



Synergistic Carbon-Biodiversity Gains in Diversified Rubber Agroforestry Systems, Southern Thailand

Uraiwan Tongkaemkaew^{1*}, Suchawadee Bowchaay¹, Benchawan Bua-Khwan², Kamolpatara Kasikam³, Robbe Verhofste⁴, Michael Commons⁴, Chooi Lin Phooi⁵, Elisa Azura Azman⁵, and Roslan Ismail⁵

¹ Faculty of Agriculture, Thaksin University, Phatthalung, 93210, Thailand; <https://orcid.org/0000-0002-6490-0337>

² The Institute for Southern Thai Studies, Thaksin University, Songkhla, 90000, Thailand

³ Wanakaset Symbiosis Network, Thailand

⁴ Terra Genesis International PBC, Freeville, New York 13068 USA

⁵ Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Malaysia

* Correspondence: uraiwan@tsu.ac.th

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Abstract: Rubber plantations cover millions of hectares across Southeast Asia, yet their capacity to deliver both agricultural production and ecological benefits depends heavily on how they are managed. This study compared six rubber management systems in southern Thailand, a conventional monoculture (RM) and five progressively diversified rubber-forest systems (RF1 to RF5), to quantify how increasing structural complexity affects biodiversity, soil health, and carbon storage. Field inventories documented tree species composition, natural regeneration, and deadwood; soil samples characterized organic carbon, nitrogen, and nutrient status; and aboveground biomass and carbon stocks were estimated using the TGO national carbon accounting tool. The most diversified system, RF5, supported 6.4 times more tree species than monoculture (205 vs. 32 species ha⁻¹) and three times the seedling regeneration (7,725 vs. 2,471 seedlings ha⁻¹). Soil organic carbon nearly doubled (from 1.44% to 2.49%), and total nitrogen increased significantly. Total non-rubber aboveground biomass rose nearly fourfold (from 622.64 to 2,348.57 kg ha⁻¹), and the associated carbon stock reached 4.04 tCO₂e ha⁻¹ compared to 1.20 tCO₂e ha⁻¹ in monoculture. These values represent only the diversified vegetation component; whole-system carbon stocks, including the rubber trees, are substantially higher. These gains moved together across the management gradient rather than independently, with soil fertility, biodiversity, and carbon accumulation reinforcing one another. Although diversified intensity and stand age co-vary in this cross-sectional design, the consistent coupling of ecological indicators across the gradient indicates that structural complexity is a primary organizing factor. Diversified rubber agroforestry systems offer a practical pathway for tropical smallholders to improve ecological function and contribute to climate mitigation.

Keywords: Rubber agroforestry; carbon stocks; conservation biodiversity; carbon sequestration; ecological indicators

1. Introduction

Carbon moves continuously between the atmosphere, living biomass, soil, and oceans. Of these pools, the atmosphere holds a relatively small fraction, CO₂, which represents just 0.04% of the gaseous mixture, but it is the pool from which photosynthetic organisms draw the carbon that builds plant tissue, feeds soil organisms, and drives terrestrial food webs [1]. Plant biomass accumulates this

carbon during growth, then returns it to the atmosphere through respiration, decomposition, and fire [2]. Forests are among the most important sites of carbon accumulation on land. Their biomass spans living trees, understory vegetation, deadwood, litter, and soil organic matter, each representing a distinct pool with different residence times and vulnerabilities [3]. Aboveground biomass is particularly exposed: deforestation and land degradation can release decades of stored carbon within years, making accurate biomass quantification essential for understanding carbon fluxes and designing effective climate policy [1, 3]. Belowground, soil organic matter holds carbon in forms that are both stable and biologically active. It regulates nutrient availability, builds cation exchange capacity, and shapes the physical structure on which plant roots depend [4].

Across the humid tropics of Southeast Asia, rubber plantations (*Hevea brasiliensis*) have become a linchpin of both livelihoods and regional carbon dynamics. Thailand, the world's top natural rubber producer, has turned tracts of land into rubber monoculture estates. While this boom fuels the economy, it also sets off alarm bells over shrinking biodiversity, deteriorating soils, and the overall resilience of ecosystems [5]. Empirical carbon stock data for rubber systems in Thailand are limited in the peer-reviewed literature, though national accounting frameworks, such as the TGO carbon sequestration tool, provide standardized approaches for biomass estimation. Empirical evidence from previous studies indicates that rubber plantations can store substantial amounts of carbon, with 14-year-old stands reported to contain approximately 135-153 tC ha⁻¹ [6]. These findings highlight the potential of rubber systems as significant carbon sinks, although carbon storage varies with stand age, tree density, and management practices. Integrating agroforestry into rubber farms is emerging as a strategy for sustainable intensification. By combining rubber trees with timber, fruit-bearing species, and/or shade-tolerant understory crops, growers can improve soil health, reduce income volatility, and diversify revenue streams [7]. Ground data from Thailand reveal that rubber-centric agroforestry arrangements (RAS) not only cushion farmers against the ebb and flow of market prices but also reinforce key ecological functions. Smallholders who embraced rubber-fruit-timber agroforestry configurations, in Phatthalung Province, enjoyed livelihood stability and extracted resources more judiciously than their peers who stuck to monoculture [8]. In a similar vein, it has been demonstrated that rubber agroforestry not only bolsters biodiversity but also enhances ecosystem services compared with conventional plantation practices [9]. RAS systems, integrating multiple species or perennial crops, achieved financial outcomes equivalent to or superior to conventional farming systems by reducing economic risk and increasing land productivity [10]. The Chinese study demonstrates that rubber forest systems improve soil health, accelerate nutrient recycling and carbon storage, and reduce atmospheric greenhouse gas emissions. Taken together, the results underscore that a diversified strategy can align carbon, biodiversity, and livelihood goals across the rubber belt [11].

Even though the biophysical and socioeconomic case for RAS is unmistakably strong, its spread remains hampered by a mix of limited support, fragile policy frameworks, and a lack of financial incentives. Many farmers still cannot access demonstration plots, receive extension guidance, or obtain credit schemes, which slows the shift toward rubber systems. To scale RAS adoption, policy measures such as embedding carbon-credit mechanisms, offering agroforestry subsidies, and delivering farmer training programs are essential. The study hypothesizes that increasing structural complexity in rubber agroecosystems generates coupled gain in aboveground biomass, species diversity, and soil carbon, rather than producing trade-offs among these outcomes [12]. This study quantifies aboveground biomass and carbon stocks across six rubber management systems, from monoculture (RM) to progressively diversified forest-garden systems (RF1 through RF5), and tests whether carbon storage, biodiversity, and soil fertility gains are coupled or independent across this management gradient [13].

