

SOIL NITROGEN STORAGE AND ASSOCIATED REGULATION FACTORS IN AN *ACACIA* HYBRID PLANTATION CHRONOSEQUENCE IN SOUTHERN VIETNAM

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(Received 25th Sep 2023; accepted 30th Oct 2023)

Abstract. The study was designed to quantify the soil total nitrogen (*STN*) storage (*SNS*) and its controlling factors in *Acacia* hybrid (*Acacia auriculiformis* × *A. mangium*) plantations in five different ages (2-, 4-, 6-, 8-, and 10-years-old) in Langa - Dongnai Forestry Company, Southern Vietnam. A total of 75 soil samples were taken at five different soil depths from 0–100 cm. The *STN* concentration, *SNS*, soil properties, and plant biomass features (i.e., understorey vegetation biomass and litter biomass) were estimated. The *STN* concentration significantly increased with increasing forest stand age, whereas significantly decreased with increasing soil depth in all stand ages. The *SNS* in the uppermost 1 m soil layer in 2-, 4-, 6-, 8, and 10-year-old stands was 6.83, 9.50, 12.30, 17.15, and 19.41 Mg ha⁻¹, respectively. The *SNS* was focused mainly on the topsoil layers (0–40 cm). The *SNS* in the upper 0–40 cm soil layer accounted for over 50% of the total soil N stocks and the accumulation revealed decreasing tendencies with an increase of soil depth in all five stands. The soil organic carbon concentration and soil particle size fractions (i.e., soil silt concentration) were detected as the leading environmental parameters modulating the *SNS* variance. Our findings illustrate that considerations of N sequestration potential in *Acacia* hybrid plantations must take stand age into account and offer significant information for a better comprehension of the key environmental parameters driving *SNS* in Southern Vietnam.

Keywords: *forest plantations, soil properties, plant biomass features, stand age, soil depth*

Abbreviations used: *PCA* (principal component analysis); *SAP* (soil available phosphorus); *SBD* (soil bulk density); *SMRA* (stepwise multiple regression analysis); *SNS* (soil total nitrogen storage); *SOC* (soil organic carbon); *STN* (soil total nitrogen); *STP* (soil total phosphorus); *SWC* (soil water content); *TOC* (tolerance); *VIF* (variance inflation factor)

Introduction

Increasing carbon dioxide (CO₂) contents and other greenhouse gas emissions into the atmosphere are becoming a crucial global threat; thus, posing serious environmental issues (Deng et al., 2017). Finding some strategies to mitigate the atmospheric concentrations of

greenhouse gases is an urgent issue (Li et al., 2019; Cuong et al., 2020). Globally, soils are the largest carbon (C) pools of the terrestrial C cycle, constituting approximately thrice the amount of C stocked in vegetation and twice the amount stocked in the atmosphere. Worldwide the upper 100 cm of soil contains 1500 Pg C (Lal, 2018). Particularly, forest soil C is a crucial component of the global C reservoir, occupying 70-73% of all soil organic C (SOC) globally (Six et al., 2002). Carbon and nitrogen (N) are essential elements for preserving soil structure, ecological function, nutrient cycling, and forest ecosystem stability (Wang et al., 2019). Soil N also plays a prominent role in C reservoirs because it interacts with C to promote ecosystem productivity and C sequestration (Ngaba et al., 2020). The dynamic change of soil N is the fundamental parameter governing C sequestration potential in forest ecosystems; for example, rising N inputs result in sustainable C sequestration (Deng et al., 2016; Wang et al., 2019). Therefore, accurate quantification of soil total N (STN) storage (SNS) and its main driving parameters in various stand ages is essential for forest sustainable management and predicting the variations of future soil C and N cycles.

Shifts in SNS after afforestation and stand age have been documented by a number of field studies. Several studies have indicated that SNS are age-independent (Sartori et al., 2007), increase with age increment (Dou et al., 2016), decrease with stand age (Smal and Olszewska, 2008; Zeng et al., 2014), or demonstrate an initial decline subsequently followed by an increase with forest stand age development (Ritter, 2007). The contradictory results distinctly reveal the necessity for mechanistic knowledge of how soil ecosystems alter with stand development in plantation ecosystems.

Acacia hybrid (*Acacia mangium* × *Acacia auriculiformis*) is a major plantation species in the Southeastern region of Vietnam due to its excellent timber, rapid growth, and high timber production. It plays a pivotal role in forestry production in Vietnam and is currently cultivated across an area of 1.5 million ha in Vietnam, accounting for approximately 32.8% of total plantation forests in the country (MARD, 2021). Apart from manufacturing wood products for industries, *Acacia* hybrid plantations contribute significantly to offering environmental services such as C and N capture and storage. Hence, a deeper knowledge of the N dynamics and its governing mechanisms in *Acacia* hybrid forest soils is of great importance for improving the C storage of soils, formulating sustainable forest management measures and strategies, and understanding their role in climate change adaptation and mitigation. Numerous researches have been conducted on biological and growth characteristics (Ngoan and Bao, 2019), wood quality (Jusoh et al., 2014), biomass and C sequestration (Hai et al., 2009), soil physicochemical features (Hung et al., 2017), biological nitrogen fixation (Dong et al., 2014) in *Acacia* hybrid forests. Information on soil N sequestration by *Acacia* hybrid plantations in Southern Vietnam, nevertheless, is limited. Thus, the study was evaluated to examine soil N in a chronosequence of five different aged *Acacia* hybrid forest stands (2-, 4-, 6-, 8-, and 10- years old) in Southern Vietnam. The particular objectives of this research were to: (i) estimate the soil total N content and storage; and (ii) ascertain the key factors controlling soil total N storage variability in *Acacia* hybrid forests over five different ages in Southern Vietnam.

