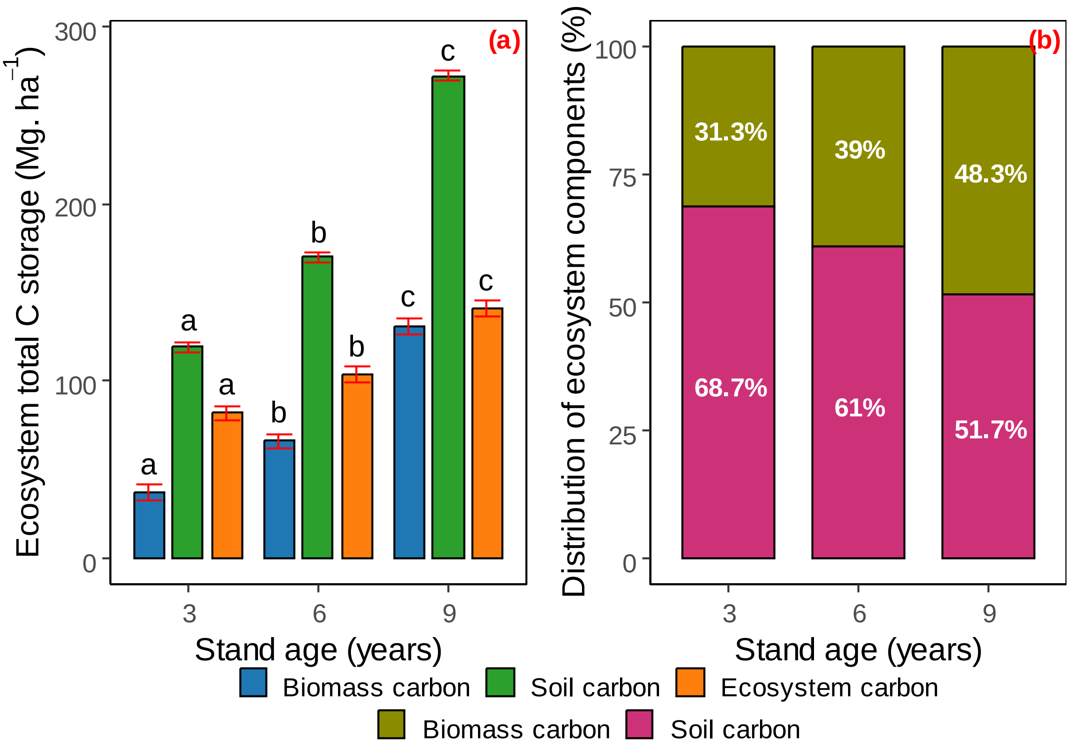

Figure 5. Carbon storage (a) and their distribution ratios (b) of ecosystem carbon storage in *Acacia* hybrid forests of three ages

Note: Different lower-case letters indicate significant differences between forest ages at p < 0.05 (one-way ANOVA and LSD test). Values represent mean ± standard deviation (SD).

CONCLUSION

The results presented in this study indicate changes in C stocks across different biomass components and soil depths in *A.* hybrid plantations at various stand ages in the Song Hinh district, Phu Yen province, Vietnam. The amount of C stored in the biomass of trees, understory vegetation, and litter on the forest floor increases with forest age. Additionally, the organic C content in the soil of *A.* hybrid forests is dependent on the age of the trees. It was observed that the amount of organic C in the soil decreases consistently with increasing soil depth. Overall, the ecosystem's C storage is largely comprised of soil organic C, accounting for 51.74% to 68.66% of the total ecosystem C. These findings suggest that *A.* hybrid is a fast-growing tree species with significant potential for C uptake.

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