



Carbon Sequestration and Carbon Stock of Agroforestry Tree Species Around Cyamudongo Isolated Rain Forest and Arboretum of Ruhande, Rwanda

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ABSTRACT

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Agroforestry (AF) is widely considered the most important tool to mitigate climate change-related issues by removing Carbon (C) Dioxide (CO₂) from the atmosphere and storing C. Therefore, this study aims to broaden current knowledge on the impact of sustainable Agroforestry (AF) on the C sequestration rate and C stock in the surroundings of Cyamudongo isolated rain forest and Ruhande Arboretum. To understand this, the permanent sample plots (PSPs) were established mainly in the four designed transects of four km long originating on the Cyamudongo isolated rain forest boundary following the slope gradient ranging from 1286 to 2015 m asl. A total number of 73 PSPs were established in the Cyamudongo study area while 3 PSPs were established in the Ruhande AF plot. The Arc Map GIS 10.4 was used to design and map the sampling areas while GPS was used for the localization of the plots. Statistical significance was analyzed through R-software. The estimated quantity of sequestered C for 2 years and 34 years of AF species was 13.11 t C ha⁻¹ yr⁻¹ (equivalent to 48 t CO₂ ha⁻¹ yr⁻¹) and 6.85 t C ha⁻¹ yr⁻¹ (equivalent to 25.1 t CO₂ ha⁻¹ yr⁻¹) in Cyamudongo and Ruhande respectively. The estimated quantity of C stored by the Ruhande AF plot is 232.94 t ha⁻¹. In Cyamudongo, the overall C stored by the AF systems was 823 t ha⁻¹ by both young tree species established by the Cyamudongo Project (35.84 t ha⁻¹) and C stored by existing AF species before the existence of the Project (787.12 t ha⁻¹). In all study areas, the *Grevillea robusta* contributed more to overall stored C. The correlation coefficients between tree diameter and living biomass ranged from moderate to very strong due to differences in terms of age, stage of growth, and tree species.

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Introduction

Background

Agroforestry (AF) has been gaining much attention due to its contribution to C sequestration and C stock. Rossetko et al. (2002) draw attention to the role of AF in C sequestration and storage as a good investment for the farmers who apply AF to their land through the Clean Development Mechanism (CDM). The AF trees and soil can contribute to the regulation of the overall climate through the removal of the CO₂ from the atmosphere and this requires the application of the best practices which rise the C stock and C sequestration (Besar et al., 2020). The AF contributes more to the reduction of the CO₂ from the

atmosphere and the importance of AF is enhanced by its ecosystem services that minimize the emissions from farming activities and the increased amount of stored C. The ecosystem service of the AF is estimated by the stored amount of C available in the standing tree biomass (Hergoualc'h, 2012).

The Kyoto Protocol of the United Nations Framework Convention on Climate Change (UNFCCC) widespread the interest in AF as a tool to overcome many problems while providing considerable importance related to various aspects of the environment especially in the third countries (Dhyani, 2014). Recently, AF has received much attention

via Kyoto Protocol as an approach to control environmental pollution through Carbon (C) capturing from the atmosphere (Nair et al., 2009). More recent evidence, Luedeling et al. (2011) suggest the change in the management of land where AF should be promoted all over the world to save our environment because of its ability to store more C through various aspects of the land. Moreover, tree planting on unused soils will improve the properties of the soils and enhance the C stock while preventing climate change-related issues (Nsabimana, 2009). However, capturing C from the atmosphere and storing it in biomass is known as C sequestration. The C stock tends to be used to refer to the amount of C stored in the reservoir (e.g., t C ha⁻¹) while C sequestration often refers to the process degree of C elimination from the atmosphere considering the aspect of time (e.g., t C ha⁻¹ year⁻¹). Mostly, similar AF practices tend to capture the same amount of C (Luedeling et al., 2011). Several factors such as rotation of crops and soil available nutrients contribute to the C capturing from AF and its contribution to soil improvement depends on the types of trees associated with crops (Nair et al., 2009).

Under favorable environmental conditions, both AF trees and soils help to capture the C from the atmosphere (Jose, 2009). The soil nutrient composition and C result from the association between trees and soil (Nsabimana, 2009).

AF for C and Climate Change Mitigation and Adaptation

C sink, C stock, and C sequestration

According to Assefa et al. (2013), a C sink is a C pool from which more C flows in than out. Forests can act as a sink through the process of tree growth and resultant biological C sequestration. The C pool is a system that can accumulate or release C. C stock is understood to mean the mass of C contained in a C pool. C stock assessment is one of the important steps to start with sustainable land use planning for low C emissions. The change in C stock with the dynamics of land-use changes may result in either C emission or sequestration. According to the International Plant Protection Convention-IPPC (2006), C pools in forest ecosystems are comprised of C stored in the living trees aboveground and belowground, in dead matter including standing dead trees, down woody debris, and litter among others.

Various approaches have been proposed under the international conventions and programs to demonstrate the role of combining trees with crops in the regulation of climate-related issues and suggested how its support should be achieved. For example, in the UNFCCC and more specifically in the Kyoto Protocol, tree plantation programs are considered to contribute to the removal of the atmospheric C and storing it in the C pool such as soil and forests (Nair and Kumar, 2011).