Material and methods

Site characteristics

The study sites were located in Langa - Dongnai Forestry Company (11°00'00" to 11°23'00"N and 107°00'00" to 107°22'00"E), Dongnai Province, Vietnam (Fig. 1). The area belongs to a tropical monsoon climate zone that has defined rainy and dry seasons. The rainy

season extends from May to November and the dry season from November to the following April. The mean annual precipitation is 3293 mm, mostly occurring from August to October. The mean annual temperature is 25°C, and the average annual relative humidity is 85% (Ngoan et al., 2023). The elevation of the research area ranges from 80 to 93 m above sea level, with slope angles ranging from 2 to 7°. Based on the FAO-UNESCO soil classification system, the primary soil type in the research area is yellowish-brown ferralitic soil, derived from shale rocks (Sam et al., 2006). The dominant woody plants are *Tectona grandis* L.f., *Sindora siamensis* Tejasm. ex Miq. var. *siamensis*, *Dipterocarpus obtusifolius* Teijsm. ex Miq., and *Hopea odorata* Roxb. Approximately 62.4% of the total forest area is made up of plantations. *Acacia* hybrid accounts for about 33.5% of all plantations in this region and is critical in the production of pulp and timber. The study site consists of a chronosequence *Acacia* hybrid stands with ages of 2-, 4-, 6-, 8-, and 10-year-old (hereafter called AH2, AH4, AH6, AH8, and AH10, respectively). All the stands were on the second rotation and were fertilized with NPK 16-16-8 fertilizer once a year for the first three years. The initial planting density of the experimental stands was recorded as 3 m × 2 m (1667 trees ha⁻¹), and thinning operations were only carried out for the stands after 4 years with thinning intensity not exceeding 30% of the standing volume. The diversity and abundance as well as the growth of understory vegetation were well-developed, especially for the 8- and 10-year-old plantations. The predominant understorey shrub and herb plant species include *Lophatherum gracile* Brongn., *Mimosa pudica* var. *tetrandra* (Willd.) DC., *Tetracera scandens* (L.) Merr., *Chromolaena odorata* (L.) R.M. King & H. Rob., *Saccharum arundinaceum* Retz., *Mallotus apelta* (Lour.) Müll. Arg., and *Chrysopogon aciculatus* (Retz.) Trin.

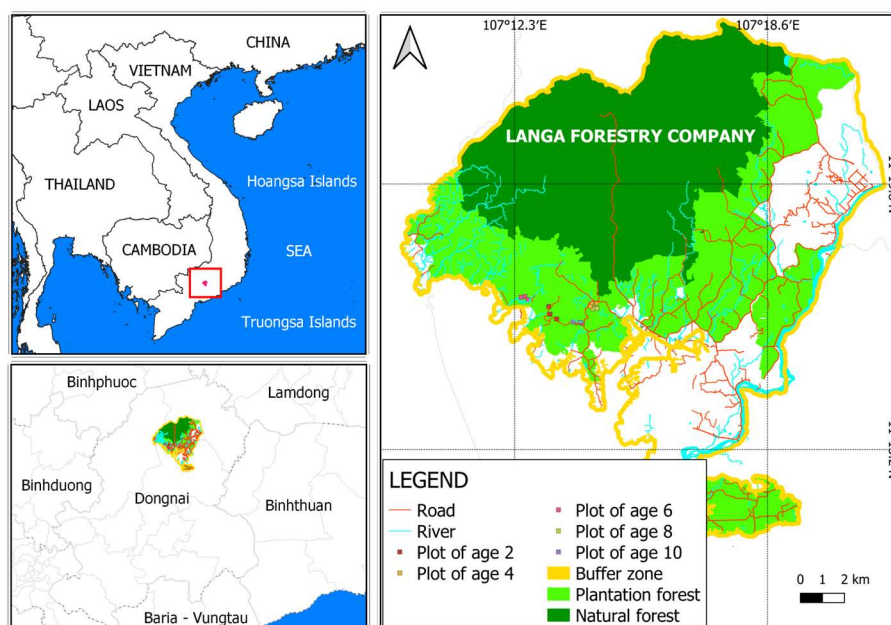


Figure 1. The location of the research sites Langa - Dongnai Forestry Company at Vietnam

Field sampling and measurements

During 10th December to 30th January in 2022, three sample plots (25 m × 20 m) were established in each *Acacia* hybrid stand (i.e., a total of 15 plots were established) (Fig. A1 in the Appendix). All five stands were no more than two km apart (Fig. 1). The tree

density, height, and stem diameter at breast height (*DBH*) of trees in the sample plots were measured. Canopy closure was estimated using a smartphone application (Gap Light Analysis Mobile Application software), and 10 points were established in each plot. More detailed information on the sampling sites is described in *Table 1*.

Table 1. Characteristics of five different aged *Acacia* hybrid forest stands

Measured variables	Stand age (years)				
	2	4	6	8	10
Average DBH (cm)	6.30 ± 0.36 ^a	12.40 ± 0.40 ^b	16.13 ± 0.32 ^c	17.80 ± 0.26 ^d	19.93 ± 0.40 ^c
Average tree Height (m)	6.17 ± 0.29 ^a	13.90 ± 0.36 ^b	17.77 ± 0.64 ^c	20.50 ± 0.50 ^d	22.63 ± 0.35 ^c
Stand density (Plants·ha ⁻¹)	1620 ± 35 ^c	1247 ± 42 ^d	980 ± 20 ^c	807 ± 12 ^b	620 ± 20 ^a
Canopy density	0.32 ± 0.02 ^a	0.55 ± 0.05 ^b	0.63 ± 0.02 ^c	0.67 ± 0.01 ^{cd}	0.69 ± 0.01 ^d
Understory vegetation biomass (Mg·ha ⁻¹)	0.86 ± 0.08 ^a	1.74 ± 0.40 ^b	2.18 ± 0.47 ^b	3.20 ± 0.29 ^c	3.52 ± 0.58 ^c
Litter biomass (Mg·ha ⁻¹)	3.08 ± 0.39 ^a	4.33 ± 0.67 ^{ab}	5.64 ± 0.56 ^b	7.33 ± 0.94 ^c	8.53 ± 1.21 ^c
Elevation (m a.s.l.)	83	86	85	95	93
Slope (°)	3	3	5	4	7

Values are means ± standard deviation (SD). Different capital letters demonstrate a significant difference between different stands ($p < 0.05$)

In each plot, the understorey biomass (including shrubs and herbs) was measured by a destructive sampling of five 2 m × 2 m quadrates. In each plot, the litter contribution was estimated by selecting five quadrates (1 m × 1 m) from each of the four corners and the plot center. All understorey biomass and litter in the samples were collected and weighed as fresh weight, taken to the laboratory, and put in a 70°C oven to dry to a constant weight. After weighing, the dry weight of understorey and litter biomass in the samples was measured, and the understorey and litter biomass amount per unit area was estimated.

Five soil pits were dug to 1-m depth from the four corners and the centre of each plot, and soil samples were collected at five depth layers (0–20, 20–40, 40–60, 60–80, and 80–100 cm) using a soil drilling sampler (5 cm diameter). Soil samples from the same depth layer in the same plot were mixed in equal volume proportions, air-dried naturally, and stored at room temperature. In total, 75 soil samples were collected (5 ages × 3 replicate plots for each age × 5 soil depths). All soil samples were sieved through a 2-mm screen before soil property analysis, and soil animals, plant roots, and other debris were removed. The soil bulk density (*SBD*) of each layer in all sampling plots was extracted using a stainless-steel cylinder with a volume of 100 cm³ of undisturbed soil samples.

