Carbon Sequestration Dynamics in Planted, Naturally Regenerated, and Old Growth Tropical Cloud Forests in the Talamanca Mountain Range, Costa Rica

Research Project 5th August- 17th November 2024

Svenja Stephenson

Abstract

Tropical cloud forests play a critical role in global carbon storage, yet their sequestration dynamics remain understudied compared to lowland tropical forests. This study investigates carbon sequestration in planted, naturally regenerated, and old-growth forest sections at Cloudbridge Nature Reserve, Costa Rica. Data were collected from fifteen 10 × 10 m plots across forest types established at different times: planted (2008, 2009, 2011), natural regrowth (1988, 2004, 2008), and old growth (last logged in the 1930s). Diameter at breast height (DBH), tree height, canopy cover, and slope were measured, and above- and below-ground biomass were estimated using allometric equations. Total carbon was calculated and converted into CO_2 equivalents. Results show that old-growth forests store substantially higher carbon (1.57 t CO_2/ha) than younger forests, reflecting their advanced structural complexity. Natural regrowth plots sequestered slightly more carbon (0.27–0.31 t CO_2/ha) than planted plots (0.25–0.27 t CO_2/ha), although planted forests displayed relatively rapid early growth. Generalized Additive Models revealed forest age as the strongest predictor of sequestration, while slope and canopy cover showed weak, non-significant effects. Limitations include small sample size, restricted plot accessibility, and exclusion of trees <10 cm DBH and soil carbon pools, likely leading to underestimates of total sequestration. This study provides baseline data for long-term monitoring of carbon dynamics in tropical montane forests and highlights the importance of both reforestation and natural regeneration in enhancing carbon storage. Continued monitoring and expanded datasets are recommended to refine sequestration estimates and guide climate change mitigation strategies.

1. Introduction

Climate change, along with the resulting global warming, stands as the central issue of the 21st century, with widespread negative effects on all life on Earth, leading to significant losses and damages to both nature and people. Global warming is altering the biophysical landscape in unprecedented ways, bringing extreme weather events, extended droughts, rising sea levels, and melting glaciers (White, 2024). These changes are undeniably caused by human activities, primarily through the emission of greenhouse gases that have subsequently caused global warming, leading to a global surface temperature increase of 1.1°C above pre-industrial levels during the period from 2011 to 2020 (IPCC, 2023).

Achieving net zero CO_2 or greenhouse gas (GHG) emissions necessitates significant and rapid reductions and cuts in CO_2 and non-CO_2 GHG emissions. These measures aim at slowing down the rate of global warming and mitigating further negative impacts. Equally important is the support of carbon sinks and sequestration methods—natural processes, technologies, and practices that absorb and store carbon dioxide from the atmosphere (IPCC, 2023).

Reforestation stands out as one of the most effective natural climate solutions due to its significant carbon-sequestration potential (Cerasoli et al., 2021). Forests, as the largest terrestrial carbon reservoirs, play a vital role in mitigating global warming by absorbing approximately 2.6 billion tons of carbon dioxide annually—around one-third of the CO_2 emissions generated from burning fossil fuels (Xie et al., 2024). By serving as substantial carbon sinks, reforestation efforts directly mitigate emissions, supporting both short- and long-term climate change goals (Zhang et al., 2023). Beyond carbon storage, reforestation helps cool land and air surfaces by increasing precipitation and atmospheric moisture (Benetó, 2022). In contrast, deforestation and poor forest management contribute to climate change by releasing stored carbon, disrupting plant diversity, and reducing ecosystem services (Yadav et al., 2022). Reforestation not only addresses these environmental challenges but also promotes ecological stability and biodiversity, making it a critical tool in the fight against climate change (Zhang et al., 2023).

Costa Rica as a country has been widely recognised for its successful reforestation efforts, which have led to the reversal of deforestation and the restoration of its natural ecosystems. Over the last 15 years the country made significant strides in land restoration, increasing its forest cover from just 25% to 57% through reforestation efforts (Copernicus EU, 2023). A reserve that was created to restore and protect cloud forest habitats in Costa Rica that have been degraded by agriculture and logging, is Cloudbridge Nature Reserve. Established in 2002, the reforestation and conservation project is located in the Talamanca Mountains of Costa Rica (Cloudbridge, n.d.) Their efforts to protect and restore tropical forests are crucial because tropical regions in particular have a significant carbon sequestration potential. According to Paulick et al. (2017, more than 50% of the carbon stored in aboveground vegetation is assumed to be located in the tropics. This is due to their dense, fast-growing trees, driven by warm temperatures and abundant rainfall, as well as their rich plant biodiversity (Artaxo, 2022).

While all tropical forests are important for their ecological roles, cloud forests, such as the one Cloudbridge has reforested, have unique characteristics and play critical roles in biodiversity, water regulation, and carbon sequestration. These forests, typically found in tropical and subtropical mountainous regions, are particularly sensitive to climate change, logging, land-use change, making reforestation critical (Karger et al., 2021). Tropical montane forests have received less research attention compared to tropical lowland forests, and their role in carbon storage remains poorly understood. This underscores the need for further studies to clarify their contribution to carbon sequestration.

Cloudbridge Nature Reserve has reforested former cattle and crop lands, resulting in three distinct forest types: replanted areas, naturally regenerated areas, and old-growth forest (Cloudbridge, n.d.). This research seeks to examine the carbon sequestration rates across three distinct forest types, with a particular focus on sections planted in different years.The replanted forest includes sections from 2008, 2009, and 2011, while the natural regrowth areas are from 1988, 2004, and 2008. The old-growth forest, last logged in the 1930s, represents a single time point for comparison. By analysing total carbon sequestration rates at these available time points, the study reveals how these rates evolve over time within each forest type, reflecting the unique successional stages of each forest. The goal is to assess sequestration dynamics within each forest type and establish baseline data for long-term monitoring. This foundational information enhances the understanding of carbon storage in these ecosystems and supports conservation strategies aimed at mitigating climate change, particularly in Cloudbridge's efforts to enhance its overall carbon storage.

The research addresses two key questions:

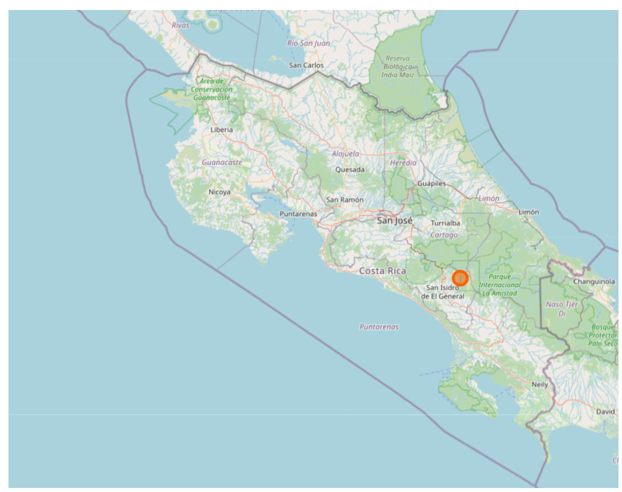
1. How do carbon sequestration rates vary and change over time between planted, naturally regenerated, and old-growth tropical cloud forest at Cloudbridge Nature Reserve?

2. How are total carbon sequestration rates influenced by slope, canopy cover, and age?

2. Methodology

2.1 Study area

Figure 1- *Location of Cloudbridge.*
Note. Derived from *(openstreetmap.org).*



Cloudbridge Nature Reserve is situated in the south-central region of Costa Rica, on the southern Pacific slopes of the Talamanca mountain range, adjacent to Chirripó National Park, a UNESCO World Heritage Site (United Nations Educational, Scientific and Cultural Organization, (n.d.)).
Talamanca Range–La Amistad Reserves / La Amistad National Park [World Heritage List No. 205]. UNESCO World Heritage Centre. Retrieved September 8, 2025, from UNESCO websiteThe reserve spans 280 hectares of tropical premontane and montane cloud forest habitat, with elevations ranging from 1500 to 2650 metres. While only small portions of the reserve contain old growth forest (around 50 ha), the majority of the land consists of abandoned or repurposed pastures that are in various stages of natural succession, gradually returning to their original climax forest state. The planted areas were established in sections over different years, with the oldest sections now reaching up to 16 years. The naturally regenerated areas vary significantly in age, ranging from approximately 15 to 40 years. Any area older than 70 years is classified as an old growth forest (Cloudbridge, n.d.).

2.2 Field data collection

To calculate and understand how carbon sequestration rates change over time in each forest type at Cloudbridge, it was essential to sample the trees present in all areas; the old growth forest, the manually replanted areas and naturally regenerating zones. For this research, randomly selected plots were selected from each forest type based on the available planting years. In the planted forest sections, two plots each were measured for the years 2008, 2009, and 2011. In the natural regrowth sections, two plots each were marked for the years 1988, 2004, and 2008. The old-growth forest, which has been undisturbed since the 1930s, includes three plots representing a single age group as no other time point was available in this forest type. Each plot covered a 10-metre by 10-metre area, and all trees within the plots were tagged with a unique identifier. For each tagged tree, the diameter at breast height (DBH), the tree height, the average canopy cover and slope were recorded. Trees partially within a plot were included if at least half of the trunk fell inside the boundary. Dead and diseased trees were also measured to ensure a comprehensive assessment of carbon sequestration, capturing all sources of carbon storage and release. This analysis was conducted between August and October 2024.

2.2.1 Plot selection

Plots were selected using a randomised pattern generated in QGIS. First, a raster layer was added, which served as the basis for selecting random points across the forest area. From these randomised points, only those accessible by trails were chosen as plot locations. A GPS device was then used to locate these points in the field, where plots were established.
In cases where multiple plots needed to be established within a restricted area due to available forest age groups, the same randomisation procedure in the given age section was applied to ensure unbiased placement. This approach ensured that plots were distributed randomly while also meeting the study's requirements for age-based grouping within each forest type.

Figure 2 depicts each of the plots chosen at Cloudbridge Reserve. More specifically, for the planted sections two plots were selected on El Jilguero from the year 2008, two plots on the Los Quetzales trail planted in 2009 and two plots on El Jilguero on from the 2011 sections. Within the Natural Regrowth section, two plots were selected in the 1988 section on the El Jilguero trail, two plots on Montaña being regrown since 2004, and two more on the 2008 section on El Jilguero. Three Old Growth Plots were chosen on El Jilguero, Sentinel and Montaña.

Table 1: Map codes for Figure 2

Abbreviated Name on Map	**Trail Name**	**Forest Type**	**Age of Forest**
EJ PLA 2008A	El Jilguero	Planted	2008A
EJ PLA 2008B	El Jilguero	Planted	2008B
LQ PLA 2009A	Los Quetzales	Planted	2009A
LQ PLA 2009B	Los Quetzales	Planted	2009B
EL PLA 2011A	El Jilguero	Planted	2011A
EL PLA 2011B	El Jilguero	Planted	2011B

EJ NR 1988A	El Jilguero	Natural Regrowth	1988A
EJ NR 1988B	El Jilguero	Natural Regrowth	1988B
MON NR 2004A	Montaña	Natural Regrowth	2004A
MON NR 2004B	Montaña	Natural Regrowth	2004B
EJ NR 2008A	El Jilguero	Natural Regrowth	2008A
EJ NR 2008B	El Jilguero	Natural Regrowth	2008B
EJ OG	El Jilguero	Old Growth	1930
SENTI OG	Sentinel	Old Growth	1930
MON OG	Montaña	Old Growth	1930

The exact coordinates for each plot can be found in Appendix Table A1.

On the map, yellow areas represent planted forests, light green indicates naturally regenerated forests, and dark green denotes old-growth forest. Dark blue dots mark the locations of the research plots along the designated trails, with white labels specifying the trail name, forest type, and corresponding forest age.