2. Materials and Methods

2.1 Study Site

The research was carried out in Thailand, spanning three districts: Thepha (Songkhla Province) and Tamot and Sri Banpot (Phatthalung Province) (Figure 1). These locations were deliberately chosen to showcase a spectrum of rubber agroforestry development stages, each overseen by farmer groups linked to the Regenerative Rubber Alliance. The participating farmers work hand in hand with Thaksin University and

Terra Genesis International, which supply long-term data and grant field access. The climate is humid tropical, with mean annual rainfall of 1,900-2,200 mm and mean annual temperature of 27-29 °C. Soils are predominantly Ultisols and Acrisols derived from sandstone and shale parent materials. They are commonly used for cultivating crops across the area.



Figure 1. Sri Banpot, Tamot, and Thepa: rubber farming communities in Thailand's southern Phattalung and Songkhla provinces.

2.2 Experiment Design

To ensure transparency in treatment classification, the six management levels (RM, RF1–RF5) were defined using a structured agroforestry complexity gradient that integrates vegetation composition, vertical stratification, and time since diversification. Specifically, RM (Level 0) represents rubber monoculture with a single canopy layer and minimal understory vegetation. In contrast, RF1–RF5 represent progressively diversified rubber-based systems characterized by increasing plant species richness, multi-layered canopy structure (ground cover, shrubs, and timber trees), and longer durations of agroforestry establishment. The classification criteria combined (i) number of plant species, (ii) presence of vertical canopy strata (as illustrated in Figure 2A), (iii) proportion of non-rubber vegetation, and (iv) years since diversification to agroforestry. Tree structural attributes, including height, were measured using hypsometric techniques based on horizontal distance and vertical angle measurements across varying terrain conditions (Figure 2B and 3a-e). These attributes were synthesized into an ordinal biodiversity score ranging from 0 to 5, where higher values indicate greater structural and composition complexity, following an established agroforestry classification framework [5, 13]. To minimize pseudo-replication and ensure statistical independence, replicate plots were separated by at least 250 m within each district, consistent with ecological field sampling recommendations for landscape-level studies. Each 400 m² plot (10 x 40 m) was georeferenced using GPS, and no two plots shared overlapping environmental conditions at the microsite level. Replicates were distributed across heterogeneous microhabitats within the district to capture within-treatment variability while maintaining independence among sampling units (Figure 3).

2.3 Data Collection and Analysis

2.3.1 Soil Sampling and Analyzing

To keep edge effects from biasing the data, a 2 m buffer was left around each plot (Figure 3f). Within each plot, fifteen random points were chosen using XY coordinates, and composite soil samples from the 0-15 cm layer were collected and processed according to protocols. Soil organic carbon (SOC) and organic matter (OM) were quantified with the Walkley method, while total nitrogen (N) was measured via Kjeldahl digestion and distillation. Available phosphorus was determined using the Bray II extraction method. The exchangeable cations potassium, calcium, and magnesium were quantified via the ammonium acetate (NH₄OAc) method.

The analyses were carried out at the Soil and Residue Analysis Laboratory, Faculty of Technology and Community Development, Thaksin University, and the Central Analytical Laboratory, Prince of Songkla University. Soil carbon data are therefore reported as concentrations (%OC) rather than as areal stocks (Mg C ha^{-1}). This limits direct comparison with carbon accounting benchmarks that require mass-per-area estimates, though the treatment comparisons remain valid as relative differences.

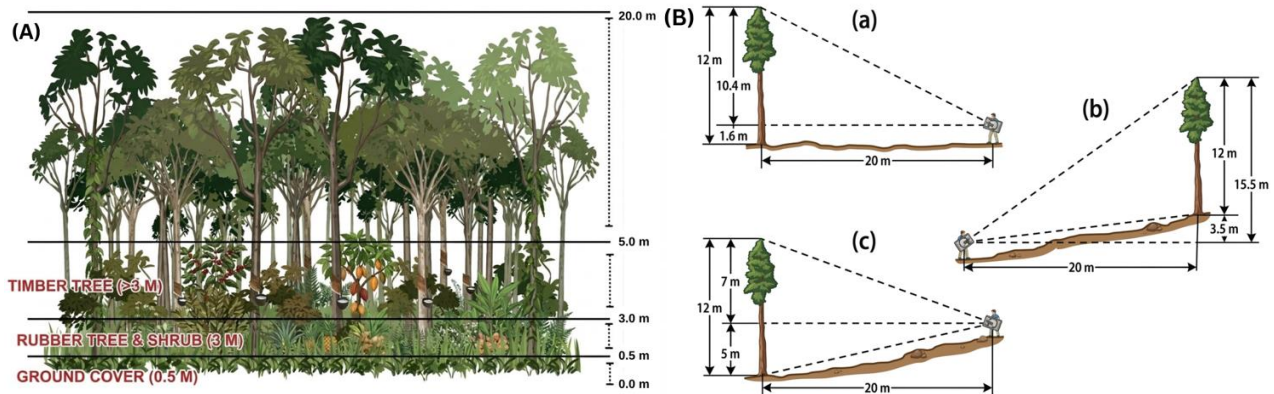


Figure 2. The diagram illustrates the vertical stratification of an agroecosystem, categorized by height (A); Hypsometry techniques used to calculate total tree height using horizontal distance and vertical angle (B); (a) level ground, (b) sloping ground (observer below), and (c) sloping ground (observer above).