Soil sample analysis

Basic soil characteristics were assessed using standard methods. *SBD* for each soil depth was measured by drying the core soil samples at 105°C for more than 24 hours until constant weight (Blake and Hartge, 1986). Soil particle size fractions, including clay content (<0.002 mm), silt content (0.02–0.002 mm), and sand content (0.02–2 mm) were measured by the pipette method (Van Reeuwijk, 2002). Soil water content (SWC, %) was

determined gravimetrically using 20 g soil samples dried in an oven at 105 °C for 24 hours (Cuong et al., 2022b). The H₂SO₄-K₂Cr₂O₇ oxidation procedure was used to measure soil organic carbon (*SOC*) concentration (Nelson and Sommers, 1982). The determination and calculation of soil total nitrogen (*STN*), total phosphorus (*STP*), and available phosphorus (*SAP*) concentrations were referred to Vietnam National Standard procedures (TCVN 6498:1999; TCVN 8940:2011; and TCVN 5256:2009) adopted by Cuong et al. (2022b). In particular, as follows: *STN* concentration was digested by a mixture of C₇H₆O₃ and H₂SO₄, and then the modified Kjeldahl procedure was used to measure *STN* content; *STP* content was tested using the colorimetrically after the sample was broken down with HClO₄ and H₂SO₄; *SAP* concentration was extracted with NaHCO₃ and measured using the molybdenum antimony colorimetric method. Soil *pH* was assessed with a pH meter at 1:2.5 soil water suspension (Cuong et al., 2022b).

Computation of soil total nitrogen storage

Soil nitrogen storage was computed from the *STN*, *SBD*, and soil horizon thickness. In the soil samples, coarse fractions (>2 mm) were very rare. Thus, the study used the following equation to compute *SNS* at each soil depth (Wang et al., 2019; Cuong et al., 2022a):

$$SNS = STN \times SBD \times SHT \times 10^{-1} \quad (\text{Eq.1})$$

where *SNS* denotes soil total nitrogen stocks (Mg N ha⁻¹); *STN* denotes the total nitrogen concentration (g kg⁻¹); *SBD* denotes the soil bulk density (g cm⁻³); and *SHT* denotes the soil layer thickness (cm).

Statistical analysis

We used the SPSS 25.0 (IBM Corp, 2017) and R version 4.2.0 (R Core Team, 2022) software programs to perform all statistical calculations. All of the data were tested for normality of distributions and homogeneity of variances before analysis. Comparisons of *STN*, *SNS*, and soil features among various stand ages and soil depths were conducted using a one-way analysis of variance (ANOVA) followed by the LSD test (*p* < 0.05). Pearson's correlations analysis was conducted to identify the relationships between *SNS* and measured environmental factors, including plant biomass features parameters (i.e., understorey vegetation biomass, and litter biomass), and soil physical (i.e., *SBD*, *SWC*, and soil texture) and chemical (i.e., *pH*, *SOC*, *STP*, and *SAP*) characteristics factors. The environmental parameters with high weighted parameter loadings were identified by minimizing the dimension of environmental parameters using Principal Component Analysis (PCA); the outcome of a possible linear correlation between variables was also eradicated (Fan et al., 2018; Cuong et al., 2022b). It should be noted that only principal components (*PCs*) having eigenvalues greater than 1 (i.e., >10% of the total variance explained) and parameters having highly weighted parameter loading (i.e., eigenvectors > ± 0.7) were retained for stepwise multiple regression analysis (*SMRA*) (Fan et al., 2018; Cuong et al., 2022b). We conducted *SMRA* using the filtered variables as inputs to figure out the significant variables that affect *SNS*. The data were all expressed as means ± standard deviation (SD) (n = 3).

Results

Soil physical properties in *Acacia* hybrid plantations

The soil textural feature parameters in the study area demonstrated that the sand content decreased while clay and silt contents increased with soil depth in stands of all ages (Fig. 2a-c). There were no statistically significant differences ($p>0.05$) in soil clay, silt, or sand fractions between different soil layers in the same forest age. AH2 showed the highest sand percentage for each soil depth ($p<0.05$), whereas AH10 had the highest percentages of clay and silt for all soil depths ($p<0.05$).