The above-ground biomass (AGB) and belowground biomass (BGB) are made by different components of vegetation and soil (Hairiah et al., 2010). More C can be removed from the atmosphere and stored through the use of trees of high rotation periods in the management of land (Nair and Kumar, 2011). The AF systems capture and store a considerable amount of C both below and above the soil surface (Ramachandran Nair et al., 2010). The amount of C stored by the AF system is more than the amount which

can be fixed by a land use management system without a tree component (Nair and Kumar, 2011). The C stored by any AF system is found in various components of the system such as woody components, crops, and soil either below ground or above ground. Besides, the C stored by trees is in different proportions in various tree parts such as stems, branches, leaves, roots, flowers, and fruits among others and total C estimation should consider all parts and components within the system (Nair and Kumar, 2011).

Scientists have always seen the measurement and estimation of C stored by forests and AF systems as an appropriate approach to mitigate climate change-related issues. Various researchers have developed several approaches and models wherever the world. These models are being applied in the environmental conditions through which they have been developed. Until now, the forest has been considered the major C sink of terrestrial ecosystems. The amount of C stored depends on the type of contributing C sink and how was managed. Among developed best models which can fit both climate and forest types in the tropics with tree size between 5-60 cm in diameter were developed by Chave et al. (2014) and was used in this study. Similarly, a few models for AF tree species and multi-stem trees or shrubs especially for the small trees with < 5 cm diameter at ground level (D₃₀) were developed and the one of Mokria et al. (2018) was followed and applied to young AF species.

In the literature, C sequestration usually refers to the process of eliminating C from the atmosphere and loading it in one or more C pools (Jose, 2009; UNEP, 2017). In AF, the term C sequestration has been applied to refer to the removal of CO₂ from the atmosphere via the system composition and deposit it into the reservoir for a long period (Ramachandran Nair et al., 2010). The amount of C removed from the atmosphere by an AF system varies according to the status and the nature of the system, ecological factors, and how the system is maintained (Jose, 2009). The C sequestration is an asset to the third-world farmers who apply AF practices as the captured C amount may be sold to industrialized countries (Ramachandran Nair et al., 2010). Widely, AF has been receiving much attention due to its ability to capture CO₂ from the atmosphere (Nair and Kumar, 2011). Ultimately, AF contributes to C sequestration, increases the range of regulating ecosystem services, and enhances biodiversity (Kay, 2019).

The Climate Change and Mitigation Measures

In the environment, several definitions of climate can be found. The term climate has been used by Assefa et al., (2013) to refer to “the weather at a location over a long time; a minimum recording period of 30 years is deemed necessary to account for normal variation”. Climate change is described as “a change of climate which is attributed directly or indirectly to human activity that alters the composition of the global atmosphere and which is in addition to natural climate variability observed over large time” (UNFCCC, 2015).

The most current accepted serious environmental issue affecting human lives on a global scale is global climate change commonly called global warming (Nair et al., 2009). In recent years, there have been considerable environmental-related problems due to global warming (Nair and Kumar, 2011). It has been demonstrated that

changing the forested areas to other land uses and their poor management resulted in ozone layer exhaustion (Sobola and Amadi, 2015).

Various approaches have been hypothesized to solve this issue. The establishment of forest and AF practices under various international conventions especially those attached to the UNFCCC such as Land Use, Land Use Change, and Forestry (LULUCF), Kyoto Protocol, and Clean Development Mechanism (CDM) has been receiving much attention due to the capacity of trees to sequester more C from the atmosphere (Nair et al., 2009)

The vegetation is a large C pool than the atmosphere and its changes affect the global environment (Schaaf, 2009). The forest has been identified to control global C and plays an important role to the people. Pan et al. (2011) identified that change with time of the forest resources must be well mastered to know how they contribute to climate regulation. It has now been suggested that environmental-related issues can be addressed by AF (Agevi et al., 2017). AF has been demonstrated to play several advantages including environmental, economic, and social at different scales (Sobola and Amadi, 2015). Conversely, the AF has to be well managed to maximize the intended target (Mbow et al., 2014).

In Africa, C sequestration is generally considered a co-benefit of strategies to support sustainable livelihoods and adapt to climate change (Mbow et al., 2014). The people's views on tree species to be used for the interventions related to the C sequestration are taken into consideration for better achievement of the project goal especially in the countries of sub-Saharan Africa (Dimobe et al., 2018). The significance of AF with regards to C sequestration and other CO₂ mitigating effects is being widely recognized, but there is still a scarcity of quantitative data on specific systems (Albrecht and Kandji, 2003).