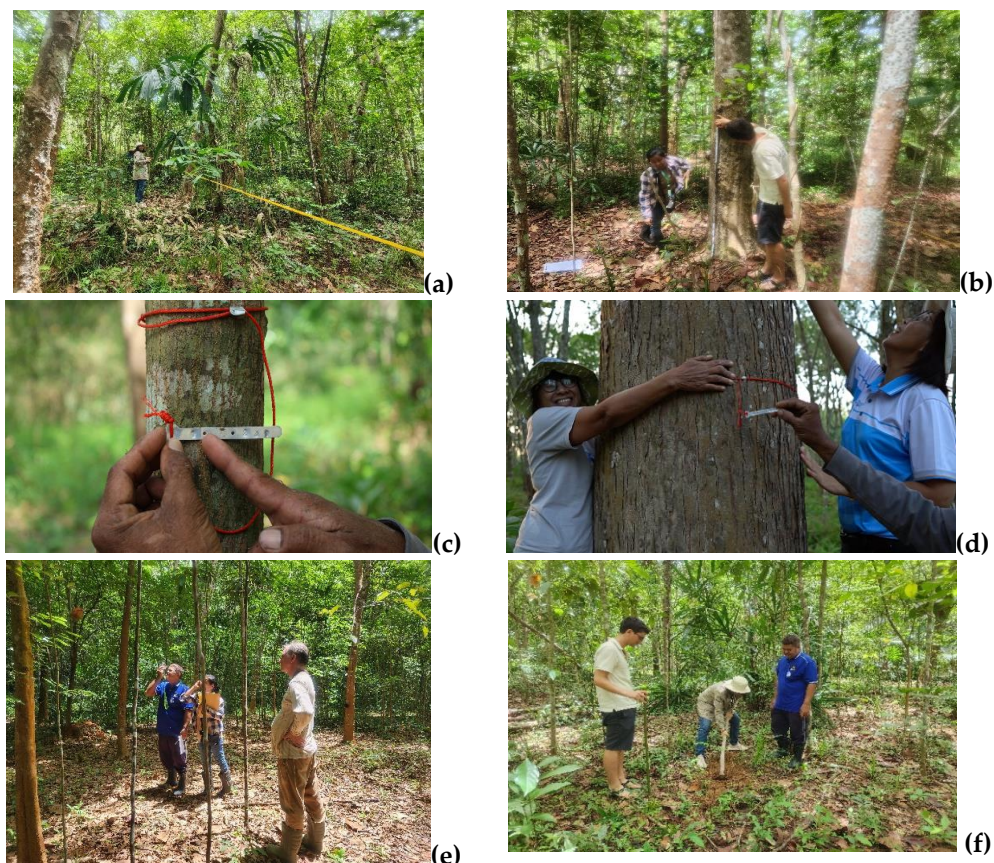


Figure 3. Field measurement and sampling of rubber trees for biomass and carbon assessment. (a) Plot establishment and tree tagging within 10 × 40 m sampling plots; (b) Tree height measurement using a clinometer; (c) Tree identification and tagging; (d) Girth at breast height (GBH) measurement at 1.3 m above ground; (e) Diameter at breast height (DBH) measurement using a diameter tape; and (f) Soil and root sampling for belowground biomass estimation.

2.3.2 Estimating Plant Community Structure

Vegetation was divided into four layers: ground cover, shrub, canopy, and emergent. Within each 400 m² plot, every plant species was identified, counted, and assigned to a growth form category: tree, shrub, vine, epiphyte, or ground cover. Species density was expressed as the number of individuals per plot, and any specimens that could not be identified on the spot were photographed for expert verification. The tally of deadwood, both stems and downed timber, was recorded using the same diameter class intervals applied to live trees, then extrapolated to a per-hectare figure by applying a factor of twenty-five.

2.3.3 Estimating Biomass

Girth at breast height (GBH) was measured at 1.3 m above ground for all trees and shrubs with a stem circumference of 15 cm or greater, from which DBH was calculated by dividing by π . Tree height (H) was measured using a Suunto PM-5/1520 hypsometer (Figure 2B). Aboveground biomass was estimated using the carbon sequestration calculation tool developed by the TGO (T-VER-TOOL (HOL-FOR/AGR-01) Version 01) [14]. Field measurements, including DBH and H, were used as input parameters for the model to estimate biomass components (stems, branches, and leaves) using standardized allometric relationships appropriate for tropical forests and agroforestry systems in Thailand.

2.3.4 Estimating Carbon Stock

Aboveground carbon stock (AGC) was estimated using the same carbon sequestration calculation tool developed by TGO (T-VER-TOOL (HOL-FOR/AGR-01) Version 01) [14]. The aboveground biomass (AGB) derived from field measurements (DBH and H) was used as input to estimate carbon stocks using standardized biomass-to-carbon conversion procedures embedded in the TGO model. This approach ensures methodological consistency between biomass and carbon estimation and aligns with national carbon accounting practices for tropical forest and agroforestry systems in Thailand.

2.4 Statistical Analysis

Descriptive statistics, mean, deviation, minimum, and maximum were tallied for every variable, including DBH, height (H), stem weight (SW), branch weight (BW), leaf weight (LW), total tree biomass (WT), below ground biomass, total biomass, carbon content, and carbon stock. To probe differences among the six agroforestry levels, a one-way ANOVA was conducted in SAS v9.0, treating agroforestry treatment as a fixed factor and location as a random factor. When the results crossed the significance thresholds ($p < 0.05$, $p < 0.001$), we used Tukey's Honest Significant Difference (HSD) test for post hoc comparisons. Correlation and regression models were then run to tease the links between traits and carbon metrics, such as DBH versus biomass or height versus carbon stock. Finally, Principal Component Analysis (PCA) was employed to uncover multivariate patterns linking soil fertility, vegetation attributes, and carbon accumulation across the systems.

3. Results and Discussion

3.1 Plant Species and Density

The chronologies of intercropping across the systems show a mix of start dates and lengths (Figure 4). Both RF5 and RF4 were launched in 1999; RF5 persisted until 2019, whereas RF4 was discontinued in 2014. The longest stretch belongs to RF2, which ran from 2002 through 2023. RF3 entered the scene in 2007. RF1 covered the interval from 2012 to 2022. These contrasts in age and duration matter for interpreting outcomes because a longer diversification window tends to amplify litter inputs, strengthen stratification, and boost recruitment. Rubber tree density was essentially the same across the systems ($p = 0.066$), implying comparable stand occupancy. By contrast, the large-tree layer with DBH > 15 cm and height over 3 m responded dramatically to diversification: both abundance and species richness surged along the RF gradient ($p < 0.001$) with RF5 occupying the highest Tukey group and RM the lowest (Table 1). Trees of size (DBH > 15 cm, height 1.3-3 m) showed no difference ($p > 0.10$). Small trees (DBH < 15 cm) grew more species-rich as diversification rose ($p < 0.05$, $p < 0.01$). The RF4-RF5 treatments consistently outperformed both the RM and the earlier RF stages. The understory mirrored this pattern: other plants and seedlings were both more abundant and more diverse in the RF systems ($p < 0.05$, $p < 0.01$), with RF4-RF5 leading the pack (Table 2). Dead-tree abundance also varied significantly ($p < 0.05$), with the highest count recorded in RF5, suggesting that the structurally

complex stands experience stronger self-thinning and turnover than a simple chronic die-back. Elevated deadwood in RF5 is consistent with the self-regulatory dynamics of structurally complex systems, in which competitive sorting channels resources toward the most viable individuals while returning biomass to decomposition pathways and the soil carbon pool. This functional turnover distinguishes diversified systems from monocultures, where deadwood more commonly reflects pathological decline. Overall, the diverse rubber forest gardens (RF5) showed the greatest vertical stratification, the richest understory diversity, and the strongest regeneration potential, reinforcing earlier findings that diversified rubber systems boost biodiversity, improve soil fertility, and increase ecosystem resilience [15, 16].

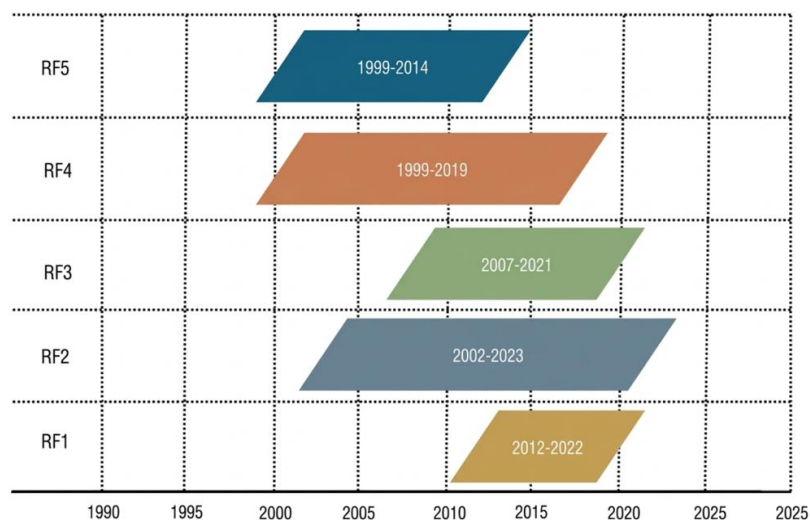


Figure 4. Observed period of intercropping for rubber agroforestry system (RF1-RF5), indicating start and end years of secondary crop/ species integration.