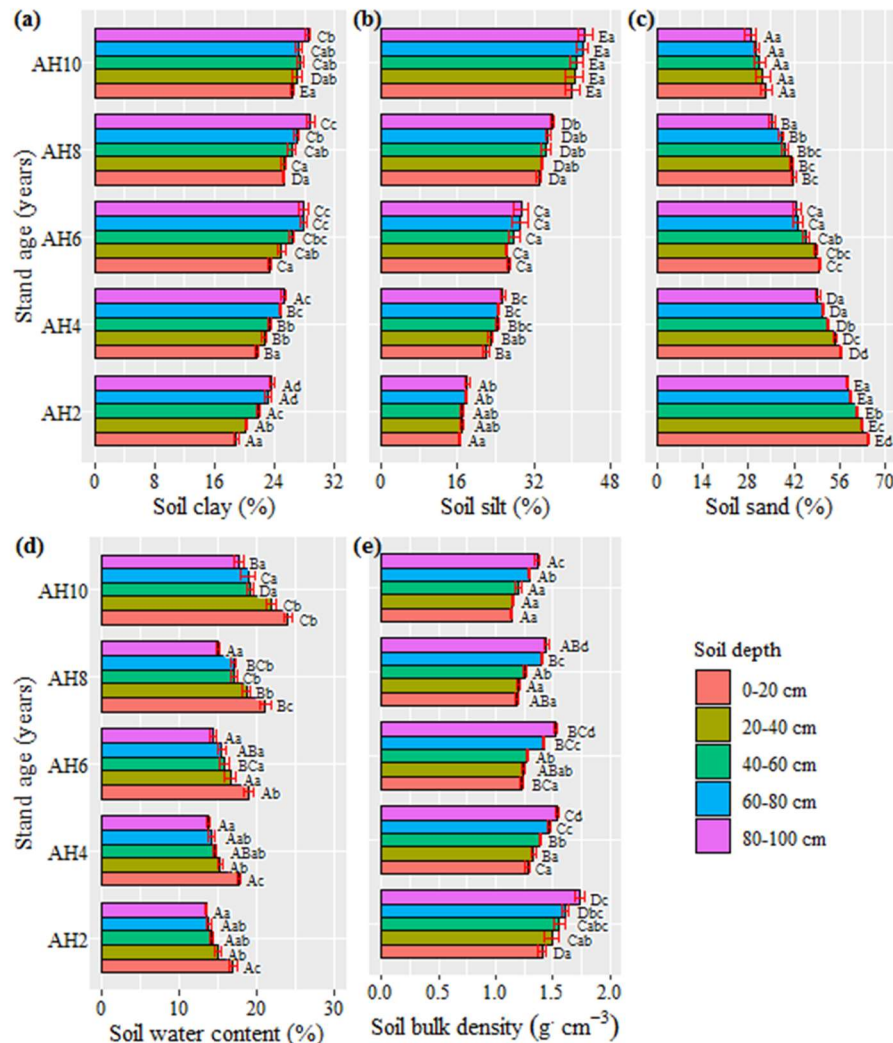


Figure 2. Soil clay (a), silt (b), sand (c), water content (d) and bulk density (d) in different soil depths *Acacia* hybrid forests. * Error bars represent standard deviation (SD). Different capital letters indicate significant differences between the same soil layers in the different stand ages ($p<0.05$). Different small letters indicate significant differences between the sampled soil layers within the same stand age ($p<0.05$)

The *SWC* was observed to decrease with increasing soil depth over the five stand ages, with the maximum found in 0–20 cm depth ($p<0.05$), ranging from 16.82% to 23.98% along forest stand ages (Fig. 2d). The *SWC* in AH10 was the highest among all stand ages

($p < 0.05$), whereas AH8, AH6, AH4, and AH2 did not differ significantly ($p > 0.05$), and this tendency was recorded at all soil depths.

The *SBD* changed in the opposite sense with both stand age and soil depth (Fig. 2e). The *SBD* value was significantly higher ($p < 0.05$) at the 80–100 cm depth than at the 0–20, 20–40, 40–60, and 60–80 cm depths for all stand ages. The *SBD* values at the AH2 were significantly greater ($p < 0.05$) than those at the other four stand ages for all soil depths. However, there was no significant distinction ($p > 0.05$) in *SBD* among stand ages (i.e., AH10, AH8, AH6, and AH4) at the five soil depths.

Soil chemical properties in Acacia hybrid plantations

Figure 3 displays the statistical comparison of soil chemical properties (soil *pH*, *SOC*, *STP*, and *SAP*) between five different aged stands at five soil depths. Soil nutrient concentrations (*SOC*, *STP*, and *SAP*) decreased with rising soil depth along the five stands, with the superficial soil depth (0–20 cm) being larger ($p < 0.05$) than in the other four deeper soil layers (20–40, 40–60, 60–80 cm, and 80–100 cm) (Figs. 3b–d). Statistically significant differences ($p < 0.05$) in soil nutrient values were found among various soil depths in the different aged stands, with the exception of *SOC* between the AH6 and AH4 at the 0–20 cm soil depth ($p > 0.05$), and the *STP* between the AH8, AH6, AH4, and AH2 at the 0–20, 20–40, 40–60 and 60–80 cm soil depths ($p > 0.05$). The concentrations of soil nutrients increased with stand age, being the highest in AH10 and the lowest in AH2, and this tendency was apparent at the five soil depths. Contrary to the patterns detected in soil nutrient contents, soil *pH* value decreased across the forest stand ages for all soil layers (Fig. 3a). Nevertheless, there was no significant variability ($p > 0.05$) in soil *pH* across forest stand ages. Soil *pH* values increased with increased soil depth for all ages. However, there was no statistically significant ($p > 0.05$) difference among different soil depths at the same forest age, except for the between soil depths 40–60 and 60–80 cm at the 2-year-old stand, and the between soil depths 0–20 and 20–40 cm, 40–60 and 60–80 cm at the 10-year-old stand ($p < 0.05$).

Soil nitrogen content in Acacia hybrid plantations

The *STN* concentration in the different soil layers increased significantly along forest stand ages ($p < 0.05$; Fig. 4a). At depths of 0–20 cm, 20–40 cm, 40–60 cm, 60–80 cm, and 80–100 cm, *STN* concentrations in AH2 were 0.81, 0.61, 0.49, 0.26, and 0.23, respectively, and *STN* concentrations in AH10 were 2.04, 1.82, 1.62, 1.38, and 1.16, respectively. The *STN* concentration of the surface soil layer (0–20 cm) was the greatest in all five stands, with values of 0.81, 1.11, 1.19, 1.81, and 2.04 g kg⁻¹, respectively (Fig. 4a). In all five stands, the results also demonstrated a significant decreasing trend of *STN* concentration with rising soil depth ($p < 0.05$).

Soil nitrogen storage in Acacia hybrid plantations

Figure 4b displays trends in the soil layer *N* stocks across the age sequence of *Acacia* hybrid stands. An apparent accumulation of *SNS* was detected ranging from 6.83 Mg ha⁻¹ in AH2 to 19.41 Mg ha⁻¹ in AH10. Soil *N* storage was significantly higher in all layers along with the age gradient ($p < 0.05$). Soil *N* storage decreased with soil layers deepen in five forest stand ages, with the maximum value measured in 0–20 cm depth, ranging from 2.26 Mg ha⁻¹ to 4.64 Mg ha⁻¹ along forest stand ages. Over 50% of *SNS* was stored at the

deepening upper soil layer of the 0-40 cm depth and the accumulation revealed decreased tendencies with increasing soil depth in the five stands.