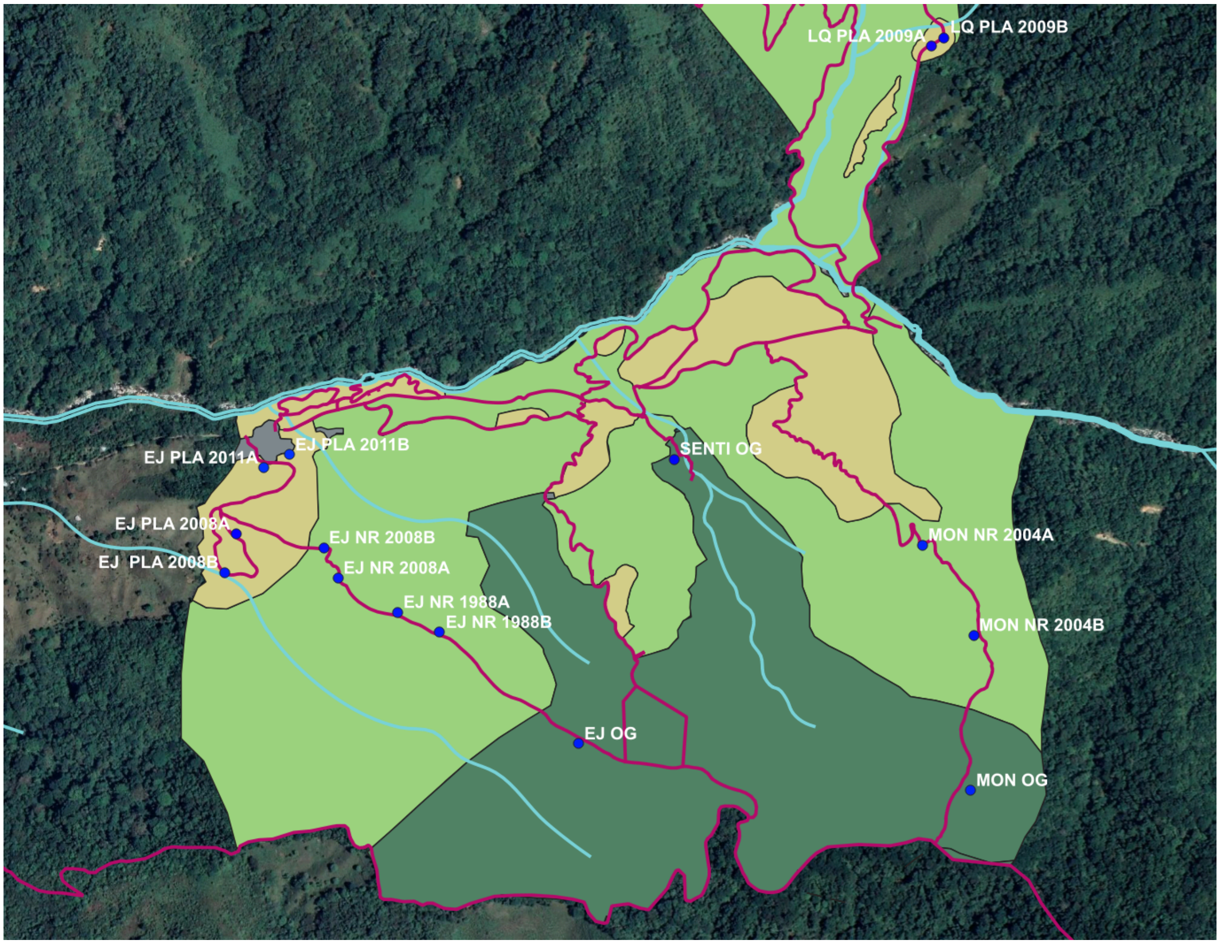


Figure 2- *Cloudbridge Map with Chosen Plots.*
Note. Derived from QGIS November 2024

2.2.2 DBH (Diameter at Breast Height) measurements

DBH (Diameter at Breast Height) measurements were conducted by classifying tree size at 1.3 metres from the ground, approximating the breast height of an average person. Measurements were taken for trees with a minimum diameter of 10 cm. To ensure consistency, a bamboo pole was marked at 1.3 metres and used as a reference for measuring tree diameters with a diameter tape. For trees too large for the diameter tape, the circumference was measured with a standard measuring tape and subsequently converted to diameter. On sloped terrain, measurements were taken from the upslope side. If a tree trunk split into multiple stems below the DBH mark (multi-stem), all stems were measured at breast height.

2.2.3 Tree Height Measurements and Calculations

Tree height was measured using the Suunto Height Meter PM-5/1520 clinometer. The procedure involved positioning the observer 15 or 20 metres from the tree, uphill, and using the clinometer's scale to take two readings. The first reading measured the distance from the observer's eye level to the top of the tree, and the second from the observer's eye level to the base. The total tree height was calculated by adding these two values together (ManulsLib, (n.d.). In cases where terrain or obstacles made it impossible to maintain a 15 or 20-metre distance, a suitable random distance was chosen, and the percentage scale on the clinometer was used. From this distance, two percentage readings were taken: from eye level to the top and from eye level to the base. These percentages were then summed and applied to the following formula:

Tree Height = (Percentage/10) × Distance

This equation accounts for the total tree height (Williams et al., 1994). Figure 3 visualises the instructions for both uphill and downhill measurements.

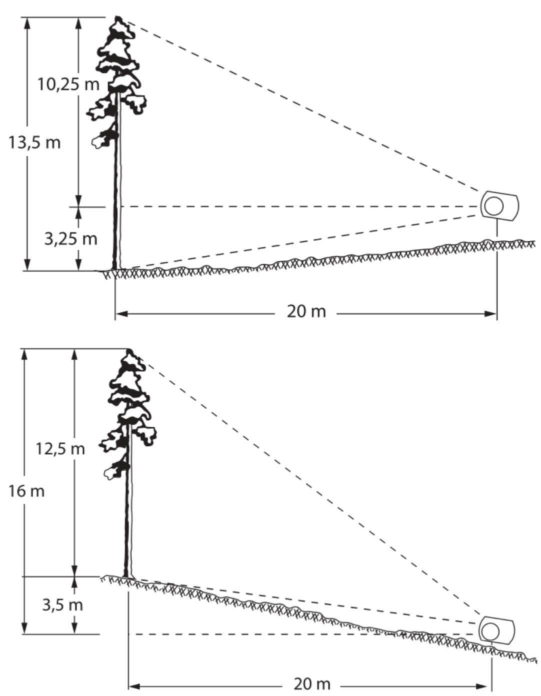


Figure 3- *Height Measurement Instruction.*
Note. Derived from Suunto PM-5/ PM-5/ 1520 User's Guide.

2.2.4. Canopy cover measurement

To assess light availability, canopy cover measurements were taken with a spherical densiometer at all four edges of each plot, oriented in each of the four cardinal directions. The average canopy cover for each plot was calculated and subsequently compared across all plots. Measurements were consistently taken in the morning to ensure similar weather conditions and light intensity, minimising variability due to time of day.

2.2.5. Slope

To obtain slope rates, a Digital Elevation Model (DEM) of the study area was sourced, providing a 3D representation of the terrain. The DEM was imported into QGIS, allowing for spatial analysis. This process enabled the extraction of slope values corresponding to each specific measurement point within the study plots.

2.2.6 Species identification

For biomass allometric equations requiring wood density data, accurate species identification for trees in each plot is ideal. However, due to the complexity of cloud forest ecosystems, where trees are diverse and often very tall, detailed species identification is challenging ,just as at the study sites in Cloudbridge. Therefore, a practical approach was employed: identifying the most common species in each forest type, recording their wood density values, and calculating an average wood density for each forest type.

2.3 Data analysis

Generally, all data analysis, calculations and statistical testings with respective graphs were run in R Studio. For each graph, the colours of the forest types were chosen respectively to the colours of those in the Cloudbridge map. Yellow depicts the planted forest, light green the natural regrowth sections and dark green stands for the old growth forest.

2.3.1 Rationale and approach behind analysing carbon sequestration trends and their influences

This study investigates how carbon sequestration rates vary across each of three distinct forest types, reflecting their unique successional stages. Due to differences in age and ecological conditions, direct comparisons across types could introduce biases related to species composition and growth dynamics. Instead, the analysis focuses on total sequestration rates at multiple time points within the planted and natural regrowth section, and one time point in the old growth section, capturing within-type variations without confounding factors from mixed comparisons of planted, naturally regenerating, and old-growth areas.

As this research does not allow directly answering the question of whether one forest type sequesters more carbon than another, due to limited possibilities and ecological differences across forest types, it instead captures the sequestration dynamic within each type. Follow-up research with extended observations over multiple years could provide a more direct comparison of carbon sequestration dynamics across forest types. However, it is important to account for various ecological factors, such as species composition, which may vary between the forest types and influence the results.

The research aims to establish baseline data on sequestration patterns within each forest type, which will support future assessments as additional data points are gathered. By exploring how environmental factors like slope, canopy cover, and age correlate with sequestration rates, the study provides valuable insights into the sequestration potential of each forest type. Ultimately, this foundational data aims to inform conservation and management strategies to enhance carbon storage and support climate change mitigation efforts.

2.3.2 Carbon sequestration calculations
2.3.2.1 Above-ground carbon calculation

In forest carbon research, biomass allometric equations are essential tools for estimating above-ground carbon storage. Two influential models used for calculating above-ground biomass (AGB) include the Brown et al. (1989) and Chave et al. (2005) equations. The Brown model, among the earliest generalised allometric models, estimates biomass based solely on DBH (Diameter at Breast Height). Its simplicity and adaptability across forest types have contributed to its widespread adoption. However, some researchers have noted that this model may lack precision when applied to forests with diverse species and varying wood densities (Walker et al., 2017).

To enhance precision, Chave et al. (2005) introduced a model incorporating wood density alongside DBH, aiming to account for species-specific traits that influence biomass estimates. This model is often considered more accurate in mixed-species and tropical forests due to its inclusion of wood density as an additional variable (Huy et al., 2016; Chave et al., 2015). Nonetheless, others argue that this added complexity may not substantially improve accuracy in studies where species data is incomplete (Pati et al., 2022).

The highest adjusted R^2 value (0.97) for allometric equations estimating above-ground biomass in wet tropical forests, as provided by Brown et al., is achieved with the following equation (Brown et al., 1989; Walker et al., 2017):

$$AGB = exp(-3.1141 + 0.9719 * ln(D2 * H))$$

where D represents diameter at breast height (DBH), and H is tree height.

The second used above-ground biomass equation is by Chave et al., which have an adjusted R^2 value of 0.99 for tropical forests, are as follows (Chave et al. 2015; Walker et al. 2017):

$$AGB = exp(-2.977 + ln(\rho * D^2 * H))$$

where D represents diameter at breast height (DBH), H is tree height and ρ stands for wood density.

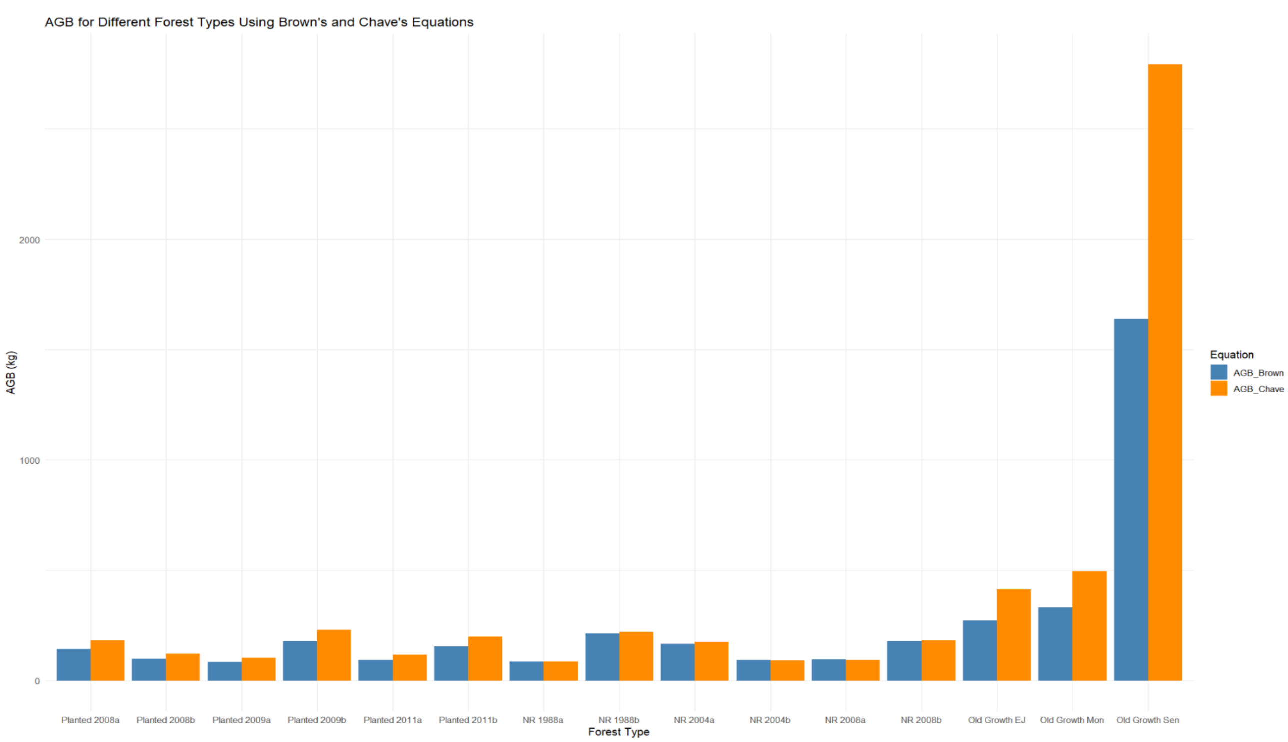


Figure 4- *Difference between AGB values for all data sets of both allometric equations.*
Note. Derived from R Studio.