3.2 Current Soil Nutrient Status

Soil chemistry shifted among the systems (Tables 3 and 4). Total nitrogen varied significantly ($p < 0.01$), peaking in RF5 ($0.203 \pm 0.040\%$) compared to $0.123 \pm 0.015\%$ in RM, a 65% increase consistent with intensified nitrogen cycling under more diverse canopies with greater litterfall diversity and root turnover. The implied carbon-to-nitrogen ratio for both RF5 and RM is approximately 12, which falls within the expected range for tropical systems with active decomposition and indicates that carbon and nitrogen inputs scaled proportionally across the management gradient. Total and available phosphorus did not differ among systems ($p > 0.05$), and exchangeable potassium, calcium, and magnesium were broadly comparable, aside from a notably higher magnesium concentration in RF4.

Soil organic matter (%OM) and soil organic carbon (%OC) differed significantly among the rubber systems ($p < 0.05$). Both parameters followed a similar upward trend with increasing system diversity; RF5 exhibited the highest values ($3.87 \pm 0.05\%$ for OM and $2.49 \pm 0.30\%$ for OC), which were significantly greater than those observed in the RM system ($2.22 \pm 0.55\%$ and $1.44 \pm 0.50\%$, respectively). Although LOI (Loss on Ignition) captures the full spectrum of organic compounds, including recalcitrant structural residues, and the Walkley-Black method primarily recovers the readily oxidizable fraction, both methods demonstrated a significant positive response. This indicates that highly diversified litter inputs effectively enhance both the labile carbon pool and the accumulation of broader, decomposition-resistant organic compounds across the treatments.

Cation exchange capacity peaked in the RF3-RF4 range (4.36-4.94 meq per 100 g) but was lower in RF5 (3.48 meq per 100 g), a non-monotonic pattern that likely reflects soil type differences across the three study districts rather than a direct management effect. Soil pH remained stable across all systems (4.73-4.92; $p > 0.05$). Electrical conductivity was significantly higher in RF4 and RF5 (2.53-2.59 mS cm^{-1} ; $p < 0.05$), consistent with greater soluble nutrient availability driven by higher organic matter inputs and litterfall mineralization.

The overall pattern of elevated %OM, increased total nitrogen, and higher electrical conductivity under the more diversified systems is consistent with the expected agroforestry signature of amplified litterfall, active root turnover, and reduced soil disturbance [16, 17]. The PCA results show that soil fertility indicators (%OM, CEC) and carbon stock load together on the same principal components (Figure 5), consistent with reports of soil-carbon coupling under diversified systems in Southeast Asia [18]. The PCA, however, groups samples primarily by study site rather than by management system, so these associations reflect site-level co-variation rather than a confirmed treatment effect. The treatment-level evidence for management effects on individual soil variables comes from the ANOVA results, not from the PCA.

Soil samples were collected from the 0-15 cm depth, which captures the biologically active surface layer and is standard for soil health assessment. For carbon stock comparisons against regional and global benchmarks, however, 0-30 cm is the more common reference depth. The values reported here, therefore, likely underestimate total soil carbon stocks across all systems. More importantly, the treatment differences observed at the surface may not fully represent what is happening deeper in the profile. In RF4 and RF5, where fruit trees and timber species contribute diverse root architectures at depth, carbon inputs from root turnover and rhizodeposition likely extend well below 15 cm. This means the soil carbon advantage of more diversified systems may be greater than surface measurements alone suggest. Future studies should sample to at least 30 cm, and ideally to 60 cm in systems with deep-rooted species, to provide a more complete picture of soil carbon stock differences across management gradients.

3.3 Growth characteristics, biomass components, and carbon stocks

Tree growth characteristics, biomass components, and carbon stocks varied significantly across rubber management systems (RM and RF1-RF5), demonstrating strong effects of system diversification on ecosystem structure and function (Table 5). Trees in highly diversified systems (RF3-RF5) exhibited significantly greater height, GBH, and DBH than those in monoculture and low-diversity systems (RM-RF2). Tree height increased from 3.68 ± 0.12 m in RM to 10.45 ± 5.52 m in RF5 ($p = 0.0025$; Table 5), reflecting enhanced vertical stratification and improved light interception in a structurally complex system. This pattern is consistent with ecological theory, which holds that multi-layered canopies promote more efficient resource capture and niche complementarity, ultimately enhancing stand-level productivity [19, 20].

Biomass accumulation increased markedly along the diversification gradient. Total aboveground biomass rose from 524.33 ± 55.52 kg ha⁻¹ in RM to 1920.22 ± 75.20 kg ha⁻¹ in RF5 ($p < 0.0001$; Table 5), while total biomass (including belowground components) increased nearly fourfold, reaching 2348.57 ± 66.67 kg ha⁻¹ in RF5. This pattern is consistent with the role of species diversity and structural heterogeneity in amplifying biomass production. Notably, the simultaneous increase in biodiversity, soil fertility, and carbon storage across the gradient indicates that these are not independent responses to diversification but coupled outcomes of increasing ecological organization. Systems with greater structural complexity appear to create conditions, including diversified litter inputs, complementary root architectures, and reduced competitive overlap, that simultaneously reinforce carbon accumulation and biological diversity. Mechanistically, such increases are likely driven by complementary resource use (e.g., light, water, and nutrients) and facilitative interaction among species, which reduces competitive overlap compared to monoculture systems [21, 22]. It should be noted, however, that the six systems in this study differ simultaneously in species composition, canopy structure, and the number of years since diversification. Systems RF4 and RF5 have been diversified since 1999, giving them over two decades of additional litterfall, root turnover, and canopy development compared to RF1, which was established only in 2012. The observed biomass and carbon differences, therefore, reflect both the effects of diversification and time, and these two influences cannot be fully separated in this cross-sectional design. Longitudinal monitoring of the same plots over time would be needed to isolate the contribution of diversification independent of stand age. It is also important to note that the carbon stocks reported here represent only aboveground live biomass, as estimated using the TGO tool. Soil organic carbon, deadwood, litter, and fine root carbon pools are not included in these figures. This accounts for the difference between the values reported here and published whole-system carbon stocks for mature rubber plantations, which typically include multiple pools and report substantially higher totals. Comparisons across studies should account for these methodological differences in the inclusion criteria for the pool.

Table 1. Structural composition of rubber trees and associated tree categories under different rubber systems (per hectare).