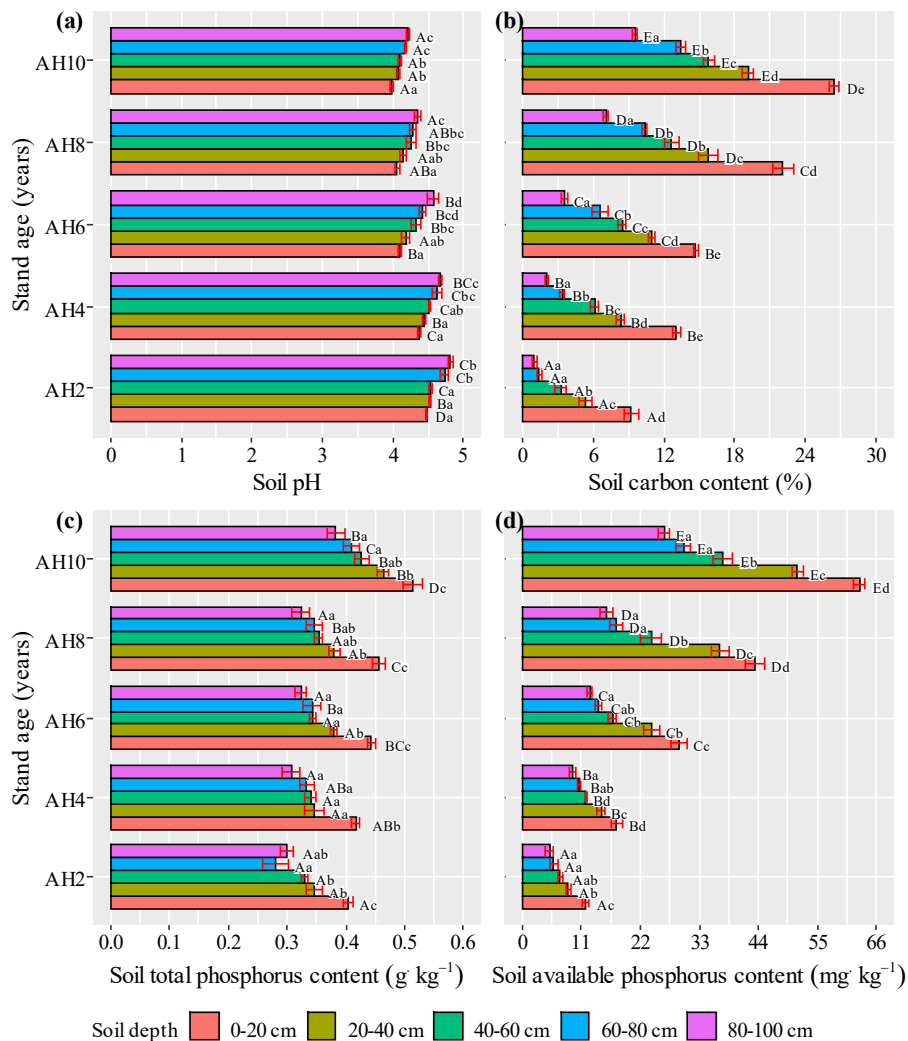


Figure 3. Soil pH (a), carbon (b), total phosphorus (c), available phosphorus concentrations in different soil depths *Acacia* hybrid forests. * Error bars represent standard deviation (SD).

Different capital letters indicate significant differences between the same soil layers in the different stand ages ($p < 0.05$). Different small letters indicate significant differences between the sampled soil layers within the same stand age ($p < 0.05$)

Effect of environmental factors on soil total nitrogen storage

The analysis of the correlation between the SNS and the numerous environmental parameters (Fig. 5). Soil total N storage substantially and positively associates with soil clay ($r = 0.43$, $p < 0.001$), silt ($r = 0.76$, $p < 0.001$), *SWC* ($r = 0.89$, $p < 0.001$), *SOC* ($r = 0.95$, $p < 0.001$), *STP* ($r = 0.79$, $p < 0.001$), *SAP* ($r = 0.96$, $p < 0.001$), but is significantly negatively associated with *SBD* ($r = -0.86$, $p < 0.001$), *pH* ($r = -0.94$, $p < 0.001$) and sand ($r = -0.72$, $p < 0.001$). Soil total N storage is also significantly positively related to biomass of plant components, including UVB ($r = 0.82$, $p < 0.001$) and litter biomass ($r = 0.85$, $p < 0.001$).

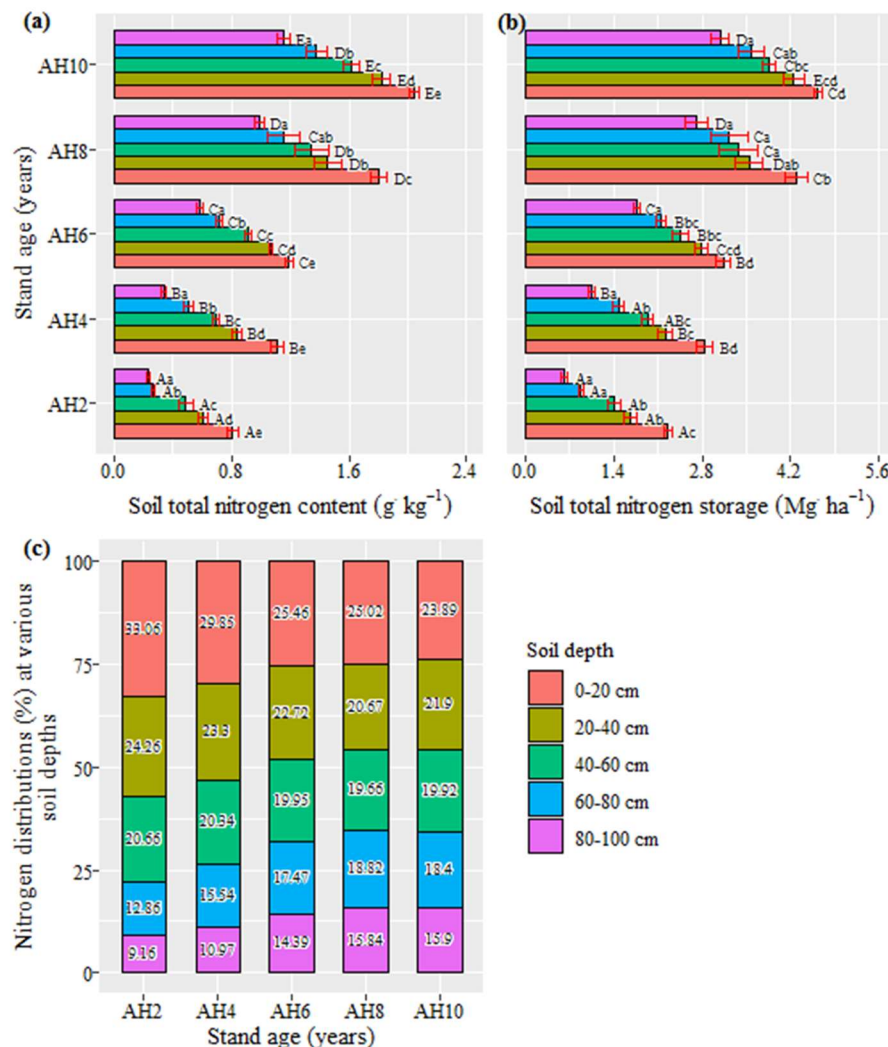


Figure 4. Soil total nitrogen concentration (a), soil total nitrogen stocks (b), and soil total nitrogen storage distribution (c) in different soil depths *Acacia* hybrid forests. * Error bars represent standard deviation (SD). Different capital letters indicate significant differences between the same soil layers in the different stand ages ($p < 0.05$). Different small letters indicate significant differences between the sampled soil layers within the same stand age ($p < 0.05$).