In this study, both Brown's and Chave's equations were applied to estimate carbon sequestration across plots. The varying above-ground biomass (AGB) levels are shown in Figure 4, with Brown's equation represented in blue and Chave's in orange. As shown, incorporating wood density values for the most common species in each forest type significantly increased AGB estimates when using Chave's equation. However, for the natural regrowth plots, Brown's and Chave's estimates were similar when averaged wood densities for the dominant natural regrowth species were applied. While Chave's equation is considered more technically accurate, it would have been ideal to use it consistently across all datasets. The challenge, however, lies in the incomplete species distribution data across forest types and gaps in wood density values for certain species, which introduces potential biases and uncertainties when applying Chave's model. Given these limitations, and despite Chave's model's technical advantages, Brown's equation, tailored for tropical wet forests, was ultimately deemed more appropriate for this dataset and was thereby used for all following carbon equations. This decision aimed to reduce uncertainty by selecting a model better suited to the regional forest characteristics, given the incomplete species-level wood density information.

Table A2 in the appendix mentions the most commonly observed species in each forest type and their respective wood density values.

2.3.2.2 Below-ground carbon calculation

Root biomass, often referred to as below-ground biomass (BGB), is a key component of carbon storage in forests. Due to the labour-intensive, challenging, and resource-demanding nature of directly measuring BGB, it is commonly estimated indirectly through equations that predict root biomass based on shoot (above-ground) biomass. Mokany et al. (2006) proposed the following BGB equation for tropical or subtropical moist forests and plantations (Mokany et al., 2006; Walker et al., 2017):

$$BGB = 0.205 \times AGB$$

2.3.2.3 Conversion of total biomass to carbon

After summing AGB and BGB, it is essential to convert the total biomass to carbon. Most current estimates for tropical forest carbon pools and fluxes assume that all tissues (wood, leaves, roots) are composed of 50% carbon on a dry mass basis. However, according to Martin and Thomas (2011), a biomass-to-carbon conversion factor of 47.4% is considered the most reliable, analytically supported value for the carbon content of tropical hardwoods in natural forests (IPCC, 2006; Martin & Thomas, 2011).

$$Total\ Biomass = AGB + BGB$$

$$Total\ Carbon = Total\ Biomass \times 47.4\%$$

2.3.2.4 Total carbon sequestration calculation

To calculate the total carbon sequestration rates, representing the CO_2 equivalent sequestered by a tree over its lifetime, an additional equation is required. Given that CO_2 comprises one carbon atom and two oxygen atoms, with atomic weights of 12 u for carbon and 16 u for each oxygen atom, the mass ratio of CO_2 to carbon is 44/12, or roughly 3.67. Therefore, to estimate the total amount of carbon dioxide sequestered by a tree, the tree's carbon weight was multiplied by 3.67 (Fransen, 2024).

Total CO2 Weight = Total Carbon × 3.67

By calculating the carbon content for each individual tree and summing these values within each plot, the total carbon sequestration rates can be compared between plots.

2.3.2.5 Annual carbon sequestration calculation

This study also initially aimed to estimate the annual sequestration rates across the three forest types. However, due to restrictions in the available data and limitations with the two main calculation approaches, the results were not included in this report. With additional data collected in the future, the following two approaches for calculating annual sequestration could be considered.

Firstly, the average annual rate, calculated by dividing total carbon by forest age, assumes a constant sequestration rate over time. However, carbon sequestration rates typically vary, with younger forests sequestering carbon more rapidly during growth phases and slowing down as they mature (Grebner et al., 2022). This method oversimplifies these dynamics, failing to capture variations in sequestration across different forest types. Calculating annual sequestration rates by dividing total carbon sequestration by forest age only gives an average, not the actual rate at each specific time point. For this study this approach was neglected because it does not accurately reflect the temporal changes in sequestration, especially for younger forests where growth rates are not uniform (Ghazoul & Sheil, 2017).
Secondly, the year-to-year rate method, on the other hand, was not suitable due to the limited number of data points available for certain forest types. With only three time points for planted forests and one for old-growth forests, calculating year-to-year differences would result in unreliable estimates, especially with such sparse data. This method was not used because it would introduce significant uncertainty and could lead to misleading interpretations. Given these limitations, more frequent data collection across a longer time span is necessary to obtain more reliable annual sequestration values (Penn State Extension, 2023).

2.3.3 Statistical testing

In terms of statistical testing, key variables, including DBH and tree height, were summarised through descriptive statistics to provide an overview of forest structure across all datasets and the three forest types, establishing baseline characteristics. Boxplots were generated to visually compare these measurements, highlighting variations in height and DBH among planted, natural regrowth, and old-growth forests to assess structural differences. Normality tests were then conducted on each dataset to assess data distribution and inform the selection of appropriate statistical methods. Total carbon sequestration values were subsequently calculated and plotted by time point and forest type to identify trends in sequestration over time within each forest category. Canopy cover and slope were analysed across forest types, with averages calculated and slope variability explored to gain further insights into how these variables vary across the different forest types. Finally, a Generalised Additive Model (GAM) was applied to investigate relationships between carbon sequestration and predictor variables, such as canopy cover, slope, and age, in order to identify significant patterns and nonlinearities influencing carbon sequestration.

3. Results and Discussion

3.1 Descriptive Statistics

3.1.1 Summary statistics of DBH and Total Height

Providing a table of summary statistics of each dataset provides the first numerical view of the of key statistical measures, including median, means, and quartiles. This gives a quick snapshot of each dataset's central tendency, making it easier to compare them across different tree measurement variables. Since DBH and Height are the key variables in each data set, emphasis was placed on them.

Dataset	DBH_Min	DBH_1st_Qu	DBH_Median	DBH_Mean	DBH_3rd_Qu	DBH_Max	Height_Min	Height_1st_Qu	Height_Median	Height_Mean	Height_3rd_Qu	Height_Max
<chr>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
El_Jilguero_Planted_2008a	10.4	11.5250	16.90	17.55000	23.075	26.8	9.30	13.6250	16.375	16.49000	19.9375	23.00
El_Jilguero_Planted_2008b	10.5	11.9000	12.50	15.82857	20.600	22.8	6.50	11.2500	13.000	13.60714	16.6250	20.00
Los_Quetzales_Planted_2009a	10.0	13.0000	14.70	15.21818	16.700	23.7	10.00	12.2500	14.000	14.22727	16.0000	18.50
Los_Quetzales_Planted_2009b	13.6	14.3000	16.20	18.81429	20.300	32.7	15.00	16.5000	18.500	18.64286	19.2500	25.50
El_Jilguero_Planted_2011a	10.1	10.9000	13.10	14.90909	17.300	28.1	8.25	12.3750	17.250	16.97727	20.2500	27.50
El_Jilguero_Planted_2011b	10.3	12.4000	18.00	17.04444	20.400	24.4	13.70	14.5000	19.750	19.55556	21.7500	34.50
El_Jilguero_NR_1988a	10.2	12.8125	13.75	18.71250	15.575	68.0	2.50	9.2500	14.500	12.66667	16.2500	19.00
El_Jilguero_NR_1988b	10.1	12.4500	22.25	22.17500	29.625	39.5	9.00	12.9000	15.500	15.82500	19.0000	23.00
Montaña_NR_2004a	10.1	11.3250	14.90	17.80714	19.550	46.5	10.50	12.3125	13.500	14.87500	16.4375	25.25
Montaña_NR_2004b	10.9	12.2000	14.30	15.18182	17.500	21.9	10.50	13.8750	16.500	16.00000	17.8750	21.00
El_Jilguero_NR_2008a	10.0	12.9750	15.00	16.45000	20.850	22.0	11.00	12.0000	13.000	13.87500	14.7500	21.00
El_Jilguero_NR_2008b	10.2	14.6000	22.20	20.80000	24.200	33.4	7.00	12.0000	16.000	14.94444	17.0000	22.00
El_Jilguero_Old_Growth	10.4	11.8000	15.20	19.93077	26.100	57.1	5.40	11.1000	15.800	16.41923	18.7000	29.25
Montaña_Old_Growth	10.6	13.3000	22.50	22.98235	29.400	46.8	7.75	16.7500	21.700	20.44118	24.0000	32.05
Sentinel_Old_Growth	16.0	26.9500	30.75	39.05000	31.625	99.2	7.75	14.6875	22.875	22.95833	24.8750	46.75

Table 1- *Table of Summary Statistics for each Data Set.*
Note. Derived from R Studio.

As depicted in Table 1, this dataset offers a comprehensive view of tree structural attributes—diameter at breast height (DBH) and height—across planted, naturally regenerating, and old-growth forests in various stages of development. The planted plots (2008, 2009, and 2011) show relatively uniform DBH values, ranging from 10 to 32.7 cm, with maximum heights around 25.5 metres. This suggests younger, actively growing trees. In contrast, natural regrowth plots that have more age variance between the years 1988, 2004, and 2008, display a broader DBH range, with some trees reaching up to 68 cm, indicating older, more established trees with more advanced structural development. Old-growth forests exhibit the most considerable size diversity, especially in Sentinel, where DBH reaches 99.2 cm and tree heights extend up to 46.75 metres. These old-growth metrics indicate significant structural complexity and biomass accumulation, critical for carbon sequestration. The differences in DBH and height hint at each forest type's unique growth patterns and stages of development.

3.1.2 Boxplot Overview of DBH and Total Height

To further get insights into outliers and general tendencies and dispersion measures, box plots between all datasets for the variables DBH and Tree Height were created.

For DBH:

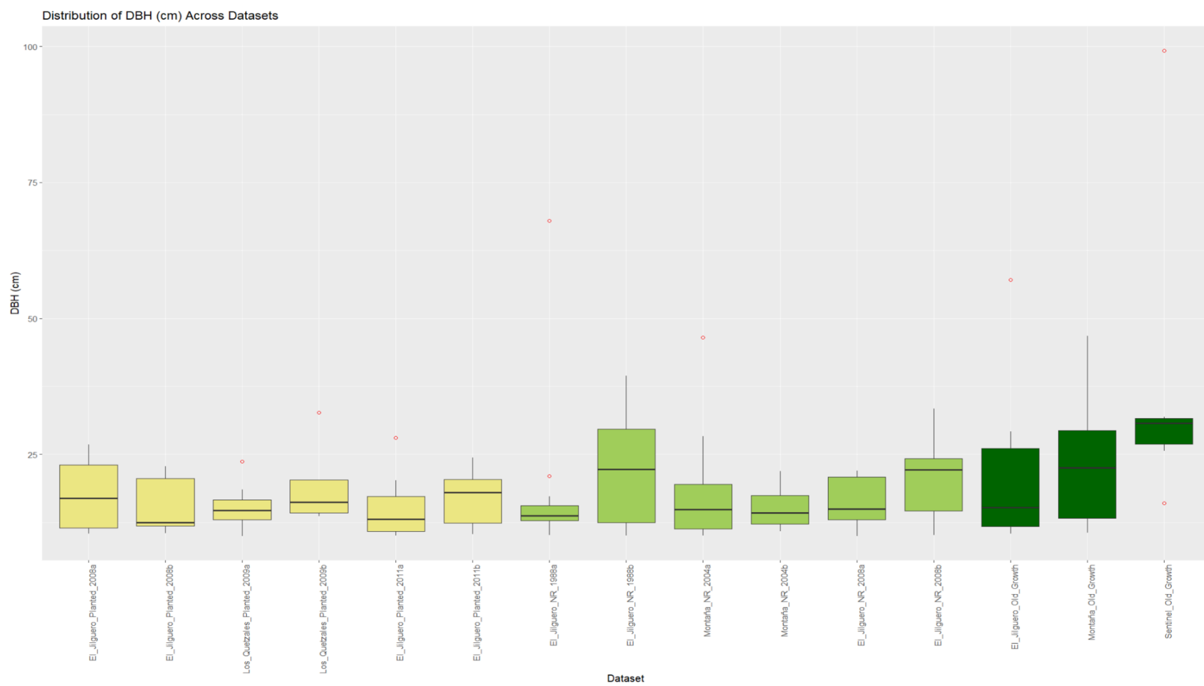


Figure 5- *Box Plots for DBH Values for each Data Set*.
Note. Derived from R Studio.