AF contributes to the improvement of people's well-being, biological resources, and environmental conditions (Lasco et al., 2014). The C stock of the land depends on the land use management practices applied and the amount of C stored differ from one land use to the other. There should be a consideration of all fixed quantities of C for better estimation at a large scale (Tomich et al., 2004). Hairiah et al. (2010) reported that the amount of C fixed or C removed from the atmosphere depends on the forest maturity. Mature forest stores more C while sequestering a small amount of C compared to the newly established plantation. Further, they asserted that more C is in AGB than in the subsoil. The C storage depends on several factors including climatic, edaphic, and socio-economic conditions. Perennial systems like home gardens and agroforests can store and conserve considerable amounts of C in living biomass and also in wood products (Albrecht and Kandji, 2003). Tree biomass varies from one region to the other and is dependent on species density, age, climatic factors, and soil factors. In Sub-Saharan Africa, it ranges from 0.29-15.2 Mg C ha⁻¹ (Agevi et al., 2017). The allometric equations can be used to evaluate the AGB. In the estimation of tree biomass, the use of allometric equations is the most appropriate since it is non-destructive (Hairiah et al., 2010).

The Government of Rwanda recognizes the importance of forestry resources for providing various ecosystem services including C sequestration. Rwanda adopted the

implementation of various international agreements (e.g., UNFCCC and Paris Agreement) related to climate change mitigation issues and has put in place specific programs and policies (Green Growth and Climate Resilience (GGCRS), Forestry Sector Strategic Plan (FSSP, 2018-2024), National forestry policy of 2018 contributing to the mitigation and adaptation to climate change (REMA, 2018). Long-term management of natural resources contributing to the mitigation of climate change issues is among Rwandan core interventions (MINILAF, 2018).

This study firstly estimated the C sequestration rate and C stock of AF tree species throughout the time in two ecological conditions of Cyamudongo and Ruhande on the following seven AF tree species: *Cedrela serrata*, *Croton megalocarpus*, *Grevillea robusta*, *Markhamia lutea*, *Maesopsis eminii*, *Podocarpus latifolius*, and *Polyscias fulva*. Further, this study determined the correlation between tree diameter at breast height (DBH) for big trees and diameter at thirty centimeters above ground (D_{30 cm}) for young or newly established tree species and biomass. The research hypothesis stated that there is a significant difference between the amount of the fixed C in the biomass of AF trees species established by the project and the existing AF trees species of the study areas. The research questions were expressed in line with the objectives of the study as follows:

- What is the amount of AGB, BGB, and above and below ground biomass (ABGB) for each of the AF tree species under study?
- What is the amount of C for each of the AF tree species under study?
- What are the AF tree species, which sequester and store more C?
- What are the factors that contribute to more C sequestration and C stock?
- What are correlation coefficients for various AF tree species as far as tree diameter and biomass are concerned?

Materials and Methods

Description of Study Areas

The Rusizi and Huye districts have been selected to estimate the C stock and C fixation rate of AF tree species. Rusizi District is located in the South-West of Rwanda and is one of seven districts of the Western Province. Two study areas are characterized by different environmental conditions. The area of the Rusizi district is 959 km². In its south, it is bordered by two countries including the Democratic Republic of Congo (DRC) and the Republic of Burundi whereas, in its north, it is bordered by Nyamasheke and Nyamagabe districts. Furthermore, in its east, it borders with Nyamagabe and Nyaruguru districts. The estimated population density is 420 inhabitants km⁻². Three sectors (Figure 1) of Rusizi district including Gitambi, Nkungu, and Nyakabuye of Rusizi District located in the community around Cyamudongo isolated forest were selected because they were the main intervention area of the Cyamudongo Project. Cyamudongo fragmented rain forest (02°33.12'S 28°59.49'E) is a small dense forest patch (300 Ha) around 8 km away from Nyungwe National Park (NNP).