Rubber System	Rubber trees	Big trees (>15 cm DBH, H>3 m)		Medium trees (>15 cm DBH, H 1.3-3 m)		Small trees (<15 cm DBH)	
		No. of tree	No. of Species	No. of tree	No. of Species	No. of tree	No. of Species
RM	1536.42 ± 496.47	140.27 ± 75.16 d	32.10 ± 6.95 d	0.00 ± 0.00	0.00 ± 0.00	261.0 ± 137.07 c	42.0 ± 11.31 c
RF1	1095.86 ± 92.36	246.62 ± 86.12 d	27.06 ± 12.22 d	16.67 ± 28.87	0.67 ± 1.15	847.0 ± 741.04 bc	108.0 ± 71.55 bc
RF2	1306.88 ± 39.69	521.52 ± 110.22 c	87.05 ± 14.67 c	5.67 ± 9.29	0.67 ± 1.15	1622.0 ± 1027.47 b	217.0 ± 112.09 b
RF3	966.28 ± 239.84	1171.19 ± 370.15 b	54.61 ± 5.32 c	0.00 ± 0.00	0.00 ± 0.00	1005.0 ± 807.71 bc	178.0 ± 83.27 b
RF4	903.34 ± 212.58	1246.39 ± 465.27 a	225.92 ± 24.57 a	1.00 ± 1.73	0.67 ± 1.15	1896.0 ± 1101.67 b	483.0 ± 203.65 a
RF5	971.83 ± 158.34	1581.65 ± 352.10 a	205.73 ± 18.91 b	2.67 ± 4.62	0.67 ± 1.15	4545.0 ± 4677.36 a	505.0 ± 193.04 a
p-value	0.066	< 0.001	< 0.001	0.12	0.21	< 0.05	< 0.01

Note: Values represent mean ± standard deviation (SD). Different lowercase letters within the same row indicate significant differences among treatments according to Tukey's HSD test. Levels of significance: $p \geq 0.05$ = not significant; $p < 0.05$ = significant; $p < 0.01$ = very significant; $p < 0.001$ = highly significant.

Table 2. Diversity and regeneration of other plants, seedlings, and dead trees under different rubber systems (per hectare).

Rubber system	Other plants		Seedling plants		No. of Dead trees	
	No. of plants	No. of Species	No. of plants	No. of Species	No. of plants	No. of Species
RM	1118.33 ± 1013.29 c	297.33 ± 154.24 c	2471.00 ± 2597.64 c	194.33 ± 72.45 c	44.33 ± 37.78 b	44.33 ± 37.78 b
RF1	820.67 ± 207.69 c	361.00 ± 159.47 bc	3516.00 ± 1682.36 bc	211.33 ± 124.71 bc	29.00 ± 24.05 b	29.00 ± 24.05 b
RF2	1053.33 ± 376.71 c	428.33 ± 194.43 b	5311.00 ± 3426.05 b	314.00 ± 122.89 b	22.00 ± 13.00 b	22.00 ± 13.00 b
RF3	1274.00 ± 889.50 bc	452.00 ± 158.18 b	3651.00 ± 1796.17 bc	353.33 ± 84.55 b	7.33 ± 7.02 b	7.33 ± 7.02 b
RF4	1913.00 ± 1208.75 ab	482.00 ± 141.66 ab	5558.00 ± 3114.47 b	382.33 ± 95.64 ab	16.67 ± 14.43 b	16.67 ± 14.43 b
RF5	1562.33 ± 958.36 ab	506.00 ± 179.94 a	7725.00 ± 3837.77 a	456.67 ± 129.55 a	97.00 ± 96.00 a	97.00 ± 96.00 a
p-value	< 0.05	< 0.01	< 0.01	< 0.01	< 0.05	< 0.05

Note: Values represent mean ± standard deviation (SD). Different lowercase letters within the same row indicate significant differences among treatments according to Tukey's HSD test. Levels of significance: $p < 0.05$ = significant; $p < 0.01$ = very significant

Table 3. Total and available nutrient contents (N, P, K, Ca, Mg) of soils under different rubber systems.

Rubber system	Total N (%)	Total P (mg/kg)	Bray II (mg/kg) Available P	NH ₄ OAc Extract (mg/kg)		
				K	Ca	Mg
RM	0.123 ± 0.015 b	231.61 ± 221.87	12.18 ± 7.61	38.13 ± 10.10	119.56 ± 57.60	32.75 ± 9.36
RF	0.140 ± 0.000 b	70.39 ± 21.63	15.72 ± 12.94	34.07 ± 2.34	90.79 ± 23.44	28.72 ± 11.22
RF2	0.123 ± 0.015 b	95.33 ± 65.00	9.97 ± 5.51	39.49 ± 8.48	123.82 ± 130.69	34.99 ± 21.17
RF3	0.137 ± 0.012 b	93.90 ± 24.90	10.78 ± 7.44	39.37 ± 14.40	124.21 ± 107.24	41.17 ± 25.09
RF4	0.143 ± 0.025 b	111.88 ± 56.42	13.04 ± 9.10	37.33 ± 7.45	109.94 ± 28.46	43.18 ± 27.31
RF5	0.203 ± 0.040 a	213.81 ± 112.14	11.28 ± 9.90	104.17 ± 123.48	58.33 ± 35.74	28.68 ± 24.27
p-value	<0.01	0.36	0.97	0.54	0.87	0.93

Note: Values are presented as mean ± SD. Different lowercase letters within the same row indicate significant differences in soil nutrient contents among rubber systems according to Tukey's HSD test. Levels of significance: $p \geq 0.05$ = not significant; $p < 0.01$ = very significant.

Table 4. Soil organic carbon, organic matter, cation exchange capacity, pH, and electrical conductivity of soils under different rubber systems.

Rubber system	%OC	%OM	CEC (meq/100g soil)	pH	EC (mS/cm)
RM	1.44 ± 0.50 b	2.22 ± 0.55 d	3.68 ± 0.89 d	4.78 ± 0.27	1.99 ± 0.33 b
RF	1.62 ± 0.89 b	2.38 ± 0.56 d	3.90 ± 1.91 c	4.73 ± 0.29	1.91 ± 0.38 b
RF2	1.53 ± 0.64 b	2.48 ± 0.56 c	4.19 ± 2.32 b	4.90 ± 0.32	1.62 ± 0.32 b
RF3	1.76 ± 0.61 b	3.12 ± 0.88 c	4.94 ± 2.18 a	4.91 ± 0.11	1.83 ± 0.25 b
RF4	2.69 ± 0.29 a	3.21 ± 0.52 b	4.36 ± 1.88 a	4.92 ± 0.04	2.59 ± 0.22 a
RF5	2.49 ± 0.30 a	3.87 ± 0.05 a	3.48 ± 0.91 c	4.73 ± 0.20	2.53 ± 0.40 a
p-value	< 0.05	< 0.05	< 0.05	≥ 0.05	< 0.05

Note: Different lowercase letters within the same row indicate significant differences in soil properties among rubber systems according to Tukey's HSD test at levels of significance: $p \geq 0.05$ = not significant and $p < 0.05$ = significant.