This overview of boxplots as seen in Figure 5, further confirms that natural regrowth sections and old growth forests tend to have larger DBH values and more variability, particularly in older datasets (e.g., El Jilguero Natural Regrowth 1988b and Montaña Old Growth), indicating older, mature forests with greater diameter diversity. The Sentinel data shows the highest DBH median, emphasising that the old growth sections generally have the trees with the biggest diameters. Planted datasets show smaller DBH values, with more uniform growth. Significant outliers occur in each forest type.

For the Total Height:

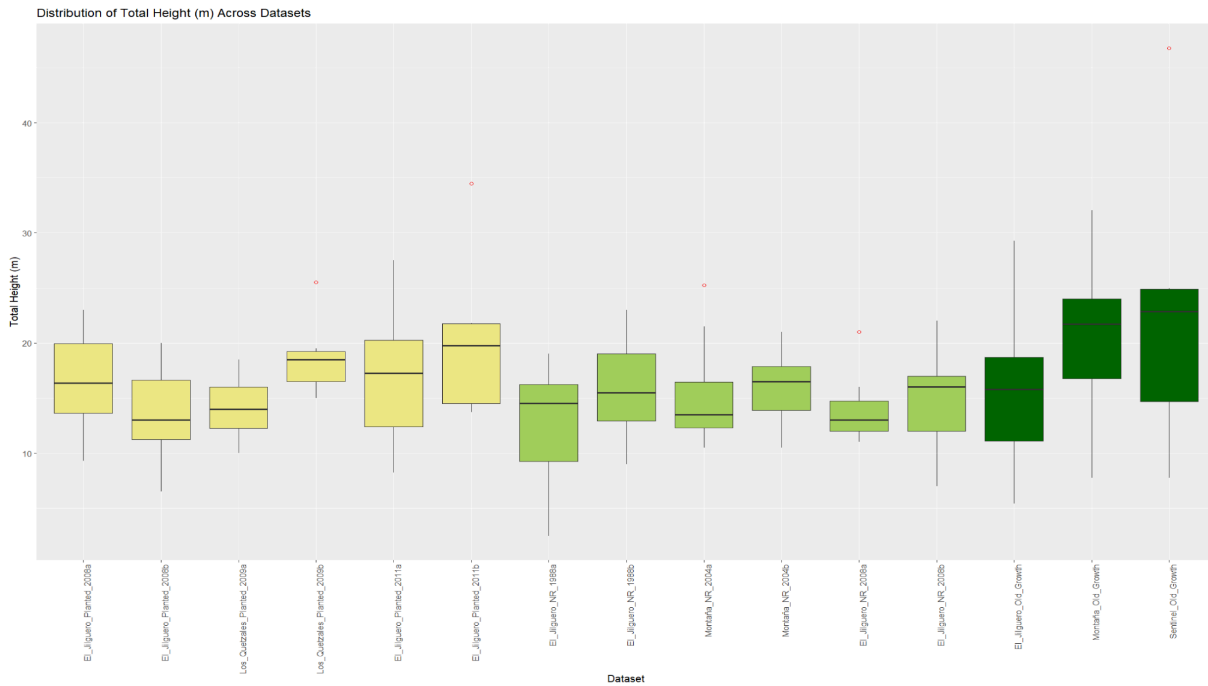


Figure 6- *Box Plots for Total Height Values for each Data Set.*
Note. Derived from R Studio.

In this boxplot summary, of tree heights across various datasets, shown in Figure 6, distinct patterns appear among the three forest types. Overall, tree height variability in both planted and natural regrowth sections is comparable, although average tree heights in planted sections are slightly higher than in natural regrowth. This is somewhat unexpected, as the 1988 section of natural regrowth is considerably older than any planted or younger regrowth sections, suggesting it should contain taller trees. These results indicate successful growth in the hand-planted trees and suggest that natural regrowth may take longer to achieve similar heights when left undisturbed. Old-growth forests (e.g., Montaña Old Growth, Sentinel Old Growth) show the tallest trees, with higher medians and wider interquartile ranges, characteristic of mature ecosystems where trees have had ample time to reach impressive heights.

3.2 Normality test

A normality test was conducted on each dataset to assess the distribution of the data, which facilitated the selection of appropriate statistical tests for subsequent analysis. Table 2 provides all Shapiro Wilk and p-values calculated.

Data Set / Time Point	**Forest Type**	**Results for Shapiro-Wilk Normality Test**
El Jilguero 2008a	Planted	Shapiro-Wilk W = 0.5687994 p-value = 6.729475e-12
El Jilguero 2008b	Planted	Shapiro-Wilk W = 0.5646377 p-value = 1.376453e-10
Los Quetzales 2009a	Planted	Shapiro-Wilk W = 0.6138226

		p-value = 4.313711e-12
Los Quetzales 2009b	Planted	Shapiro-Wilk W = 0.7380707 p-value = 1.671192e-07
El Jilguero 2011a	Planted	Shapiro-Wilk W = 0.7088794 p-value = 9.763122e-11
El Jilguero 2011b	Planted	Shapiro-Wilk W = 0.6848404 p-value = 1.110733e-09
El Jilguero 1988a	Natural Regrowth	Shapiro-Wilk W = 0.5141508 p-value = 5.835884e-14
El Jilguero 1988b	Natural Regrowth	Shapiro-Wilk W = 0.7117214 p-value = 1.351572e-08
Montaña 2004a	Natural Regrowth	Shapiro-Wilk W = 0.6105091 p-value = 9.198453e-14
Montaña 2004b	Natural Regrowth	Shapiro-Wilk W = 0.512578 p-value = 1.123528e-13
El Jilguero 2008a	Natural Regrowth	Shapiro-Wilk W = 0.6101491 p-value = 1.024028e-12
El Jilguero 2008b	Natural Regrowth	Shapiro-Wilk W = 0.6831589 p-value = 1.035435e-09
El Jilguero	Old Growth	Shapiro-Wilk W = 0.6264999 p-value = 1.319382e-12
Montaña	Old Growth	Shapiro-Wilk W = 0.7435051 p-value = 3.444568e-12
Sentinel	Old Growth	Shapiro-Wilk W = 0.7101948 p-value = 2.413505e-07

Table 2 - *Results of Shapiro-Wilk Test on each Data Set.*
Note. Derived from R Studio.

The Shapiro-Wilk test results indicate that for each dataset (both planted and natural regrowth at various time points, as well as old growth), the p-values are all very low ($<< 0.05$). This suggests that none of the datasets are normally distributed. The low p-values (much smaller than 0.05) furthermore indicate that the null hypothesis of normality for each time point and forest type. The Shapiro-Wilk W statistic varies, but overall, values below 0.75 often indicate strong deviations from normality, as seen in many of these given results.

Regarding further implications on statistical testing, since none of the datasets meet normality assumptions, non-parametric tests for further tests are more suitable than parametric tests (Wasserman, 2006). The lack of normality for those datasets, typically seen in ecological and environmental datasets can be explained through various factors, such as species diversity, age and disturbance within the forests, environmental gradients, or localised conditions such as slope and canopy cover (Patil & Rao, 1994).

3.3.Carbon Sequestration Analysis
3.3.1 Total Carbon Calculations

After applying the given calculations for obtaining the AGB and BGB, and merging both data sets for each time point, the following total CO2 weights for the time points were calculated.

Forest Type and Time Point	**Total Carbon Sequestration Values**
Planted forest from 2008	0.2514053 t CO2/ha
Planted forest from 2009	0.2733983 t CO2/ha
Planted forest from 2011	0.2599537 t CO2/ha
Natural Regrowth Forest from 1988	0.3128579 t CO2/ha
Natural Regrowth Forest from 2004	0.2724769 t CO2/ha
Natural Regrowth Forest from 2008	0.2851661 t CO2/ha
Old Growth Forest from 1930	1.565948 t CO2/ha

Table 3 - *Total Carbon Sequestration Values for each Time Point.*
Note. Derived from R Studio.

Visualised in Bar Graphs these values are depicted as such:

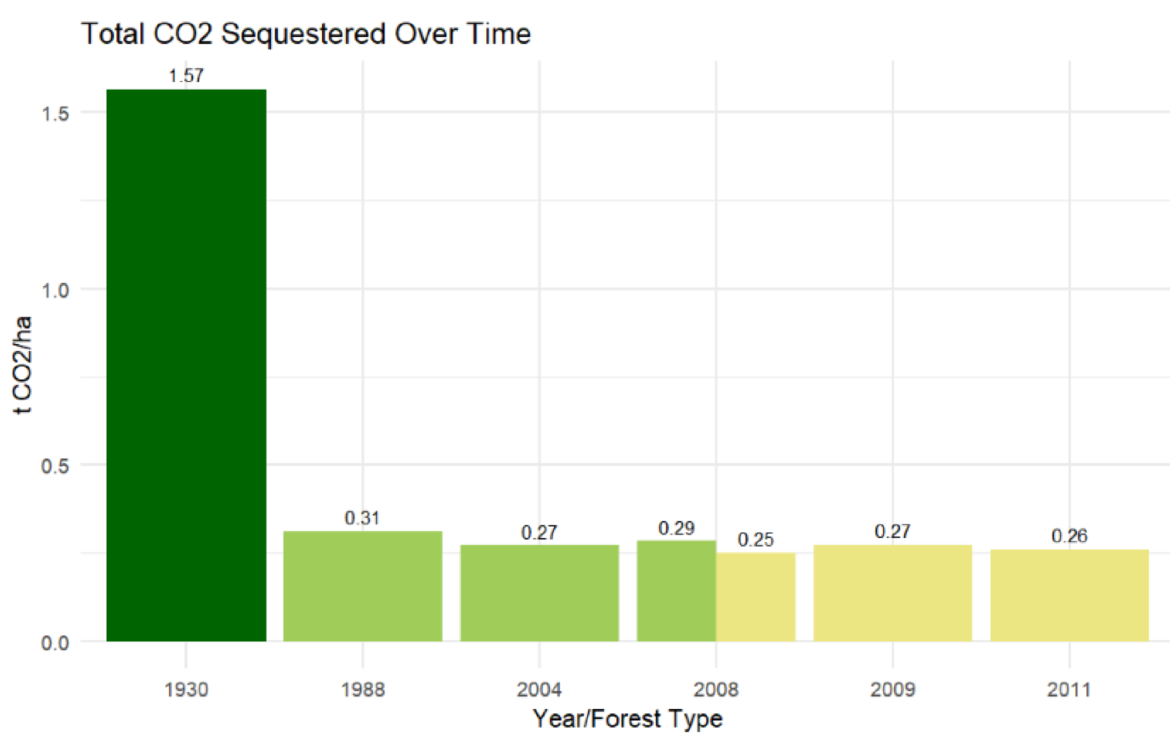


Figure 7 - *Total Carbon Sequestration Values for each Time Point in Bar Graphs.*
Note. Derived from R Studio.