The PCA results illustrated two *PCs* with eigenvalue > 1 , representing 91.02% of the variance (Fig. 6). The first *PC1* explained 72.85% of the total variation, whereas the *PC* interpreted 18.18% of the variation. The varimax rotation factor loadings revealed that *SOC*, *STP*, *SWC*, *SAP*, *SBD*, and *pH* were the factors under *PC1*. However, multivariate correlation values indicated *SOC* was strongly related to *STP*, *SWC*, *SAP*, *SBD*, and *pH* (Fig. 5). Hence under *PC1*, *SOC* with 0.938 loading value was remained as the indicator parameter for *SMRA* to avoid redundancy. Under *PC2*, soil clay, silt, and sand are all significantly correlated ($p < 0.01$) with each other but only the soil clay with the highest loading value (0.937) is selected for *SMRA* (Fig. 5).

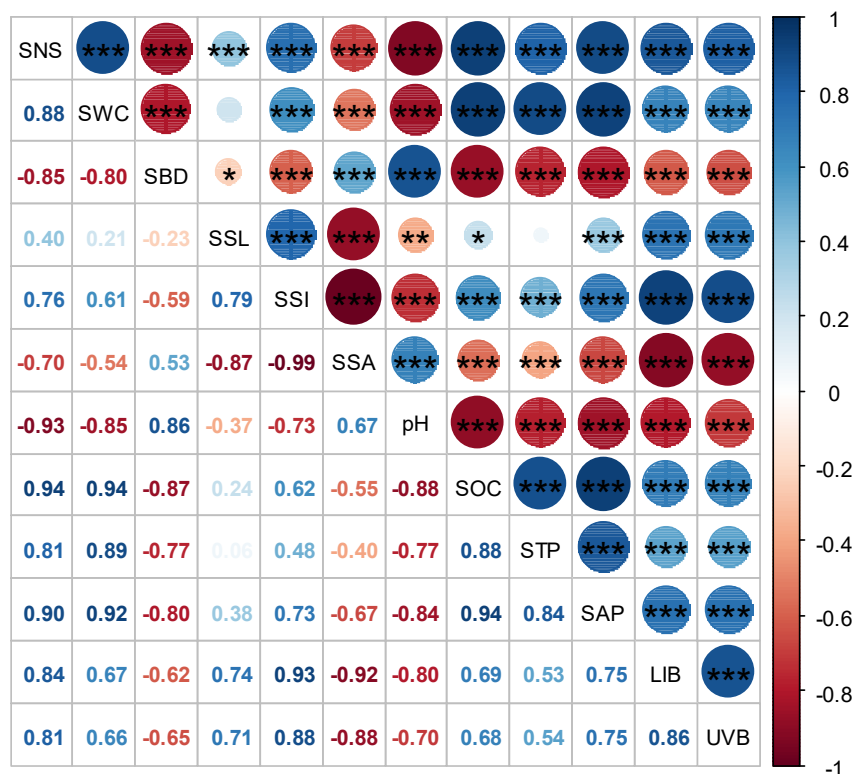


Figure 5. Correlation analyses between soil total nitrogen stocks and environmental parameters in *Acacia* hybrid forest stands. SNS, soil total nitrogen storage; SWC, soil water content; SBD, soil bulk density; SSL, soil clay; SSI, soil silt; SSA, soil sand; SOC, soil organic carbon; STP, soil total phosphorus; SAP, soil available phosphorus; UVB, understorey vegetation biomass; LIB, litter biomass. *** Correlation is significant at the 0.001 level; ** Correlation is significant at the 0.01 level; * Correlation is significant at the 0.05 level

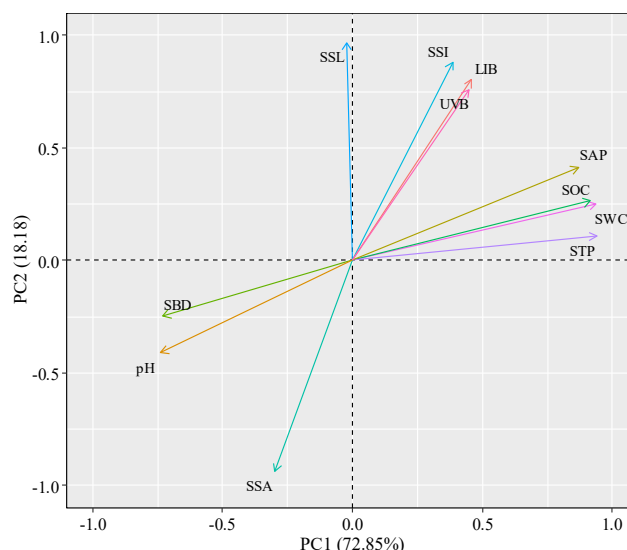


Figure 6. Principle component analysis (PCA) of the environmental parameters. * SWC, soil water content; SBD, soil bulk density; SSL, soil clay; SSI, soil silt; SSA, soil sand; SOC, soil organic carbon; STP, soil total phosphorus; SAP, soil available phosphorus; UVB, understorey vegetation biomass; LIB, litter biomass

To detect the greatest predictive parameters that impact *SNS*, we performed *SMRA* with *PCI* (*SOC*), and *PC2* (Clay) as independent parameters and *SNS* as the dependent parameter. Results by multiple linear regressions revealed that the *SOC* and soil clay were the two most predominant parameters governing *SNS* and both factors had a substantial positive influence (*Table 2*). The tolerance (*TOL*) of these 2 variables was all greater than 0.1, and the variance inflation factor (*VIF*) was all less than 10, demonstrating that there is no multicollinearity between the two environmental variables.

Table 2. Results of stepwise multiple linear regression analyses demonstrating the relationship between soil total nitrogen storage and environmental factors

Dependent variable	Explanatory variable	Coefficient estimate	SE	t-value	p-value	R ²	TOL	VIF
SNS	Constant	-0.859	0.370	-2.322	0.023	0.911	0.944	1.059
	SOC	0.153	0.006	24.725	0.000			
	Soil silt	0.077	0.015	5.122	0.000			

SNS, soil total nitrogen storage; *SOC*, soil organic carbon; SE, standard error of the coefficient estimate; TOL, tolerance; VIF, variance inflation factor. The values of TOL > 0.10 and VIF < 10 denote there is no multicollinearity to the research data