Carbon content and carbon stock followed a similar trajectory, increasing significantly with system complexity. Carbon stock increased from 1.20 ± 0.32 tCO₂e ha⁻¹ in RM to 4.04 ± 0.33 tCO₂e ha⁻¹ in RF5 ($p < 0.0001$; Table 5), indicating a substantial enhancement in carbon sequestration capacity. This strong relationship between biomass and carbon stock supports the principle that woody biomass accumulation is a primary driver of carbon storage at the ecosystem scale [23]. However, the observed carbon values fall within the lower range of published estimates for the mature rubber system, likely due to differences in stand age, site conditions, and measurement approaches [24, 25]. This suggests that the systems studied are still in a developmental stage and have not yet reached their full carbon sequestration potential.

ANOVA confirmed strong treatment effects across all variables (Table 6). Among structural traits, DBH showed the sharpest response ($F = 111.58$, $p < 0.0001$), which reflects its central role in allometric biomass equations — small differences in trunk diameter produce disproportionately large differences in estimated biomass. The very high F-values for carbon content ($F = 1,428.46$) and total biomass ($F = 352.19$) partly reflect this allometric amplification across the hectare scale. Residual normality and variance homogeneity should be verified to confirm that ANOVA assumptions hold at these effect sizes.

A focused comparison between monoculture rubber (RM) and highly diversified agroforestry (RF5) further highlights the ecological advantages of diversification (Table 7). RF exhibited significantly higher values across all ecological and biophysical indicators, with species richness increasing by 6.4-fold and

seedling regeneration by 3.1-fold ($p < 0.01$; Table 7). These findings indicate enhanced habitat heterogeneity and regeneration capacity, which are critical for long-term ecosystem resilience and biodiversity conservation [20].

Soil properties were also significantly improved in RF5, with significantly higher organic matter content and total nitrogen. In contrast, soil organic carbon showed a positive trend that did not reach statistical significance across treatments ($p = 0.51$), suggesting more efficient nutrient cycling and improved soil quality. These improvements are likely driven by increased litter input, root biomass turnover, and microbial activity in a diversified system [21]. Such processes play a fundamental role in sustaining long-term productivity and ecosystem functioning.

In addition, total biomass in RF5 was 3.5–4.2 times higher than in RM, accompanied by a substantial increase in carbon stock ($p < 0.001$; Table 7). This reinforces the conclusion that diversified rubber agroforestry systems not only enhance biodiversity but also significantly improve ecosystem productivity and carbon sequestration potential. However, increasing system complexity may also introduce trade-offs, such as intensified competition for resources or greater management complexity, which should be considered in future research [19].

Belowground biomass was estimated using a fixed root-to-shoot ratio, applied uniformly across all treatments and species. In more diverse systems, including fruit trees and timber species with varying rooting depths and architectures, this approach is likely to introduce error into belowground estimates. Species-specific root:shoot ratios or direct root sampling would improve accuracy in future studies.

3.4 Carbon Stock Distribution and Relationships with Tree Density Across Management Systems.

Analysis revealed a highly stratified distribution of carbon stock across the rubber systems. The overall distribution of carbon stock exhibited a distinct multimodal pattern (Figure 5A), which can be directly attributed to the strong regulatory effect of management. As demonstrated in the boxplot and mean analysis (Figure 5C, 5F), carbon accumulation is strictly hierarchical: RM yielded the lowest baseline (approximately $1.1 \text{ tCO}_2\text{e ha}^{-1}$), while RF5 demonstrated the highest sequestration capacity, consistently exceeding $4.0 \text{ tCO}_2\text{e ha}^{-1}$ with minimal intra-group variance.

Tree density and carbon stock decouple within system cohorts (Figure 5B). While the global correlation matrix (Figure 5E) suggests a strong positive relationship between tree density and carbon stock ($r = 0.89$), a granular examination of the clustered scatterplot (Figure 5D) provides a more nuanced ecological insight. The data indicated that high carbon stock is primarily governed by system-level factors rather than by an absolute increase in tree density. Within the highest-performing cohort (RF5), tree density ranged from approximately 50 to over 120 trees per plot. Yet, the corresponding carbon stock remained relatively stable near its maximum, suggesting that factors beyond tree density — species composition, stand age, and management history — are the primary drivers of carbon accumulation in these systems. This indicates that system-level organization, including species composition, stand structure, and management history, governs carbon accumulation more strongly than tree density alone. Within individual management systems, adding more trees does not proportionally increase carbon stocks, but shifting the system toward greater structural complexity does. This distinction has direct implications for how carbon incentive programs should evaluate agroforestry: rewarding complexity and ecological function rather than simple tree counts. The negative correlation observed within the RM system ($r = -0.68$) likely reflects density-dependent competition dynamics: in high-density monoculture stands, individual tree growth and biomass accumulation are slower due to resource competition, resulting in lower per-tree and per-hectare carbon stocks at higher tree densities. This pattern is consistent with self-thinning theory in even-aged plantations.

The stability of carbon stocks across sites, regardless of aboveground tree density, points to a significant contribution from belowground biomass pools. This highlights how roots act as linchpins for long-term carbon stabilization through rhizodeposition and turnover [26]. Consequently, the evidence bolsters the case for folding both aboveground and below-ground carbon pools into accounting frameworks and for leveraging RF systems to underpin mitigation pathways such as REDD+ or credit-based schemes [27].

Table 5. Tree growth characteristics, biomass components, and carbon stock of rubber under RM and RF systems (RF1–RF5).