To further visualise the total carbon sequestration values in a line graph, better depicting the time difference between the time points, this graph shows further insights:

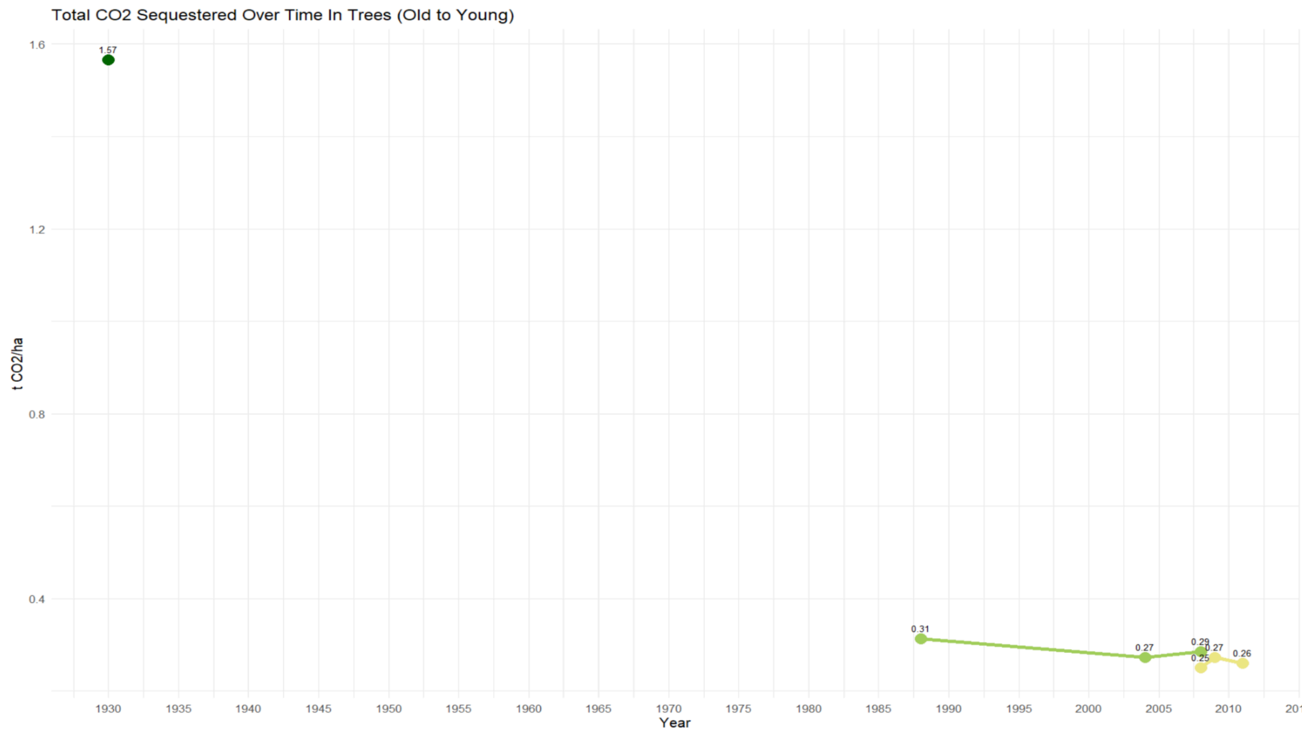


Figure 8 - *Total Carbon Sequestration Values for each Time Point in Line Graphs.*
Note. Derived from R Studio.

As shown in Table 3 and Figures 7 and 8, the old-growth forest exhibits the highest carbon sequestration at 1.5659 t CO2/ha, reflecting its mature and extensive biomass characteristic of well-established ecosystems. This observation aligns with findings that mature, stable forest ecosystems, like old-growth forests, are highly efficient at long-term carbon storage due to their complex structures and biodiversity (Grebner et al., 2022).

In comparison, natural regrowth forests demonstrate slightly higher carbon sequestration rates than the planted sections, with the 1988 natural regrowth forest having the highest sequestration at 0.3129 t CO2/ha. This is followed by the 2008 and 2004 natural regrowth forests, with values of 0.2852 t CO2/ha and 0.2725 t CO2/ha, respectively. Notably, the 2008 section of natural regrowth demonstrates a successful performance, nearly matching the sequestration levels of the much older 1988 section, suggesting particularly effective growth during its early stages. The lower carbon sequestration rate in the 1988 section, despite its age, is notable and may be attributed to the slower establishment commonly observed in naturally regenerating forests. Without the benefit of planting or human intervention, natural regrowth must contend with more competition from pre-existing shrubs and other early colonising plants, which can slow early-stage tree growth and delay substantial carbon accumulation (Ghazoul & Sheil, 2017).

For planted forests, the 2008, 2009, and 2011 sections sequester between 0.25 to 0.27 t CO2 per hectare, with the 2009 section achieving the highest value at 0.2734 t CO2/ha. This relatively consistent sequestration across the four-year span is not unexpected, given the short time frame, and suggests that significant variations in carbon accumulation are more likely to emerge as these forests mature. While the natural regrowth sections show slightly higher carbon values, the planted sections appear competitive in their carbon accumulation, especially considering their younger ages. A likely reason for this is human facilitation in planted forests, which involves selecting suitable species and minimising competition, allowing for faster growth and greater initial carbon sequestration. As the planted and natural regrowth forests progress, their carbon sequestration values are expected to rise with the maturation of their structures. In contrast, the old-growth forest has likely reached its climax stage, with little potential for further carbon accumulation (Staples et al., 2019).

Factors that are known for influencing the carbon sequestration potential of forests, and might differ between forest types at Cloudbridge, need to be studied more. Generally, abiotic factors include non-living components such as temperature, sunlight, and soil composition, which significantly affect the efficiency of photosynthesis and, consequently, carbon sequestration. The availability of sunlight is crucial for photoautotrophs to convert CO2 into organic carbon. Also, slopes impact carbon sequestration because they influence soil depth, moisture retention, and nutrient availability, all of which affect tree growth and biomass accumulation (Bonan, 2023).
Secondly, biotic factors such as living organisms, including plants and microbes, play a vital role in carbon fixation. The health and diversity of these organisms can enhance the carbon sequestration process. For instance, different plant species have varying capacities to absorb CO2, which influences overall carbon storage. Furthermore, the condition of soil is essential for maintaining carbon reserves. Healthy soils with rich organic matter can store more carbon, while degraded soils may release stored carbon back into the atmosphere. Improving soil organic carbon is crucial for the health of terrestrial ecosystems. Moreover, climate change with altered climatic conditions can influence the effectiveness of carbon sequestration processes. Increased temperatures and altered precipitation patterns can affect plant growth and microbial activity, thereby influencing the overall carbon capture capabilities of ecosystems (Bonan, 2023).
For this research, the variables that emerged as the two most influential factors affecting carbon sequestration that could be analysed within the reserve, were canopy cover (availability of sunlight) and the slope, given their known impact on carbon storage levels. However, further research should be conducted on any of the other environmental factors possibly influencing carbon to possibly inform the reserve on what conditions and environments Cloudbridge can support to foster a steady carbon accumulation.

3.4 Canopy Cover and Slope Analysis

Before understanding and testing for the influences the variables of canopy cover and slope have on carbon sequestration, firstly an overview of the average canopy cover and slope were provided, and subsequently graphs of the individual slope values per data set were visualised.

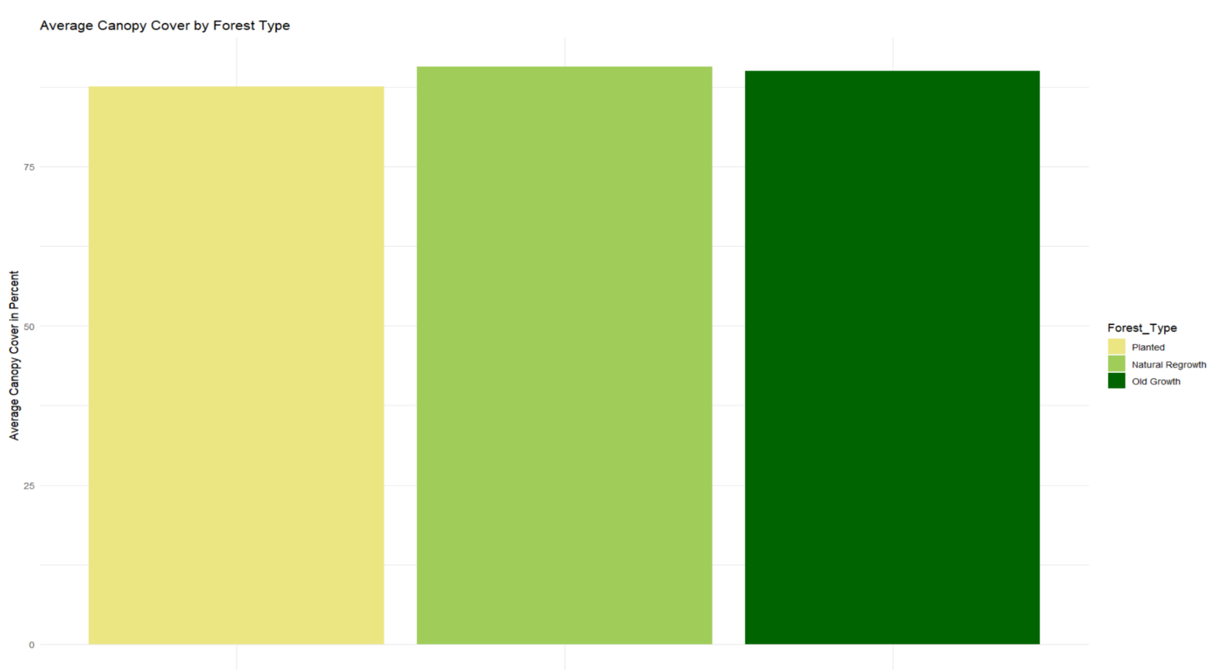


Figure 9 - *Average Canopy Cover Values by Forest Type.*
Note. Derived from R Studio.

As illustrated in Figure 9, average canopy cover varies only slightly among the forest types, with values of 87.62% for planted forests, 90.74% for natural regrowth, and 90.05% for old-growth. This narrow range, spanning just over 3%, reflects a generally consistent canopy density across all forest types, suggesting healthy growth overall—particularly valuable for assessing the condition of both planted and naturally regrowth areas.

While these canopy cover differences may not be statistically significant based on the current data, the marginally higher values in natural regrowth and old-growth forests could indicate more advanced structural development compared to planted forests. If further studies at Cloudbridge were to confirm this as statistically significant, even small canopy cover variations might impact factors like light availability, soil moisture, and understory growth, all of which could affect carbon sequestration rates and overall ecosystem health (Ghazoul & Sheil, 2017). Notably, canopy cover does not appear directly related to forest age among these types, age portrayed within the forest types, suggesting it can serve as an independent variable in further testing of relationships between ecological factors and carbon sequestration.

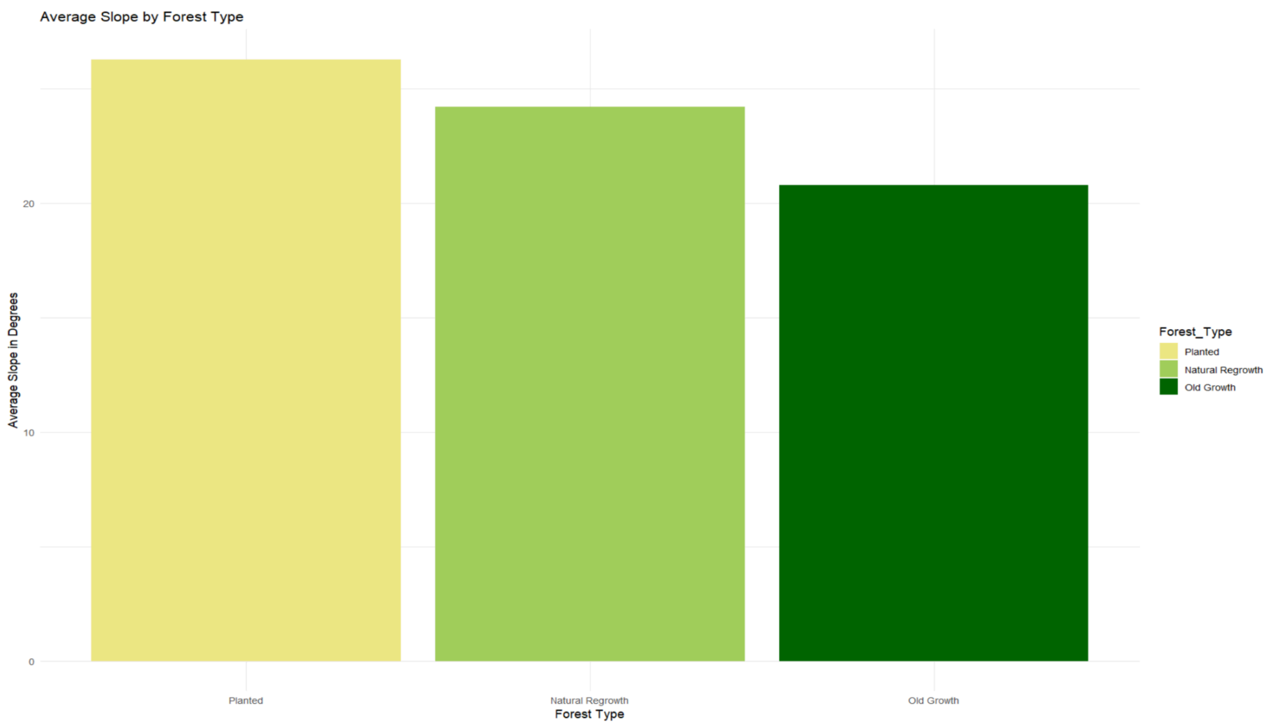


Figure 10 - *Average Slope Values by Forest Type.*
Note. Derived from R Studio.