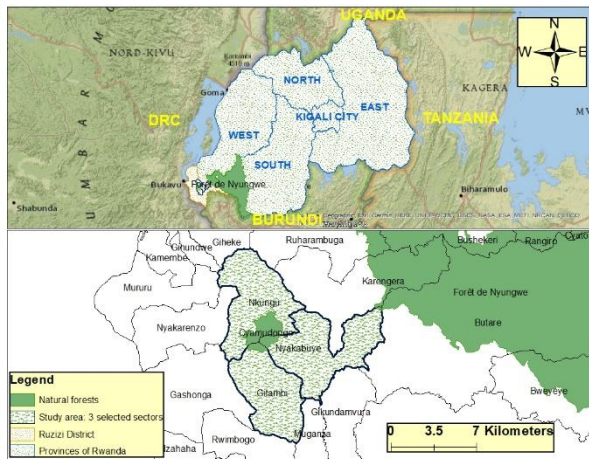


Figure 1. Location of Cyamudongo study area, in Rusizi District of Rwanda

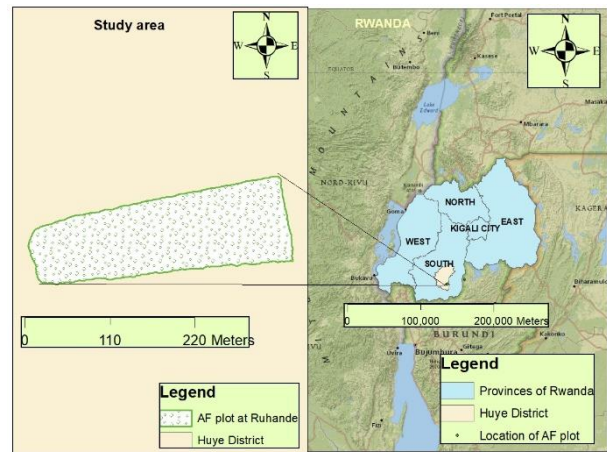


Figure 2. Location of Ruhande AF plot, in Huye District of Rwanda

Huye district is one of eight districts in the Southern province of Rwanda. It has a total surface area of 581.5 km². Huye district is bordered by Nyanza district in its North, Gisagara district in the East and South, Nyaruguru district in the South West, and Nyamagabe district in the North West. Its estimated population density is 540 inhabitants km². The Arboretum of Ruhande is located in the Ngoma sector and was established in 1934 on a total area of 200 ha with the main purpose of research, seed production, and promoting AF in Rwanda. It contains several species of conifers (56) and broadleaved (148). The climate of Ruhande is characterized by two rainy seasons and two dry seasons. The first rain season starts in March and ends in May whereas the second starts in September and ends in December. The first dry season starts in January and ends in February while the second starts in June and ends in August (Nsabimana, 2009). The study was conducted in the AF plot that was established by the University of Koblenz-Landau of Germany in October 1986 (König, 1992) as an extended area attached to the Arboretum of Ruhande with the main purpose of research in AF and soil conservation. The altitude range across the study area in that particular AF plot is 1669 to 1683 m asl.

Species Description and Purpose for Intervention

Seven AF tree species from different families were used to estimate AGB, BGB, C stock, and C sequestration rates. The targeted AF tree species in Cyamudongo were either young established AF tree species by Project or existing (mature) AF tree species in the agricultural landscape in the surroundings of Cyamudongo isolated rain forest. These AF tree species include *Cedrela serrata*, *Croton megalocarpus*, *Grevillea robusta*, *Maesopsis eminii*, *Markhamia lutea*, and *Podocarpus latifolius*, and *Polyscias fulva*. Cyamudongo Project started the plantation of AF tree species in 2017, which are considered young in this study whereas the farmers used to mix AF tree species in their lands even before the Cyamudongo Project intervention, and those species were considered mature. The farmers used to grow AF tree species using the traditional techniques such as poor management practices of trees, poor spacing management, poor plantation techniques, and poor AF tree species selection among

others, which compromised the productivity of both crops and AF products for example stakes, firewood, the fodder for livestock and timber.

On the other hand, there were only four targeted ancient AF tree species in the AF small plot (3 ha) managed by the University of Koblenz-Landau of Germany that was established in 1986 in partnership with the Government of Rwanda through the University of Rwanda former "Université Nationale du Rwanda". These AF tree species were *Cedrela serrata*, *Grevillea robusta*, *Maesopsis eminii*, and *Polyscias fulva*. Initially, the purpose of this plot was the research where its contribution to soil erosion control and the role of AF tree species in the improvement of soil productivity was investigated and published by previous researchers (for example Prof. Dr. Dieter König) of the University of Koblenz-Landau. As the AF has received too much attention in these recent years for its contribution to the removal of C from the atmosphere as a strategy for mitigation of climate change-related issues there was a need to monitor the C stored and sequestered by that AF plot.

Sampling Design

In this study, four transects were designed by the use of ArcMap software 10.4 in the way that each transect has 4 km originating from Cyamudongo fragmented rain forest boundary towards Bugarama downhill via the high mountains of Nyakabuye and Gitambi Sectors of Rusizi District (Figure 3). The four km corresponds to the width of the buffer established by the Cyamudongo Project. The distance of 600 m was kept between two consecutive transects. Systematically, the distance from one plot to the next within the transect was respected corresponding to 250 meters that were consistently measured. The plots fallen in AF land use were counted while those fallen in forest land use were removed from the scope of our study. Expectedly, there would be 68 plots if the study area were uniform i.e. $\{4000 \text{ m (length of transect)} / 250 \text{ m (distance between 2 consecutive plots)} + 1\} \times 4$ (number of transects)}. Consequently, 19 plots were found in forestry land use type which resulted in 49 plots in AF land-use type which equals 4.9 ha since the plot size is 1000 m².