Discussion

Changes in soil total nitrogen content and storage

Soil depth is a prominent factor influencing *STN* distribution (Xu et al., 2019). Our data revealed that the *STN* concentration in the mineral soil decreased with an increase in soil depth (*Fig. 4a*). Previous findings about *STN* content with soil depth obtained were the same to our results (Zhang et al., 2018a; Wang et al., 2019; Cuong et al., 2022a; Xu et al., 2023). This could be interpreted by the decreasing inputs of soil organic matter (i.e., plant litter and roots) with increasing soil depth from both above- and below-ground litterfall (Cuong et al., 2022a; Rahman et al., 2022), particularly by the declining impact of the humus layer on the soil surface (Xu et al., 2019) and the reduction activities of soil animals and microbes (Houlton and Morford, 2014). As soil depth increases, the soil permeability and root absorption reduce, and the *SBD* increases, inhibiting the input of dissolved soil organic matter by leaching (Berger et al., 2002). This suggests that the surface soil participates most actively in N sequestration (Wang et al., 2021). The N concentration in the mineral soils significantly increased with the stands age, perhaps due to the increasing litter productivity and slow decomposition in older forest stages with the development of forest stands (Wang et al., 2019). This finding was in accordance with the previous investigations (Zhang et al., 2019; Ngaba et al., 2020; Cuong et al., 2022b). These changes could be explained by the continuous N inputs from above-ground litterfall, root exudates, and root turnover outweighing N decomposition along with the forest stand development. Our study revealed that *SNS* was significantly positively correlated with the plant biomass variables (i.e., understory biomass and litter biomass) (*Fig. 5*), demonstrating *SNS* accumulation with increased plant biomass as a result of nutrient biological cycling through forest litter production (Liu et al., 2020). Several studies have indicated that litter and roots are the two main sources of soil N input by plants, which are the foundation of biogeochemistry and nutrient cycling in forest ecosystems (Zhou et al., 2015; Cao et al., 2020). As the afforestation time increases, soil

microbial and enzymatic activities are enhanced (García de León et al., 2016), thereby advocating soil N transformation and storage. Yet, there still exists a great deal of divergences among previous research findings regarding the impacts of forest stand age on SNS (Markewitz et al., 2002; Smal and Olszewska, 2008; Mao et al., 2010; Noh et al., 2010; Li et al., 2018; Wang et al., 2019). These controversial results may result from various kinds of parameters that are interpretable for restoring SNS after afforestation, such as soil physicochemical properties features, tree species planted, site management practice, disturbances, N₂ fixation, climate, land cover change, all of which may overshadow the impact of forest stand age on SNS (Mao et al., 2010; Cuong et al., 2022a). Besides the intricate relationships among soil N storage and plantation age, in terms of the vertical distribution of SNS, more than 50% of the SNS was recorded in top 40 cm of the mineral soil horizon in all five stands (Fig. 4c), illustrating that larger amounts of SNS were stored in the topsoil layer. This suggests that, despite being sensitive to human disturbance activities, soil natural erosion, and extrinsic factors, the surface soil in the research site is the prominent N pool. Therefore, the current findings suggest that focus research on the vertical variability of SNS and preservation of N in the surface soil from human disturbances, soil natural erosion, and extrinsic factors are required in the context of N sequestration.

Main environmental factors controlling soil total nitrogen storage

Soil physical and chemical characteristics and plant biomass parameters are principal controls of SNS (Li et al., 2019; Cuong et al., 2022a). The findings from this study revealed that biomass, soil physical soil chemical features significantly affected the SNS in *Acacia* hybrid plantation ecosystems (Fig. 5), with SOC and soil texture (soil clay content) being the most crucial controlling factors of SNS among the eleven factors tested (Table 2). Some researchers have demonstrated that SOC is a major index that has substantial impacts on SNS in earlier studies because soil organic matter is a crucial N sink pool and N accumulates rapidly in soil organic matter (Lewis et al., 2014). Decomposition and accumulation of organic matter in soil are critical pathways for soil N accumulation (Nie et al., 2020), which correspond with the positive relationship found between SNS and SOC (Fig. 5). As one of the most important parameters of soil physical factors, soil texture plays a significant role in modulating SNS. In the current study, the noticed variability in SNS in stands of different ages was positively correlated to both clay and silt content, while SNS was negatively correlated to sand content (Fig. 5). Other studies have revealed that SNS was highly positively linked to the clay and silt contents of soils (e.g., Hassink, 1994; Zhou et al., 2019) because clay and silt particle fraction and micro-aggregates help to absorb soil organic matter and protect it from soil microbial degradation in an anaerobic environment to varying degrees (Zhou et al., 2019). Furthermore, soil mechanical constitution could also indirectly impact SNS by regulating soil particle agglomeration, hydrology, aeration, water retention, and temperature (Saxton and Rawls, 2006). Additionally, other soil characteristics parameters (e.g., soil P, SWC, SBD, and soil pH) were the major environmental factors with high factor loadings, which may be used to explain SNS variability to some extent (Fig. 6). Our results revealed that SNS was significantly positively related to soil P concentrations (STP and SAP concentrations) (Fig. 5), demonstrating the significant role of soil nutrients in affecting the change in SNS at a local scale (Sam et al., 2006; Liu et al., 2012). Previous investigations discovered that soil P limitation might constrain N accumulation, where the connection between phosphorus and iron or aluminum oxides alleviates P availability

for microbial growth and activities (Cleveland et al., 2002; Liu et al., 2012). *Acacia* hybrid is a leguminous plant species with a high symbiotic biological N fixing capacity due to its symbiotic association with nodule-forming bacteria (Cuong et al., 2022a), and it is likely to maintain the N input sources in our areas. Meanwhile, it has been reported in earlier studies that P deficiency in the soil might affect symbiotic N₂ fixation activity (Augusto et al., 2013), ultimately affecting *SNS*. Schleuss et al. (2019) illustrated that the increase in *STP* was beneficial to the acquisition of P by plants, which can increase plant productivity and therefore strengthen the input sources of *STN*. Soil water content is another dominant soil physical indicator that has had significant effects on *SNS* because it can strongly adjust soil organic matter decomposition via soil microbial biological activity. Our findings indicated that *SWC* had a considerable positive impact on the *SNS* (Fig. 5), which is congruent with the findings from prior reports (Zhang et al., 2018c; Duan et al., 2020). That may be due to *SWC* may alter during stand development, which in turn leads to the improvement of soil biological activity (microbial biomass and enzyme activities) linked with nutrient decomposition and cycling (Zhang et al., 2019), thereby enhancing soil N transformation and storage. For example, increasing soil microorganisms and enzymes can stimulate the decomposition of soil organic (Zhang et al., 2018b). Furthermore, exogenous N derived from plant decomposition and atmospheric deposition can also be fixed in the soil by microbes (Ren et al., 2017). The *SNS* at the study site was also significantly affected by *SBD*. Our findings showed that *SBD* was significantly negatively correlated with *SNS* (Fig. 5). Previous researchers demonstrated that soils with lower *BD* accumulated more *STN* concentrations because it can be mobilized via the pores inside the soil constitution. Soils with low *BD* have more macro-pores and macro-aggregates, which can stimulate soil microbe activity, tree root development, and below-ground biological activities, consequently enhance the formation of organic matter and N content in soils (Duan et al., 2020). Soil *pH* is regularly referred to as a critical environmental element that governs the distribution of the soil microbial community involved in N cycling (Lauber et al., 2009). Our study also discovered a strong negative association between soil *pH* and *SNS* (Fig. 5). Similar findings were reported by several earlier reports (Kemmitt et al., 2006; Duan et al., 2020). Studies have pointed out that soil acidification has probable to slow soil organic matter turnover in some acid soils by changing the composition of microorganisms, hence conducive to the accumulation of *SNS* (Schmidt, 1982; Wang et al., 2018). Additionally, alternative N sources and processes may also have an influence on *SNS*. For example, atmospheric N deposition (Mao et al., 2010), and biological N fixation (Li et al., 2019) are frequently used to clarify *SNS* increase during the development process of forest. Furthermore, the stand characteristic features (e.g., stand density, canopy closure, *DBH*, and *H*) may assist in elucidating the alteration in *SNS* across various age forests (Table 1).