In terms of average slope values, as visualised in Figure 10, these values vary across the forest types: 26.28° in planted forests, 24.20° in natural regrowth, and 20.78° in old-growth forests. These differences suggest a slightly steeper terrain for the planted forests compared to the old-growth areas. Higher slopes can introduce environmental stressors, such as soil erosion and reduced water retention, potentially influencing tree growth and carbon sequestration. These conditions could also lead to differences in soil composition and nutrient availability, impacting the development and carbon storage potential of each forest type. Further statistical analysis attempts to confirm if these slope variations significantly affect biomass and carbon dynamics across the study area (Bonan, 2023).

As no significant difference in canopy cover was observed between the forest types with the current data, a detailed breakdown of canopy cover across individual datasets is not provided. However, for slope values, which show notable variation across forest types as discussed, a graph has been included to illustrate this variation within the individual datasets.

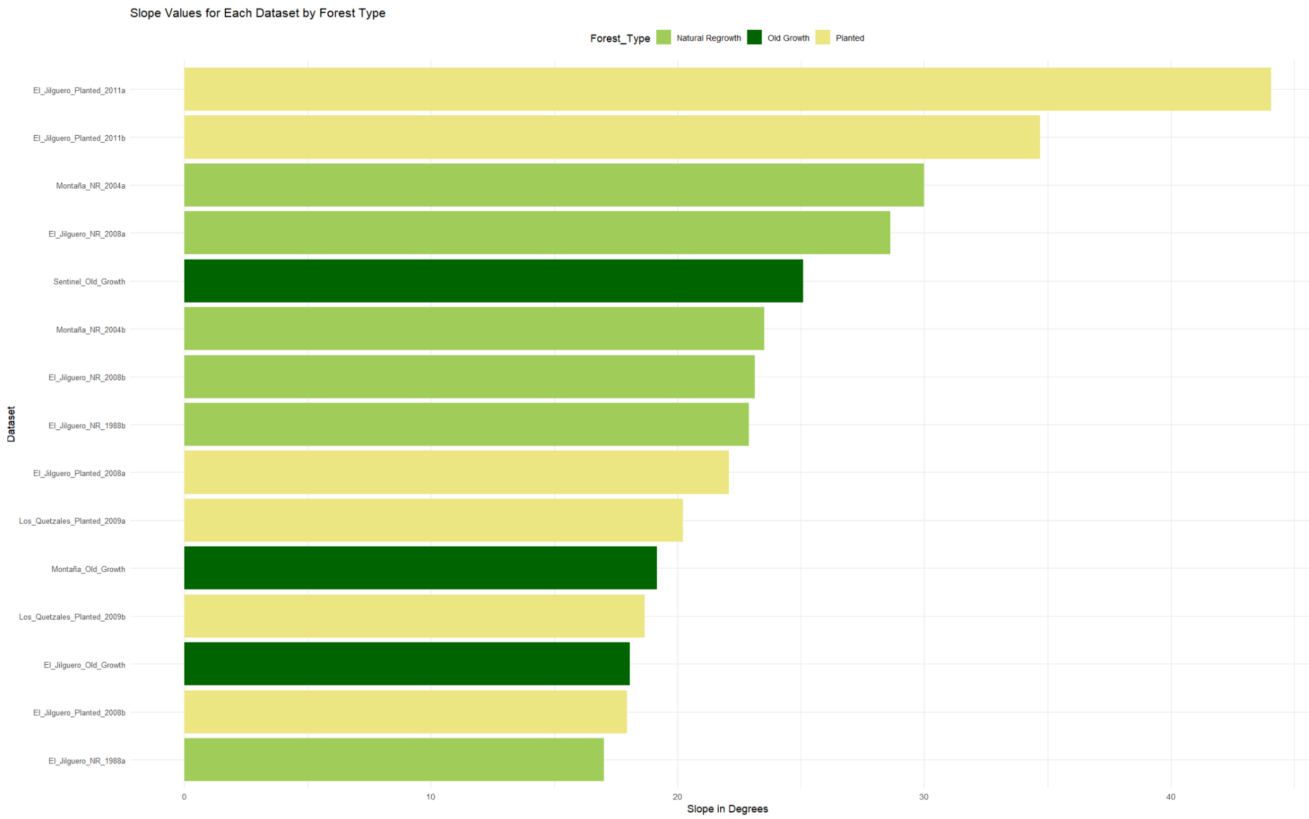


Figure 11 - *Slope Values for Each Dataset by Forest Type.*
Note. Derived from R Studio.

A visual inspection of Figure 11 indicates possible differences in slope values across datasets within each forest type, with planted forests generally showing higher slopes than natural regrowth and old-growth areas. This pattern hints at a potential systematic difference rather than purely random variation, although statistical testing would be necessary to confirm this hypothesis. However, this study has not delved further into these potential differences, as the main focus for this research is carbon sequestration.

One reason for considering this observation as likely due to random variation is that the Cloudbridge Reserve is known for its highly variable slope across all forest types. Extensive field experience has shown that steep sections exist in every forest type, suggesting that slope may not differ systematically among them. With only fifteen data points, the available sample might not adequately capture this variability and is therefore more likely to reflect random fluctuations.

Additionally, plot locations were specifically selected near existing trails due to accessibility constraints, introducing a sampling bias. Although the sampled areas show relatively lower slopes in natural regrowth and old-growth forests, other areas within these forest types are known to have steep slopes, which were not included in the sample. The plot selection based on proximity to trails may thus skew the interpretation. To accurately determine if there is a genuine difference in slope among the forest types, a more comprehensive study with additional, randomly distributed plots across the reserve would be necessary.

3.5 Relationship Analysis

Given that none of the datasets met normality as indicated by the Shapiro-Wilk test, a nonparametric approach like the Spearman correlation initially seemed appropriate for examining relationships between slope, canopy cover, age, and carbon sequestration. This could be followed by multiple linear regression to quantify these relationships further. However, both methods assume linearity or monotonicity, which may not align with the complex, non-linear nature of ecological data, such as carbon sequestration. Fitting a linear regression model to non-linear data can lead to biassed estimates and misleading conclusions (Wassermann, 2006).

For this reason, Generalised Additive Models (GAMs) were selected for their ability to handle non-linear relationships. GAMs fit smooth functions to predictors, allowing them to model complex ecological dynamics without assuming rigid linear trends. This flexibility makes them particularly useful for studying carbon sequestration. To assess how total carbon sequestration rates are influenced by slope, canopy cover, and age, a GAM was selected to analyse the relationships between these variables. Next to accommodating non-linear relationships, GAMs allow each predictor to have an independent, additive effect on the response variable—in this case, carbon sequestration. This makes GAMs a flexible choice when domain knowledge suggests that each variable contributes independently to the target variable (Gomez-Rubio, 2018).

As described above, in this study, canopy cover was found to have minimal variation between forest types, suggesting that it likely does not contribute significantly to differences in sequestration rates across these forests. Additionally, observed differences in slope between forest types appear to reflect random variation rather than systematic age-related patterns. Consequently, it was deemed appropriate to treat canopy cover, slope, and age as independent factors affecting carbon sequestration. Using a GAM enables a more refined exploration of how each of these factors individually influences sequestration rates, providing insights that might be missed by models assuming strictly linear or interactive effects. It is important to note, however, that other variables may also influence carbon sequestration, but these have not been thoroughly examined in this study.

The GAM was applied to the entire dataset, incorporating planted, natural regrowth, and old-growth forest types. This approach was selected to capture general trends across all forest types, minimising the risk of over-interpreting sparse data, particularly for old-growth forests, which only had a single time point. A forest-type-specific GAM would have been unfeasible, as it would have been limited to only three time points per forest type for planted and natural regrowth forests, and a single time point for old-growth, making it impossible to model old-growth data effectively. Analysing the data as a whole allowed for a broader understanding of how age, slope, and canopy cover influence carbon sequestration across the reserve, even though it meant that specific forest-type effects could not be fully explored, which could be focussed on in future research with more time points available.

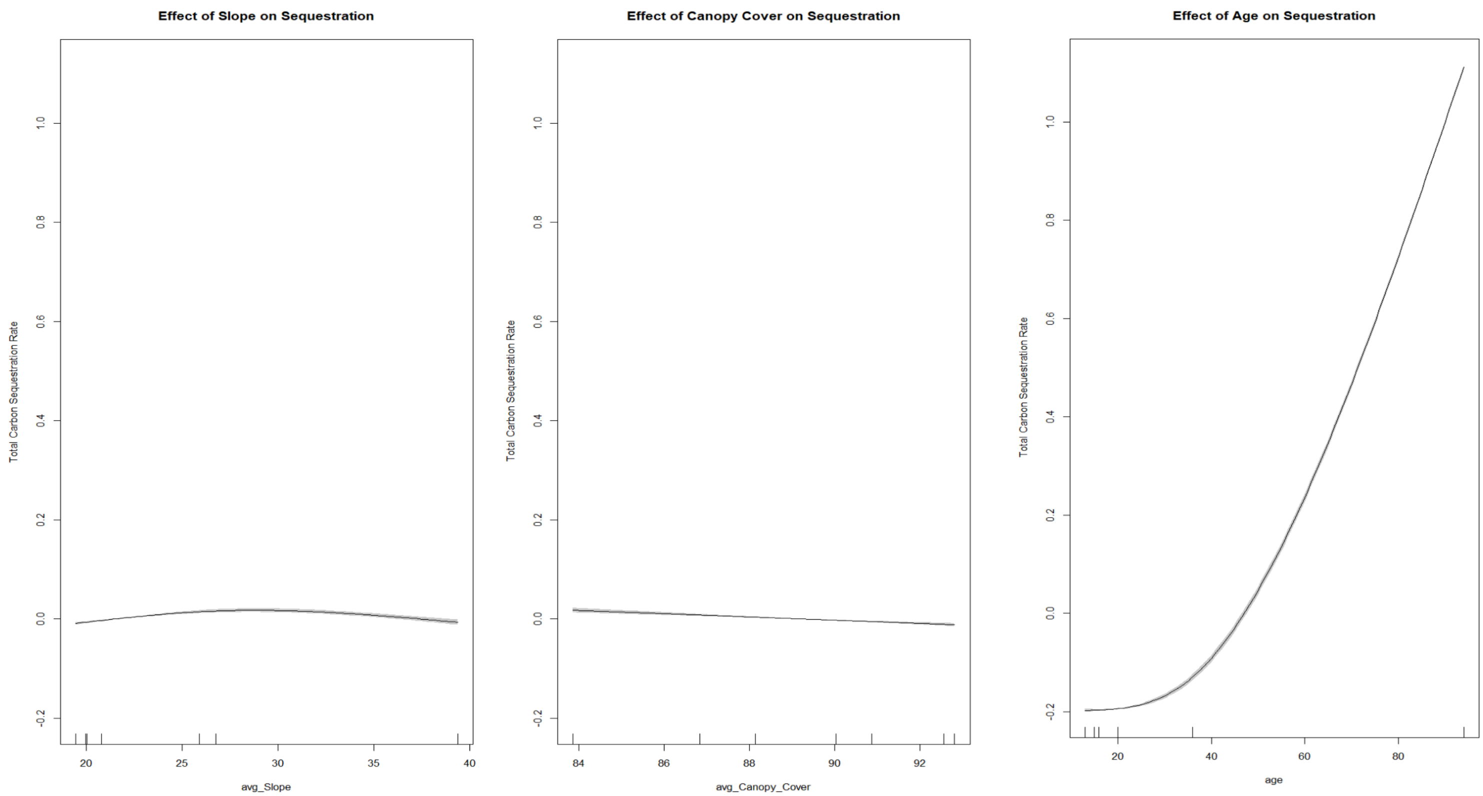


Figure 12 - *Effects of Slope, Canopy Cover and Age on Total Carbon Sequestration.*
Note. Derived from R Studio.