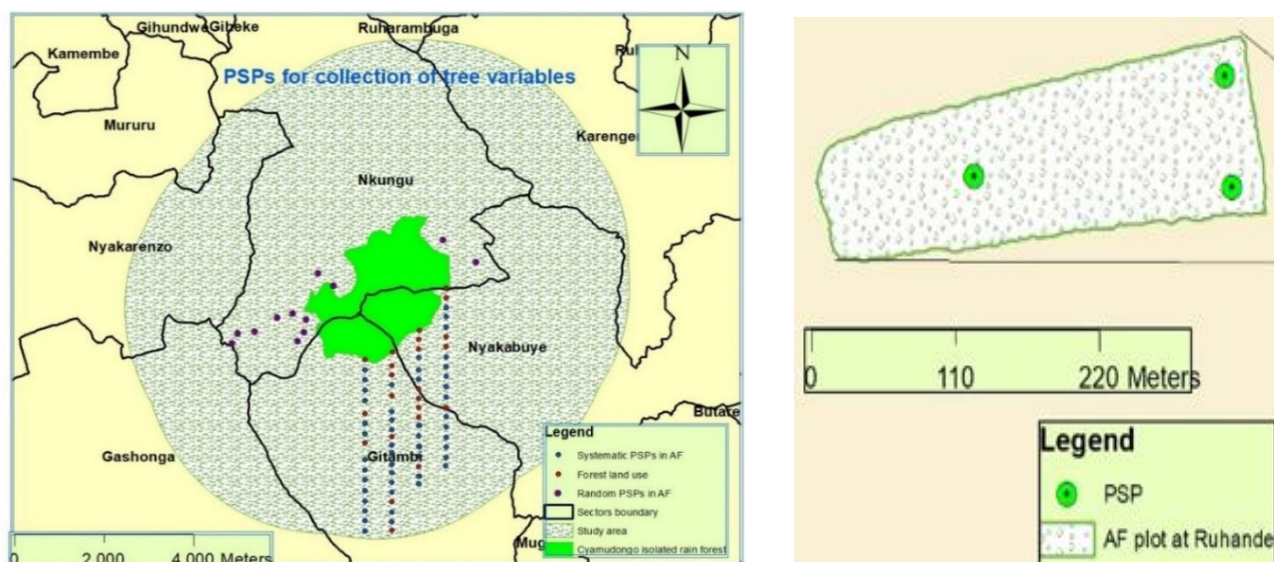


Figure 3. Designed transects and PSPs in the study areas of Cyamudongo and Ruhande

According to Nizami (2010) the sampling intensity may be reduced from the standard of 2.5 percent of the total forest area to 1.0, 0.5, and 0.25 percent. This reduction of sampling intensity does not increase the coefficient of variation or uncertainty associated with the mean estimated forest Carbon. One of the important recommendations of Nizami's study is that a 1.0% sampling intensity is adequate. Contrary to the previous statement, the size of 4.9 ha was not representative of the entire project intervention area in AF land use as it was below 1% of the total study area (6125 ha). Therefore, 12 additional plots were subjectively established out of transects to cover 1% of the total study area to determine C fixation and C stock in AF land use around Cyamudongo fragmented rain forest.

It has now been suggested various sizes of AF plots vary from small to big ones (FAO, 2018) and depend on tree density (Heiskanen et al., 2013). In this study, the large circular plots with large radius were established as the trees were established with a small density by either scattering trees on crop land (CL), or planting trees on the boundary and contour lines. The circular plots were selected because they were easy to be designed and easily established on the steep slopes found in the study areas, especially in Cyamudongo. In a recent review of the literature on circular plots, Karki et al. (2016) and FAO (2018) found that the circular plots minimize the edge and borderline trees effect which usually occurs on rectangular shapes. The number of established plots was dependent on the size of the plots and the targeted area of the study. A circular sample plot of 0.1 ha (1000 m²) in size is used for measuring the woody biomass (Heiskanen et al., 2013). The plot radius of 17.84 m (0.1 ha plots) is used on very sparse woody vegetation (Macdicken, 1997). The Land Degradation Survey Framework (LDSF), sample plot design with a sample plot of 0.1 ha (1000 m²) corresponds to the tenth of the area of the established permanent sample plots (PSP) was used in this study.

The 6.125 ha corresponds also to 61 Permanent Sample Plots (PSPs) since the plot size is 1000 m² with a circular plot radius of 17.84 m (equivalent to 1000 m²) was taken to measure above-ground tree biomass (diameter at breast height (DBH) \geq 5 cm for large trees and D₃₀ cm for small trees < 5 cm) to maximize the chance of having more individual trees within the plot for data collection since the project established AF systems with a density of about 200 trees per ha. The plot size was consistent for Ruhanda Arboretum where 3 plots corresponding to 0.3 ha represented a tenth of the study area (3 ha). Besides, the Global Positioning System (GPS) was used to take geographic coordinates of the centers of established plots and ArcMap GIS was used to design and display data on the map.

Meteorological Data

The meteo data were obtained in two ways. Primarily, the meteo stations from the automated National Meteo Agency were used. Secondary, there were established field buckets (Table 1) in the area of Cyamudongo and as the study was conducted in three sectors, there was one bucket for each. The targeted data were precipitations which were collected daily. Nyakabuye and Nyakibanda automatic meteo stations were used for Cyamudongo and Ruhanda respectively. Besides, the 3 buckets in Cyamudongo for various altitude ranges were installed in the field for rain data collection to maximize the quality of the estimate of the rainfall of the Cyamudongo region and were found within a 4 km width of buffer (Figure 3) corresponding to the boundary of the project intervention. The daily rainfall for both automated meteo station and bucket were averaged for two years. As the daily rainfall recorded in buckets was in ml per area of 63.5 cm² for the used bucket of 9 cm diameter, it was converted into comprehensively daily rainfall in mm per m². Furthermore, the yearly rainfall was obtained by summing up all the daily rainfall of the total days of the year.