Conclusions

The current study revealed the storage and dynamics of soil N in differently aged *Acacia* hybrid plantations in Southern Vietnam. Soil total N concentration significantly increased with the increase in stand ages, and considerably reduced with increased depth of soil layers across all stand ages. The mineral soil stocks of total N exhibited continuously increasing trends across stand development, and the upper 0–40 cm soil depth held more than 50% of the total soil N stocks at five stand ages. Soil organic C concentration and soil particle size fractions (i.e., soil silt concentration) were the two

most important environmental parameters modulating the *SNS*, and both parameters had a positive impact. Results from this study revealed that *Acacia* hybrid plantations had considerable soil N sequestration potential during stand development. The findings highlighted the significance of stand age in forest soil N storage assessment for policymakers when modelling global climate change scenarios.

Acknowledgments. The authors would like to thank the Langa-Dongnai Forestry Company for offering critically important information on the study area and allowing us to access the plantations for this work. We also acknowledge Dr. Nguyen Thanh Tuan for his invaluable comments and suggestions on improving our manuscript.

Funding. This research project received funding from the Fundamental Scientific Research Foundation of Vietnam National University of Forestry – Southern Campus (No. 43/QD/PHDHLN-KHCN&HTQT).

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APPENDIX

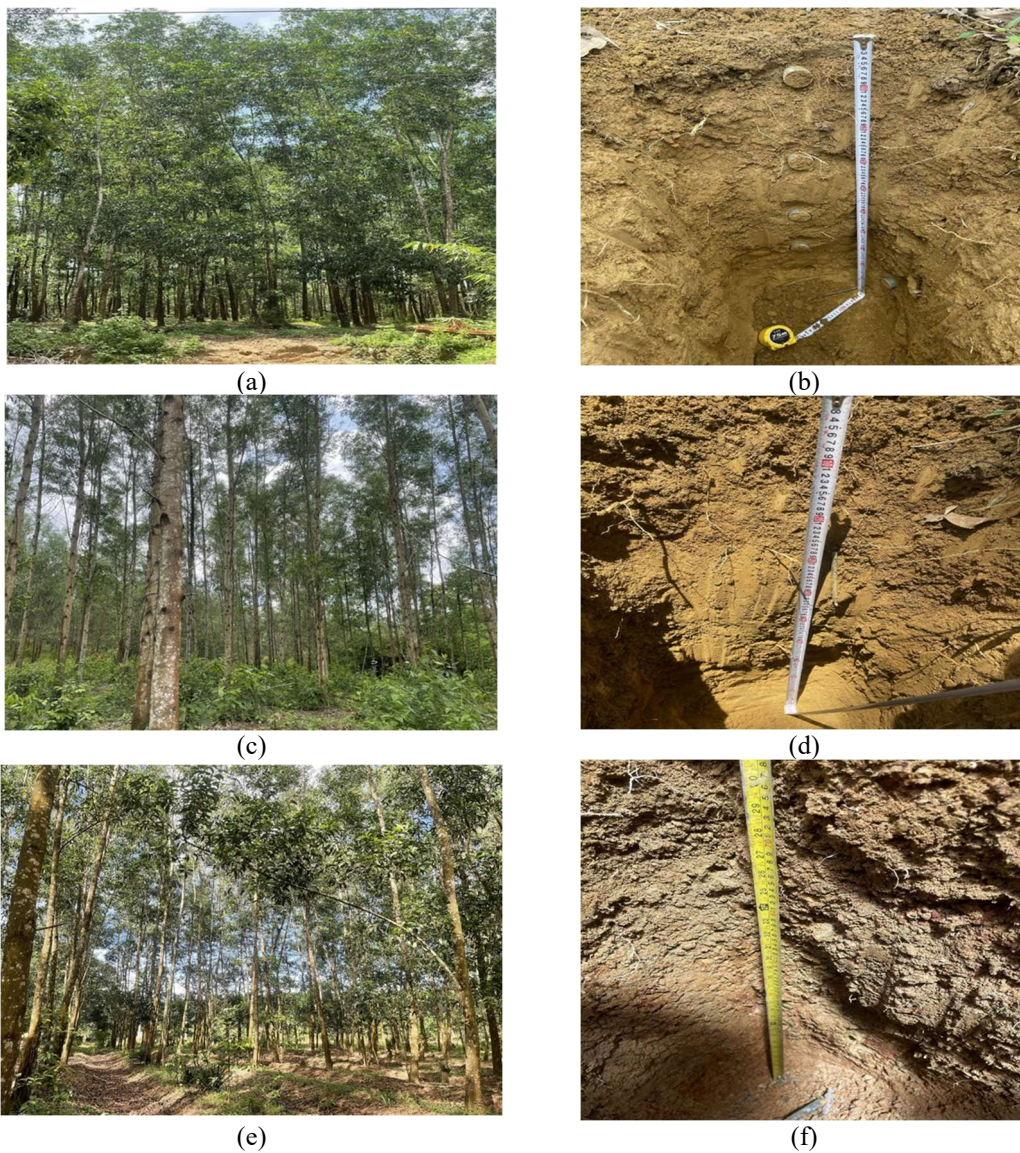


Figure A1. Some pictures of the 4-year-old (a-b), 6-year-old (c-d), and 8-year-old (e-f) *Acacia* hybrid stands