Figure 12 and the printed statistical values reveal following key insights about the model:

Slope: The relationship between slope and carbon sequestration is nearly linear (edf = 1.990), though the effect is marginally non-significant (p = 0.09788). The plot suggests that slope has a weak influence on total carbon sequestration, with a nearly flat relationship observed within the data range. With a larger dataset, slope may show stronger associations, particularly in areas with greater variation in topography.

Canopy Cover: Similar to slope, canopy cover exhibits a weak association with carbon sequestration (F-statistic = 56.61, p = 0.084), with a nearly flat relationship. However, canopy cover could become a more significant factor with a larger dataset, especially if there is greater variability in canopy density across forest types.

Age: The relationship between age and carbon sequestration is strong and non-linear (edf = 1.999, p = 0.00211). This confirms that age is a significant predictor, with older forests storing more carbon due to biomass accumulation over time. The non-linear pattern reflects rapid carbon uptake in younger forests, followed by a stabilisation as they mature.

Intercept: The intercept is statistically significant, with an estimate of 0.4601723 (p = 0.00119), representing the baseline total carbon sequestration rate when all predictors are at their reference level.

Model Fit: The model explains 100% of the variance (R-squared = 1), but given the small sample size (n = 7), this may indicate overfitting. The GAM's flexibility was constrained by setting a low number of degrees of freedom (k=3), which helps mitigate overfitting and ensures that the model focuses on broad trends rather than overly complex curves.

Although this analysis found that age was the most significant factor affecting carbon sequestration levels, other studies have highlighted the potential impact of variables like slope and canopy cover. Research indicates that canopy cover, in particular, is often positively correlated with carbon sequestration. For instance, in studies conducted in Ethiopia, although proven in a different ecosystem, increased canopy cover has been shown to enhance aboveground biomass carbon stocks (Solomon et al., 2024), suggesting that forests with denser canopies tend to sequester more carbon. Conversely, more open canopies in younger forests might suggest faster growth phases, contributing to different sequestration dynamics (Bonan, 2023).

Similarly, slope also plays a role in carbon storage, with some studies showing that both slope and elevation can significantly influence carbon sequestration rates. In general, slope can affect soil moisture, erosion, and tree stability, which all play roles in tree growth and, consequently, carbon sequestration rates. Steeper slopes may experience more runoff and soil loss, potentially reducing growth rates, while gentler slopes might retain moisture better, supporting more biomass (Grebner et al., 2022). More specifically, Singh & Benbi (2018) have proven the slope position significantly affects carbon sequestration in soils. The study found that the highest carbon were observed at hilltops and back slopes, while bottomlands showed a decrease and back slopes under erosion had the lowest values. This indicates that higher elevations tend to sequester more carbon compared to lower, eroded positions. Furthermore, the quality of organic matter also varies with slope position. Hilltop soils had a greater proportion of recalcitrant organic matter, which is more stable and less prone to decomposition. In contrast, middle slope soils had a higher proportion of labile organic matter, making them more susceptible to loss (Singh & Benbi, 2018). Although current data does not yet confirm the effects of varying slope values, further research is necessary to explore how the slopes at Cloudbridge might influence tree carbon accumulation across the reserve.

In this context, expanding the dataset at Cloudbridge could reveal stronger and more statistically significant relationships between these environmental variables and carbon sequestration. With long-term observations, more data points collected and additional variability in the data, it would be possible to explore how slope and canopy cover, alongside other factors, contribute to carbon storage across different forest types more thoroughly. This approach may also help refine understanding of how forest management practices could optimise carbon sequestration in reforestation efforts.

3.6 Limitations

The limitations in this research stem from both methodological constraints and data availability impacting the accuracy of insights into carbon sequestration patterns across forest types.

3.6.1 Data Collection

The data collection process faced several limitations that impacted the accuracy and comprehensiveness of the carbon estimates in this study. Measuring tree heights was challenging, particularly for very tall trees, as the clinometer used occasionally struggled to provide precise measurements at extreme heights and very dense canopy cover that limited visibility. This may have introduced some inaccuracies in the height data, subsequently affecting the carbon calculations for these trees.

Additionally, for the natural regrowth plots at El Jilguero (2008), the available area was so small and mostly inaccessible due to steep slopes, that setting up more than two plots would have been very difficult. This limits the data robustness for this site.

Resource limitations of the researcher also impacted the scope of data collection. To simplify

measurements within the time available, only trees with a diameter at breast height (DBH) of 10 cm or greater were included. However, both the planted and natural regrowth forests contained substantial growth below this threshold, meaning younger and thinner trees, which also sequester carbon, were excluded from analysis. This exclusion likely led to an underestimation of carbon stocks in these areas. In contrast, nearly every tree in the old-growth forest met the DBH threshold, so carbon estimates in this forest type may be more comprehensive.
This DBH threshold also excluded non-tree carbon sources, such as ground vegetation, deadwood, leaf litter, and soil organic carbon, all of which contribute significantly to total ecosystem carbon storage. Without these components, the data do not capture the full scope of the forest's carbon cycle, including processes like decomposition and nutrient cycling that affect long-term carbon sequestration.

3.6.2 Limited Data Availability

Furthermore, several data limitations affected the scope of this analysis. First, a limitation involves the varying time frames of data collection across forest types. The planted forest areas have shorter, more recent time windows than the natural regrowth sections, which span longer periods, as Cloudbridge began natural reforestation efforts earlier than active planting. These differing time frames make direct comparisons across forest types challenging. Additionally, only one data point is available for the old-growth forest, which restricts any assessment of carbon accumulation rates over time. Given that the forest was last cut in the 1930s, all measurements for old-growth forest represent a single time point.

Secondly, with the available data, it was not feasible to model future growth, which would have provided valuable insights. This analysis offers only a snapshot of current carbon stocks across different forest types, allowing for an understanding of carbon storage capacity but limiting insight into long-term growth trends. Consequently, accurately estimating future carbon sequestration rates is challenging with the limited temporal data points available.

Species identification also posed a limitation. Due to the extreme height of many trees, leaves were often difficult to observe, making accurate species identification and use of precise allometric equations for wood density challenging. This constraint could slightly reduce the accuracy of carbon estimates.

Data may also be skewed by inconsistencies in tree ages within each forest section. While each forest type generally aligns with a certain planting or growth period, some sections contain trees older than the documented start date. This variability could affect the accuracy of time-specific carbon sequestration data, as trees not representative of the designated growth period were included in measurements.

3.6.3 Data Analysis Limitations

Firstly, limited statistical expertise presented some constraints in the analysis, potentially resulting in overlooked techniques or misapplied analyses. Errors may have occurred in developing or interpreting R code, which could impact the accuracy of findings.

More specifically in regards to the GAM analysis, this approach is partly constrained by a small sample size, limiting its ability to capture complex relationships, particularly regarding slope and canopy cover variables. Additionally, the model does not adequately represent old-growth forest behaviour due to the lack of multiple time points, raising the risk of overfitting given the dataset's small size. The high R-squared value may also overstate the model's capacity to generalise beyond this dataset.

3.6.4 Geographic Limitations

Geographic limitations further constrained the study, as data collection was restricted to plots located along accessible trails. The dense vegetation and steep slopes of the forest made it difficult to access other areas, limiting the analysis to only those portions of the forest next to the trails. This restriction means that the sampled plots may not accurately reflect the growth conditions or carbon sequestration potential of the entire forest. The trees along the trails might differ in characteristics, such as growth rates and biomass, from those in the interior of the forest, potentially leading to a skewed representation of the overall forest ecosystem.

3.7 Future recommendations

Future research with more comprehensive data across all forest types will help refine these preliminary findings and offer a clearer understanding of how total and especially annual sequestration rates evolve over time in each forest type. Expanding the range of variables assessed—such as temperature, microclimate variations, humidity, soil composition, wood density, climate change impacts—could provide valuable insights into their impacts on carbon sequestration. Additionally, measuring non-tree carbon sources such as soil carbon, deadwood, and litter would enhance our understanding of the broader carbon cycle and its role in sequestration.

Establishing permanent plots at Cloudbridge would enable accurate tracking of carbon accumulation and allow for better estimation of biomass stocks and changes over time. With more data, comparisons between planted and natural regrowth forests could be improved, offering guidance on land management practices that optimise carbon sequestration—particularly relevant in the context of climate change mitigation. If permanent plots are not feasible, increasing the number of plots at each time point and expanding this approach in future years would provide more robust data on how each forest type sequesters carbon over time.

Since part of the planted area at Cloudbridge is affected by an unidentified tree disease, comparing the impacted sections—planted between 2010 and 2011—with healthy sections from the same period (e.g., El Jilguero Planted 2011A & B) would be valuable. Initial data from one affected plot indicate significantly lower AGB and carbon values, suggesting that these sections may struggle to accumulate carbon at the same rate. This finding, coupled with the tendency for monocultures and low-diversity stands to be more susceptible to pests and diseases, could inform management practices for Cloudbridge's reforestation efforts.

For future research, modelling future carbon sequestration over time would provide valuable insights. With limited time points available, particularly in the planted sections, continuing observations and incorporating data from future observations will improve the accuracy of future sequestration rate predictions. With more data, it would also be possible to fit GAMs for each forest type, allowing a deeper understanding of how various variables influence carbon sequestration within each forest type. This could complement the current GAM that examines all forest types together.

Furthermore, using advanced forest analysis techniques, such as those found in R libraries like forestSOM, FVS (Forest Vegetation Simulator), and biomass, could provide more accurate insights into carbon sequestration at Cloudbridge.

Another potential avenue for future research is the inclusion of smaller trees (those under 10 cm DBH), which could better account for carbon storage in younger forests.

Moreover, scaling plot-level measurements to the reserve's entire area would also help provide insights into the overall carbon sequestration rate at Cloudbridge. This is useful for understanding how carbon sequestration at a small, localised level (i.e., individual plots) translates to the larger, overall forest ecosystem. It also provides a more accurate estimate of the total carbon sequestration across the entire reserve and can in turn help to understand the reserve's contribution to regional carbon storage efforts. Satellite data or aerial imagery could be used to map forest cover, density, and changes over time, enabling a more comprehensive view of forest dynamics at a landscape scale.

Further statistical testing of variables such as slope and canopy cover would clarify whether within this research observed differences between forest types are statistically significant and meaningful. Additionally, accurately identifying each tree in the plots would facilitate the use of more precise allometric equations for biomass calculation, improving the accuracy of carbon sequestration estimates. Moreover, after gathering more data, exploring methods and calculating results for annual sequestration rates could provide valuable insights into how carbon is accumulated on a year-by-year basis over time.
Finally, some literature research could be conducted to understand what factors contribute to variations in carbon sequestration between old-growth, replanted, and naturally regenerated forest types at Cloudbridge. Additionally, researching what forest management practices could optimise carbon capture potential within all three forest types.

4. Conclusion

In conclusion, this study provides valuable insights into how carbon sequestration rates vary across planted, naturally regenerated, and old-growth forests at Cloudbridge Nature Reserve. The first research question, regarding the variation in carbon sequestration rates over time, reveals that old-growth forests sequester significantly more carbon than both natural regrowth and planted forests, highlighting the greater stability and carbon storage capacity of mature ecosystems. While natural regrowth sections, especially the 1988 section, show slower carbon accumulation, likely due to competition with other vegetation, planted forests seem to capture carbon more quickly in their early stages due to reduced competition and human facilitation.