Table 1. Location of meteo stations and used buckets for recording rainfall and estimated distances from study areas

| # | Meteo station/bucket | Distance (station to forest main boundary in km) | Geographic coordinates | | |
|---|----------------------|--|------------------------|-----------|----------|
| | | | Latitude | Longitude | Altitude |
| 1 | Nyakibanda meteo | 6 | 9706200.6 | 799615.16 | 1614 |
| 2 | Nyakabuye meteo | 2.5 | 9716452.2 | 725343.77 | 1216 |
| 3 | Bucket 1: Gatara | 0.3 | 9718612 | 719682.01 | 1922 |
| 4 | Bucket 2: Nyamubembe | 2 | 9718721.9 | 724872.26 | 1394 |
| 5 | Bucket 3: Njambwe | 1 | 9716772 | 717278.16 | 1703 |

Table 2. Recorded AF trees per species and location

| # | Tree species | Location | | | Total |
|-------|------------------------------|----------|------------------------|--------------------------------|-------|
| | | Ruhande | Cyamudongo (RS) | | |
| | | | Established by project | Not established by the project | |
| 1 | <i>Cedrela serrata</i> | 14 | 94 | 29 | 137 |
| 2 | <i>Grevillea robusta</i> | 53 | 429 | 248 | 730 |
| 3 | <i>Maesopsis eminii</i> | 7 | 79 | 36 | 122 |
| 4 | <i>Polyscias fulva</i> | 53 | 24 | 25 | 102 |
| 5 | <i>Croton megalocarpus</i> | NA | 4 | 2 | 6 |
| 6 | <i>Markhamia lutea</i> | NA | 36 | 202 | 238 |
| 7 | <i>Podocarpus latifolius</i> | NA | 10 | 4 | 14 |
| Total | | 127 | 676 | 546 | 1349 |

Tree Variables Measurement and Collection of the Samples of Wood Biomass

For each tree species, the stem density, DBH, average height, wood specific density (WSD), total tree biomass, C stock, and C dioxide equivalent (CO₂e) were estimated. In 2018 and 2019, DBH and total height were measured to calculate the aboveground biomass for all trees ≥ 5 cm DBH. They were measured for seven AF species under study as described in 2.2. The tree parameters were taken on 1,222 and 127 individual trees respectively from Cyamudongo and Ruhande, which make a total of 1,349 individual trees (Table 2). Vertex IV (Hypsometer) was used for tree heights and diameter tape for tree DBH. The incremental borer with an inner diameter of the bit 5.10 mm was used to collect wood biomass from the tree stems, electronic balance for fresh weights, and a ruler for sample heights. For each tree species, 3 samples were collected from 3 individual trees. For big size trees (> 10 cm DBH), the small pieces of wood were extracted from the tree to determine wood density and samples were oven-dried at 105 °C until the constant weight. For small tree species (< 10 cm DBH), the Secator was used to cut off a small branch of regular shape which was weighted and measured its total length and the mean diameter for indirect variables calculations. Thereafter, immediately the wood sample heights and diameters were bagged, labeled, and transported to the laboratory of the University of Koblenz-Landau for wood density and C determination. The study was conducted on 1349 individual trees which include 1222 in Cyamudongo and 127 in Ruhande study areas. The number of AF young tree species established by the Cyamudongo Project that has been the basis for this study was 676 while the existed AF tree species in the farms around Cyamudongo isolated rain forest was 546. These highlighted numbers of individual trees for various AF tree species varied with stand density (stems/ha). The differences also depended on which AF tree species have been appreciated by farmers because of the role they attributed to them considering the targets (e.g., firewood, stakes, fodder, erosion control, and timber among others).

The more species are appreciated by farmers; the more individual trees of that particular AF tree species are found on their lands.

Wood Samples Calculation

In this study, the green volume of each sample was calculated by the use of Huber's formula based on the circumference measured in the middle of the log.

$$(V = \frac{\pi}{4} \cdot D^2 \cdot L) \quad (1)$$

The term wood density has been used by Chave (2006) to refer to "the ratio of the oven-dry mass of a wood sample divided by the mass of water displaced by its green volume". The volume without bark was taken to check data consistency during the calculation of tree wood density. It was calculated from measurements of oven-dry weight combined with measurement of green volume. The volume of a tree core was estimated by the dimensional method as described by Chave (2006). Hence, the volume was calculated by assuming a regular cylindrical shape. This required measuring both the total length and its diameter, with a small caliper, avoiding the pressure of the caliper blades on wood. Oven dry weight was measured with a digital balance of precision of 0.01 g. The wood samples were put in the Oven-dried at 105°C until constant weight and then milled for C contents.

Tree biomass calculation

Aboveground woody biomass was estimated from the volume of trees and the average oven-dry wood density of each species. The allometric equation suggested by Chave et al. (2014) was found to be the most improved model for various types of forest, AF, and in different environmental conditions and was used in this study, particularly for trees with DBH above 5 cm.

$$AGB = 0.0673 \times (\rho D^2 H)^{0.976} \quad (2)$$

Where D (DBH) is in cm, H (tree height) is in m, and ρ (wood-specific density) is in g cm⁻³.

For young established AF tree species (trees < 5 cm diameter at 30 cm), we adopted an allometric equation developed by Mokria et al. (2018).

$$Y = 0.2567(D * H)^{1.1213} \quad (3)$$

Where Y, D₃₀, and H, are aboveground biomass (kg/plant), diameter at stump height (30 cm), and total tree height (m), respectively. The total biomass of individual trees was obtained by applying the biomass expansion factor (1.74) to the biomass of the stem as described by Brown and Lugo (1992). The following root-to-shoot ratio was indicated according to Krug et al. (2006) and IPCC (2006):

$$R = \frac{W_{\text{root}}}{W_{\text{aboveground}}} \quad (4)$$

Where: R= the ratio between a tree and root while W root = Tree root dry weight (g).