Parameter	RM	RF1	RF2	RF3	RF4	RF5	p-value
<i>Tree Growth Characteristics</i>							
H (m)	3.68 ± 0.12 b	3.52 ± 0.57 b	3.32 ± 0.83 b	8.87 ± 2.51 a	9.10 ± 2.88 a	10.45 ± 5.52 a	0.0025
GBH (cm)	12.83 ± 1.98 b	13.18 ± 4.41 b	13.94 ± 3.02 b	25.86 ± 10.42 ab	28.63 ± 13.22 a	31.67 ± 13.69 a	0.0205
DBH (cm)	4.03 ± 0.68 b	3.01 ± 0.38 b	4.17 ± 0.54 b	14.24 ± 2.52 a	12.70 ± 1.15 a	12.67 ± 2.99 a	<0.0001
<i>Aboveground Biomass Component</i>							
WS (kg ha ⁻¹)	419.89 ± 84.26 e	833.83 ± 72.95 d	837.47 ± 36.18 d	950.94 ± 75.06 c	1189.36 ± 63.08 b	1413.86 ± 39.70 a	<0.0001
WB (kg ha ⁻¹)	88.06 ± 29.23 d	155.70 ± 44.79 c	171.26 ± 29.11 c	185.78 ± 11.49 c	256.39 ± 42.18 b	323.45 ± 50.03a	<0.0001
WL (kg ha ⁻¹)	16.38 ± 3.22 d	25.79 ± 1.83 c	28.58 ± 3.16b c	34.88 ± 4.89 b	35.64 ± 7.29 b	44.35 ± 6.63 a	<0.0001
WT (kg ha ⁻¹)	524.33 ± 55.52 f	915.31 ± 59.21 e	1062.31 ± 43.21 d	1171.60 ± 85.58 c	1506.38 ± 47.53 b	1920.22 ± 75.20 a	<0.0001
<i>Belowground Biomass and Total Biomass</i>							
Underground biomass (kg ha ⁻¹)	148.32 ± 39.18 d	274.13 ± 39.07 c	300.33 ± 32.66 c	316.33 ± 23.11 c	393.22 ± 39.53 b	503.35 ± 47.05 a	<0.0001
Total biomass (kg ha ⁻¹)	622.64 ± 82.10 f	1089.45 ± 94.68 e	1362.64 ± 68.48 d	1512.93 ± 60.77 c	1924.6 ± 38.65 b	2348.57 ± 66.67 a	<0.0001
<i>Total Carbon Content and Carbon Stock</i>							
Carbon content (kgC ha ⁻¹)	327.89 ± 86.62 d	606.04 ± 86.37 c	663.94 ± 72.21 c	699.33 ± 51.09 c	869.32 ± 87.38 b	1100.88 ± 88.78 a	<0.0001
Carbon stock (kgC ha ⁻¹)	1127.27 ± 72.42 f	2147.15 ± 87.61 e	2309.45 ± 89.60 d	2639.20 ± 51.74 c	3312.49 ± 74.45 b	4311.54 ± 42.05 a	<0.0001
(tCO ₂ e ha ⁻¹)	1.20 ± 0.32 d	2.22 ± 0.32 c	2.43 ± 0.26 c	2.56 ± 0.19 c	3.19 ± 0.32 b	4.04 ± 0.33 a	<0.0001

Note: Values are mean ± SD. Different lowercase letters within the same row indicate significant differences among treatments according to Tukey's HSD test (p ≤ 0.05). WS: weight stem, WB: weight branch, WL: weight leaf; WT: weight total.

Table 6. One-way ANOVA results for tree growth traits, biomass components, and carbon metrics across management systems (RM and RF1-RF5).

Variable	F-value	p-value	Significance
Tree height (H, m)	26.58	0.0025	**
Girth at breast height (GBH, cm)	8.83	0.0205	*
Diameter at breast height (DBH, cm)	111.58	<0.0001	***
Stem biomass (WS, kg ha ⁻¹)	158.07	<0.0001	***
Branch biomass (WB, kg ha ⁻¹)	27.23	<0.0001	***
Leaf biomass (WL, kg ha ⁻¹)	49.36	<0.0001	***
Total tree biomass (WT, kg ha ⁻¹)	434.20	<0.0001	***
Underground biomass (kg ha ⁻¹)	76.03	0.0001	***
Total biomass (kg ha ⁻¹)	352.19	<0.0001	***
Carbon content (kgC ha ⁻¹)	1428.46	<0.0001	***
Carbon stock (tCO ₂ e ha ⁻¹)	42.06	<0.0001	***

Note: Factor = management system with six levels (RM, RF1-RF5); unit of analysis = per tree; replicates per level = 4. Values are F-statistics and p-values from one-way ANOVA. Significance code: *p < 0.05, ** p < 0.01, *** p < 0.001. Pairwise comparisons among systems were evaluated using Tukey's HSD test, and the results are presented in Table 5.

Table 7. Comparison of ecological and biophysical indicators between monoculture rubber (RM) and highly diversified rubber agroforestry (RF5). Data are presented as mean ± standard deviation. Total biomass and carbon stock are expressed as site-level ranges (minimum-maximum). Statistical significance between treatments was determined using appropriate comparative tests (*p* < 0.05)

Variable	RM (Monoculture)	RF5 (Highly Diversified)	p-value	Ecological Significance
Species Richness (trees, spp. ha ⁻¹)	32.10 ± 6.95	205.73 ± 18.91	<0.001	6.4-fold increase in structural complexity and habitat diversity
Seedling Regeneration (seedlings ha ⁻¹)	2471.00 ± 2597.64	7725.00 ± 3837.77	< 0.01	3.1-fold enhancement of natural regeneration potential
Soil Organic Carbon (%OC)	1.44 ± 0.50	2.49 ± 0.30	0.01	Greater long-term capacity for soil carbon sequestration
Total Nitrogen (%)	0.123 ± 0.015	0.203 ± 0.040	< 0.01	Significant improvement in nitrogen cycling efficiency
Total biomass (kg ha ⁻¹)	548.55 – 689.26	2278.74 – 2426.53	< 0.001	3.5-4.2-fold increase in total biomass accumulation
Carbon stock (tCO ₂ e ha ⁻¹)	0.78 – 1.29	3.75– 4.33	< 0.001	Substantially higher potential for climate change mitigation

Note: Ranges represent site-level minimum and maximum values.