The second research question, which examines how total carbon sequestration is influenced by factors like slope, canopy cover, and age, found that forest age is strongly linked to higher total carbon accumulation, with older forests storing more carbon overall. Although no significant impact of slope or canopy cover on carbon sequestration was detected in this dataset, canopy cover across all forest types shows similar values, indicating healthy forest development regardless of the management approach. Further research with expanded datasets and long-term monitoring is needed to explore these relationships more fully and understand how slope and other ecological factors might influence carbon dynamics over time.

The importance of further research cannot be overstated. Long-term monitoring and additional data collection are vital to deepen the understanding of carbon sequestration dynamics at Cloudbridge. Such research could provide critical insights into how different forest management practices, topographic factors, and ecological conditions influence carbon storage over time. Expanding the scope of data and incorporating more frequent, longitudinal observations will allow for a more comprehensive analysis of the factors affecting carbon sequestration. This, in turn, will enable the development of more informed forest management strategies to optimise carbon capture potential and promote ecosystem resilience. In summary, while this study provides valuable insights into the current carbon sequestration potential of various forest types at Cloudbridge, ongoing research will be essential for refining our understanding and ensuring the effectiveness of conservation and reforestation efforts in the face of climate change.

5. Acknowledgements

I would like to express my sincere gratitude to Cloudbridge Nature Reserve for giving me the opportunity to conduct my research as a research intern.
I am deeply thankful for the guidance and supervision provided by my two supervisors at Cloudbridge, Greilin Fallas Rodríguez and Madelyn Peterson. I am also grateful for the support from my university supervisor at the University of Groningen, Dr. Sarah Fenon. Their expertise and valuable advice were crucial throughout my research journey.
I would like to extend my appreciation to Edgar Madrigal Fallas for generously sharing his local knowledge of tree species and the reserve, which greatly enhanced my understanding of its ecology.
Finally, I wish to acknowledge the hard work and support of the volunteers and fellow researchers at Cloudbridge, with special thanks to Bart Ruesen for his continued support and shared ecological insights.

6. References

Artaxo, P., Hansson, H. C., Machado, L. A. T., & Rizzo, L. V. (2022). Tropical forests are crucial in regulating the climate on Earth. PLOS Climate, 1(8), e0000054. https://doi.org/10.1371/journal.pclm.0000054

Bonan, G. (2023). Carbon sequestration. In Cambridge University Press eBooks (pp. 157–167). https://doi.org/10.1017/9781108601559.014

Brown, S., Gillespie, A., & Lugo, A. (1989, December). (PDF) biomass estimation methods for tropical forests with applications to Forest Inventory Data. Forest Science 35(4). https://www.researchgate.net/publication/233643575_Biomass_Estimation_Methods_for_Tropical_Forests_with_Applications_to_Forest_Inventory_Data

Benetó, P., Moutahir, H., & Khodayar, S. (2022, July 7). *Reforestation ef orts and impact on climate change*. CO Meeting Organizer Plinius17. https://meetingorganizer.copernicus.org/Plinius17/Plinius17-90.html

Cerasoli, S., Yin, J., & Porporato, A. (2021). Cloud cooling effects of afforestation and reforestation at midlatitudes. Proceedings Of The National Academy Of Sciences, 118(33). https://doi.org/10.1073/pnas.2026241118

Chave, J., Andalo, C., Brown, S., Cairns, M. A., Chambers, J. Q., Eamus, D., Fölster, H., Fromard, F., Higuchi, N., Kira, T., Lescure, J., Nelson, B. W., Ogawa, H., Puig, H., Riéra, B., & Yamakura, T. (2005). Tree allometry and improved estimation of carbon stocks and balance in tropical forests. Oecologia, 145(1), 87–99. https://doi.org/10.1007/s00442-005-0100-x

Cloudbridge. (n.d.). About - cloudbridge nature reserve in costa rica reserva natural en Costa Rica. Cloudbridge Nature Reserve in Costa Rica Reserva Natural en Costa Rica - Open to visitors 365 days a year for hiking and wildlife tours, we enable people of all generations and all walks of life to be able to enjoy and learn about this incredible part of Costa Rica. https://www.cloudbridge.org/about/

Copernicus EU. (2023, April). The Reforestation of Costa Rica. Copernicus. https://www.copernicus.eu/en/media/image-day-gallery/reforestation-costa-rica

Fransen, B. (2024, 26 september). How to calculate CO2 sequestration. EcoMatcher. https://www.ecomatcher.com/how-to-calculate-co2-sequestration/#:~:text=The%20atomic%20weight%20of%20Carbon,in%20the%20tree%20by%203.67

Ghazoul, J., & Sheil, D. (2017). Tropical Rain Forest Ecology, diversity, and conservation: The ever changing forest: Disturbance and dynamics. Oxford University Press.

Gomez-Rubio, V. (2018). Generalized Additive Models: An Introduction with R (2nd Edition). Journal Of Statistical Software, 86(Book Review 1). https://doi.org/10.18637/jss.v086.b01

Grebner, D. L., Bettinger, P., Siry, J. P., & Boston, K. (2022). Introduction to forestry and

Natural Resources: Forest Succession. Academic Press.

Huy, B., Poudel, K., Kralicek, K., Hung, N., Khoa, P., Phương, V., & Temesgen, H. (2016). Allometric Equations for Estimating Tree Aboveground Biomass in Tropical Dipterocarp Forests of Vietnam. Forests, 7(12), 180. https://doi.org/10.3390/f7080180

Intergovernmental Panel on Climate Change [IPCC]. (2006). Guidelines for National Greenhouse Gas Inventories. Chapter 4: Forest Lands. Japan: National Greenhouse Gas Inventories Programme

IPCC, (2023). Summary for Policymakers. In: Climate Change 2023: Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, H. Lee and J. Romero (eds.)].

IPCC, Geneva, Switzerland, pp. 1-34, doi: 10.59327/IPCC/AR6-9789291691647.001

Karger, D. N., Kessler, M., Lehnert, M., & Jetz, W. (2021). Limited protection and ongoing loss of tropical cloud forest biodiversity and ecosystems worldwide. Nature Ecology & Evolution, 5(6), 854–862. https://doi.org/10.1038/s41559-021-01450-y

ManulsLib. (n.d.). PM-5/; optical height meter; instructions for use - suunto 1520 user manual [page 5]. ManualsLib. https://www.manualslib.com/manual/166835/Suunto-1520.html?page=5#manual

Martin, A. R., & Thomas, S. C. (2011). A reassessment of carbon content in tropical trees. PLoS ONE, 6(8), e23533. https://doi.org/10.1371/journal.pone.0023533

Mokany, K., Raison, R.J. and Prokushkin, A.S. (2006) Critical Analysis of Root: Shoot Ratios in Terrestrial Biomes. Global Change Biology, 12, 84-96. https://doi.org/10.1111/j.1365-2486.2005.001043.x

Pati, P. K., Kaushik, P., Khan, M. L., & Khare, P. K. (2022, June 15). Allometric equations for biomass and carbon stock estimation of small diameter woody species from tropical dry deciduous forests: Support to REDD+. Trees, Forests and People. https://www.sciencedirect.com/science/article/pii/S2666719322000966?via%3Dihub

Patil, G. P., & Rao, C. R. (1994). Environmental statistics. North-Holland.

Paulick, S., Dislich, C., Homeier, J., Fischer, R., & Huth, A. (2017). The carbon fluxes in different successional stages: modelling the dynamics of tropical montane forests in South Ecuador. Forest Ecosystems, 4(1). https://doi.org/10.1186/s40663-017-0092-0

Penn State Extension. (2023). Carbon accounting in forest management. https://extension.psu.edu/carbon-accounting-in-forest-management

Singh, P., & Benbi, D. K. (2018). Soil organic carbon pool changes in relation to slope position and land-use in Indian lower Himalayas. CATENA, 166, 171–180. https://doi.org/10.1016/j.catena.2018.04.006

Staples, T. L., Mayfield, M. M., England, J. R., & Dwyer, J. M. (2019). Comparing the recovery of richness, structure, and biomass in naturally regrowing and planted reforestation. Restoration Ecology, 28(2), 347–357. https://doi.org/10.1111/rec.13077

Solomon N;Pabi O;Annang T;Asante IK;Birhane E; (2024). The effects of land cover change on carbon stock dynamics in a dry afromontane forest in northern Ethiopia. Carbon balance and management. https://pubmed.ncbi.nlm.nih.gov/30191432/

United Nations Educational, Scientific and Cultural Organization. (n.d.). Talamanca Range–La Amistad Reserves / La Amistad National Park [World Heritage List No. 205]. UNESCO World Heritage Centre. Retrieved September 8, 2025, from UNESCO website

Walker, S. M., Murray, L., & Tepe, T. (2016, June). Allometric equation evaluation guidance document. Allometric Equation Evaluation Guidance Document. https://winrock.org/wp-content/uploads/2018/08/Winrock-AllometricEquationGuidance-2016.pdf

Wasserman, L. (2006). All of nonparametric statistics. SPRINGER Verlag (NY/NJ).

White, R. (2024). Climate change. Oxford Research Encyclopedia Of Criminology And Criminal Justice. https://doi.org/10.1093/acrefore/9780190264079.013.762

Williams, M. S., Bechtold, W. A., & LaBau, V. J. (1994). Five Instruments for Measuring Tree Height: An Evaluation. Southern Journal Of Applied Forestry, 18(2), 76–82. https://doi.org/10.1093/sjaf/18.2.76

Xie, D., Feng, L., Yan, X., Sharma, R. P., Wang, Z., Duan, G., Tang, S., Liu, S., & Fu, L. (2024). forestat: An R package for computing forest carbon sequestration and potential productivity. Ecological Indicators, 166, 112477. https://doi.org/10.1016/j.ecolind.2024.112477

Yadav, V. S., Yadav, S. S., Gupta, S. R., Meena, R. S., Lal, R., Sheoran, N. S., & Jhariya, M. K. (2022). Carbon sequestration potential and CO2 fluxes in a tropical forest ecosystem. Ecological Engineering, 176, 106541. https://doi.org/10.1016/j.ecoleng.2022.106541

Zhang, B., Lan, K., Harris, T. B., Ashton, M. S., & Yao, Y. (2023). Climate-smart forestry through innovative wood products and commercial afforestation and reforestation on marginal land. *Proceedings of the National Academy of Sciences*, *120*(23). https://doi.org/10.1073/pnas.2221840120

7. Appendix

Name of Plot	**GPS Coordinates**
EJ PLA 2008A	-83.5784°, 9.4703°
EJ PLA 2008B	-83.5784°, 9.4702°
LQ PLA 2009A	-83.5661°, 9.4788°
LQ PLA 2009B	-83.5657°, 9.4789°
EL PLA 2011A	-83.5779°, 9.4714°
EL PLA 2011B	-83.5775°, 9.4717°
EJ NR 1988A	-83.5755°, 9.4688°
EJ NR 1988B	-83.5748°, 9.4685°
MON NR 2004A	-83.5662°, 9.4700°
MON NR 2004B	-83.5653°, 9.4684°
EJ NR 2008A	-83.5766°, 9.4694°
EJ NR 2008B	-83.5768°, 9.4700°
EJ OG	-83.5723°, 9.4666°

SENTI OG	-83.5706°, 9.4715°
MON OG	-83.5653°, 9.4657°

Table A1- *GPS coordinates of each Plot.*
Note. Derived from QGIS.

Species	**Forest Type**	**Wood Density**
Quercus salicifolia	Planted	0.67
Cedrela tonduzii	Planted	0.36
Ulmus mexicana	Planted	0.55
Ocotea valeriana	Planted	~0.65
Heliocarpus appendiculatus	Natural Regrowth	0.18
Cecropia angustifolia	Natural Regrowth	~0.33
Myrsine coriacea	Natural Regrowth	0.7
Inga sp	Natural Regrowth	~0.55
Saurauia montana	Natural Regrowth	~0.43
Saurauia pittieri	Natural Regrowth	~0.45
Quercus salicifolia	Old Growth	0.67
Macrohaceltia macrotheranta	Old Growth	Unknown
Billia rosea	Old Growth	Unknown
Guatteria talamancana	Old Growth	~0.55

Table A2- Most common Species per Forest Type with Respective Wood Density Values.
Note. Derived from GlobalWoodDensityDatabase.