Hernandez (2004) reported the factors to be used to estimate the BGB from AGB for different tree species categories. The factors of 0.25 and 0.3 are to be used for coniferous and broad-leaved species respectively. The total annual biomass increment was determined by considering the data collected in 2018 and 2019 that was averaged. The obtained value was multiplied by the C concentration of the dry wood sample to obtain the C increment per hectare and year. The C stock has been computed following the procedure described by Pearson et al. (2014) where the dry wood C has to be multiplied by the wood biomass for getting the C per area unit and targeted species. The default of 0.47 was used to estimate the CO₂e as reported by Krug et al. (2006) for IPCC (2006). In this regard, the biomass value was converted into carbon concentration and carbon dioxide equivalent concerning the above procedure. The biomass stock (kg/m²) of each sampling plot was obtained by summing up all the individual biomass weights (in kilograms) of the sampling plot area. The AGB value was converted to tones per hectare by considering the total number of plots. Bismark et al. (2008) highlighted the method for estimating the C sequestration which takes into account the ratio between the molecular weight of CO₂ and the atomic weight of C (44/12) which was followed in this study.

$$\text{Sequestration of CO}_2 = \left(\frac{Mr_{\text{CO}_2}}{Ar_{\text{C}}} \right) \quad (5)$$

$$\text{Sequestration of CO}_2 = 3.67 \times \text{C content} \quad (6)$$

Where: Mr = molecule relative, and Ar = atom relative

Statistical Analysis

The R-software was used for the different statistical tests. A *Kruskal Wallis test* (the non-parametric equivalent of an ANOVA) was used to test AGB, BGB, ABGB, and C stock for various tree species in time for different study areas. As the automatic contrast procedure, which exists for ANOVA, is not developed for *Kruskal Wallis tests*, a pairwise comparison between various variables of different tree species using a separate *Mann-Whitney Wilcoxon test* was used. The regression analysis to determine the relationship among the variables of tree species was determined using the Spearman correlation test.

Results

Precipitations of the Study Areas

The monthly precipitations (Figure 4) were summed up to make the annual precipitations for different weather stations located in different agro-ecological zones of Cyamudongo (Nyakabuye weather station) and Ruhande (Nyakibanda). For the Cyamudongo study area, the daily precipitations recorded from Nyakabuye local station were added to the daily collected ones through various buckets established in different locations of the study area which were averaged to increase the quality of the precipitation estimate. The annual precipitations were 1605.8 mm and 1436 mm respectively for 2018 and 2019 in Nyakibanda while they were 1835 mm and 1638 mm in Nyakabuye respectively for 2018 and 2019.

Stand Variables, Wood Biomass, and C Content of Different AF Tree Species

Stand Variables, Wood biomass, and C Content of Different AF Tree Species Around Cyamudongo Isolated Forest

The AGB, BGB, ABGB, C stock, and CO₂e were estimated and compared between the existing AF species before the Cyamudongo Project and the species established by the project (Table 3). In 2 years of study (2018 and 2019), the existing AF species provided the highest values in terms of the aforementioned variables. Besides, the wood biomass accumulated in various AF species, C stock, and CO₂e were statistically different per category of recorded species (p<0.05). There was a marginal (close to being statistically significant) significance by comparing the two categories in time (p= 0.054). Therefore, the high amount of accumulated dry biomass and C for existing AF species is associated with the tree age, size (H and DBH), and species composition. The average value of ABGB was 52.6 t ha⁻¹ for existing AF tree species while it was 36.7 t ha⁻¹ for young species.

Table 4 summarizes the averaged AGB, BGB, ABGB, C stock, and CO₂e per hectare and AF tree species category of seven AF tree species scattered in the CL of the area around Cyamudongo isolated forest. By comparing the amount of dry biomass and C estimated for various AF species, the results show a statistically significant difference (p< 0.05). The tree biomass and C stock varied with species, species density, growth speed, and age. The AF species established by the project were chronologically ranked in terms of C stock as follows: *Grevillea robusta* > *Cedrela serrata* > *Polyscias fulva* > *Markhamia lutea* > *Maesopsis eminii* > *Croton megalocarpus* > *Podocarpus latifolius*. Similarly, the existed AF species were also ranked as follows: *Grevillea robusta* > *Markhamia lutea* > *Maesopsis eminii* > *Cedrela serrata* > *Polyscias fulva* > *Croton megalocarpus*. The obtained C stock values were averaged for all AF species to further rank them as a general estimate of the ABGB and C stock in the study area. Hence, the *Grevillea robusta* (257 t ha⁻¹) was followed by *Markhamia lutea* (94.73 t ha⁻¹), *Maesopsis eminii* (26.8 t ha⁻¹), *Cedrela serrata* (22.95 t ha⁻¹), *Polyscias fulva* (9.64 t ha⁻¹), *Croton megalocarpus* (0.195 t ha⁻¹) and *Podocarpus latifolius* (0.19 t ha⁻¹). The density of individual species per ha was 210 trees and the density of every AF species was also determined.