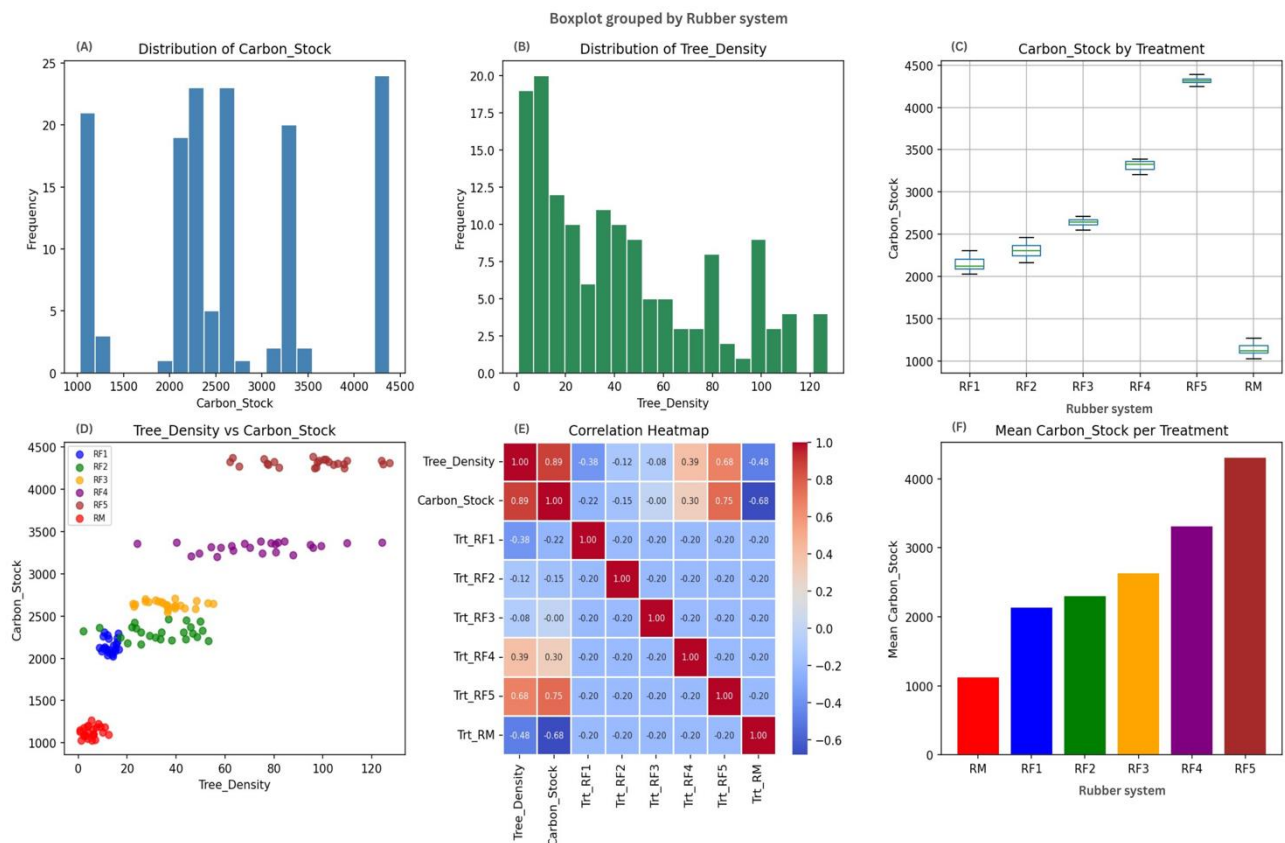


Figure 5. Comprehensive evaluation of carbon stock distribution, relationships with tree density, and management effects across non-rubber agroforestry components. (A) Frequency distribution of overall carbon stock showing a multimodal pattern, (B) Right-skewed frequency distribution of tree density. (C) Boxplot illustrating carbon stock variability across different management systems (RF1-RF5, RM). (D) Scatterplot demonstrating the clustering of tree density and carbon stock relationships by management system. (E) Pearson correlation matrix of key variables. (F) Bar chart comparing mean carbon stocks ($\pm$ SE) among the management systems.

3.5 PCA-Guided Synthesis of Soil, Vegetation, and Carbon

Principal component analysis of soil quality and carbon stock (PC1 = 47.4%, PC2 = 13.4%; cumulative = 60.8%) separated the three study sites (Figure 6A). Thepha formed a distinct cluster, while Tamot and Sri Banpot partially overlapped and showed broader spread, reflecting greater within-site variability at those locations. When soil parameters were combined with plant growth and carbon variables (PC1 = 36.1%, PC2 = 24.9%; cumulative = 61.0%) (Figure 6B), the same site-level pattern held, with Tamot and Sri Banpot showing higher internal variability than Thepha. The PCA results primarily reflect site-level differences in the combined soil-vegetation-carbon system across the three districts, rather than treatment-level separation among management systems. Within this site-level variation, soil fertility indicators (%OM, CEC) and carbon stock variables load consistently together, suggesting that where organic matter and nutrient retention capacity are higher, carbon storage also tends to be higher. This association is consistent with findings from other Southeast Asian agroforestry landscapes [28, 29], though the cross-sectional design of this study means these relationships should be interpreted as associations rather than confirmed causal pathways.

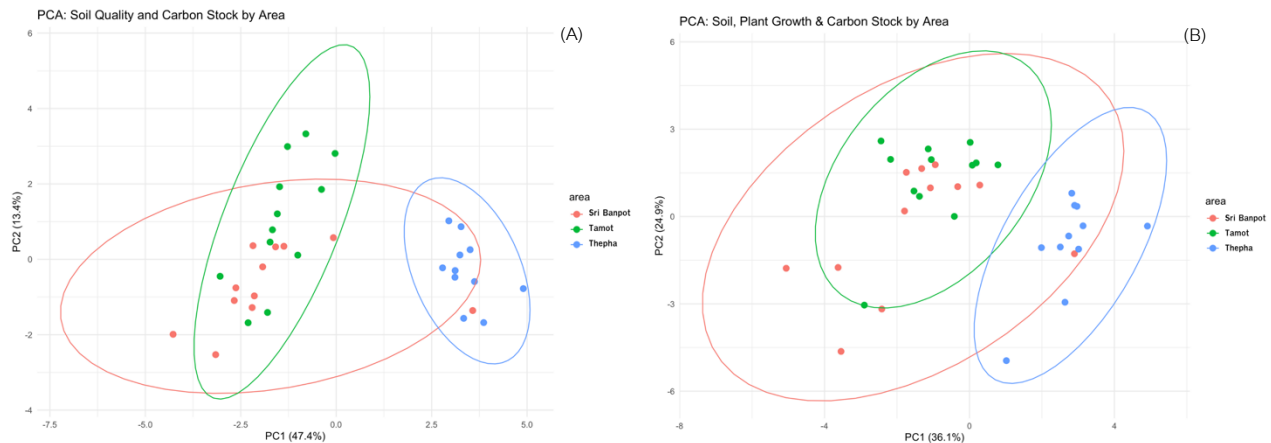


Figure 6. Principal Component Analysis (PCA) of (A) soil quality and carbon stock, and (B) soil qualities, plant growth, and carbon stock, classified by study area: Sri Banplot, Tamot, and Thepha. Each point represents a single sample from the respective area, and the ellipses indicate the confidence regions (confidence ellipses) for each group, illustrating the clustering of the samples.

4. Conclusion

Rubber plantations managed as diversified agroforestry systems outperformed monocultures across all ecological dimensions measured in this study. Species richness, seedling regeneration, soil organic matter, total nitrogen, standing biomass, and carbon stock all increased along the RM to RF5 gradient, increasing together rather than independently. The most diversified systems did not trade one benefit for another; they accumulated ecological and carbon benefits as coupled outcomes of increasing structural complexity. This coupling suggests that biodiversity, soil fertility, and carbon storage are not separate services to be optimized individually, but expressions of a single underlying process: the development of ecological organization within the system. This finding has a direct practical implication: rubber agroforestry is not a compromise between production and conservation but rather a system in which the two reinforce each other. Policies that make it financially viable for smallholders to diversify their rubber holdings — through carbon markets, payments for ecosystem services, or agroforestry subsidies — are likely to generate returns across biodiversity, soil health, and climate goals simultaneously. Future work should extend soil sampling to at least 30 cm (and to 60 cm in systems with deep-rooted species), include bulk density measurements to enable stock-change accounting, refine belowground carbon estimates using species-specific root:shoot ratios or direct root sampling, and establish long-term monitoring plots to track how carbon stocks develop as these systems continue to mature. The ecological and carbon benefits documented here accumulated over one to two decades of progressive diversification. Policy and market mechanisms designed to incentivize these systems, including carbon crediting, payments for ecosystem services, and agroforestry certification, should be designed to accommodate this developmental trajectory rather than requiring immediate, measurable returns from a single intervention.

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Conceptualization, Writing (reviewing and editing); MC: Conceptualization, Methodology, Writing (reviewing and editing); CP: Data Analysis; EA and RI: Reviewing and editing.

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