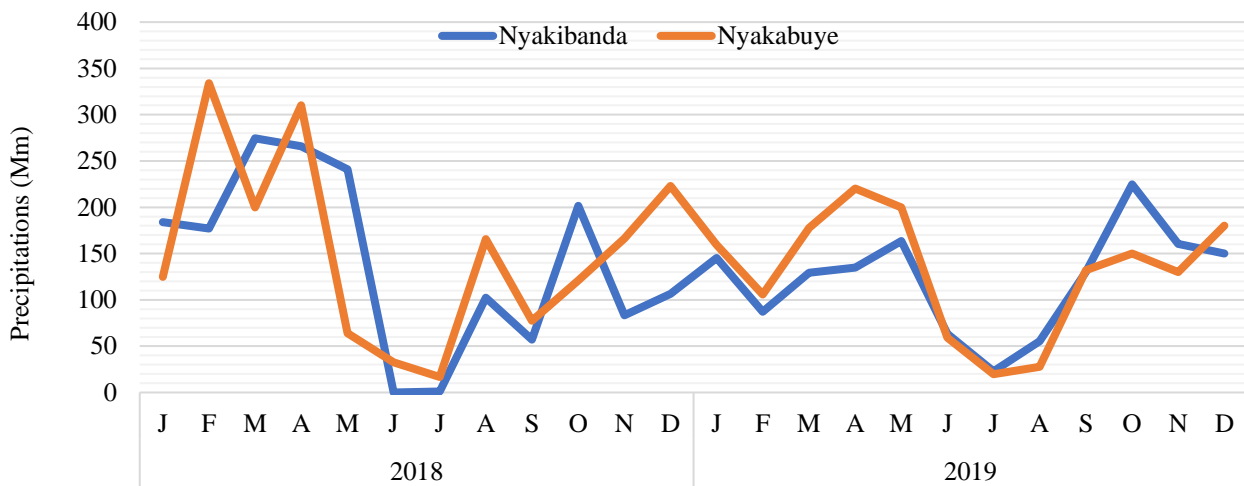


Figure 4. Monthly precipitation in Nyakibanda and Nyakabuye weather stations

Table 3. Estimated biomass and C stock per species category of AF live trees in sustainable AF of communities around Cyamudongo isolated forest.

| Category of recorded species | Year | AGB [t/ ha] | BGB [t/ha] | ABGB [t/ ha] | C stock [t/ha] | CO ₂ e [t/ha] |
|---------------------------------------|------|-------------|------------|--------------|----------------|--------------------------|
| Existed AF species before the project | 2018 | 642.6 | 192.8 | 835.4 | 381.6 | 179.4 |
| Existed AF species before the project | 2019 | 683.1 | 204.9 | 888.0 | 406.0 | 190.8 |
| Established AF species by project | 2018 | 16.5 | 4.9 | 21.4 | 9.6 | 4.5 |
| Established AF species by project | 2019 | 44.7 | 13.4 | 58.1 | 26.2 | 12.3 |
| Total | | 1387 | 416 | 1803 | 823 | 387 |

Table 4. Estimated quantity of live tree biomass and C stock per AF tree species and species category

| Category of recorded species | Species | AGB [t/ ha] | BGB [t/ ha] | ABGB [t/ha] | C stock [t/ha] | CO ₂ e [t/ha] |
|---------------------------------------|------------------------------|----------------|---------------|----------------|----------------|--------------------------|
| Established AF species by project | <i>Cedrela serrata</i> | 9.51 | 2.85 | 12.36 | 5.53 | 2.6 |
| | <i>Croton megalocarpus</i> | 0.34 | 0.1 | 0.44 | 0.2 | 0.09 |
| | <i>Grevillea robusta</i> | 38.29 | 11.49 | 49.78 | 22.62 | 10.63 |
| | <i>Maesopsis eminii</i> | 3.81 | 1.14 | 4.96 | 2.2 | 1.03 |
| | <i>Markhamia lutea</i> | 4.37 | 1.31 | 5.69 | 2.42 | 1.14 |
| | <i>Podocarpus latifolius</i> | 0.32 | 0.09 | 0.41 | 0.19 | 0.09 |
| | <i>Polyscias fulva</i> | 4.57 | 1.37 | 5.94 | 2.67 | 1.26 |
| Sub-total | | 61.21 | 18.35 | 79.58 | 35.83 | 16.84 |
| Existed AF species before the project | <i>Cedrela serrata</i> | 68.46 | 20.54 | 89 | 40.38 | 18.98 |
| | <i>Croton megalocarpus</i> | 0.33 | 0.1 | 0.42 | 0.19 | 0.09 |
| | <i>Grevillea robusta</i> | 819.96 | 245.99 | 1065.95 | 491.49 | 231 |
| | <i>Maesopsis eminii</i> | 86.92 | 26.08 | 112.99 | 51.4 | 24.16 |
| | <i>Markhamia lutea</i> | 321.87 | 96.56 | 418.43 | 187.04 | 87.91 |
| | <i>Polyscias fulva</i> | 28.14 | 8.44 | 36.58 | 16.62 | 7.81 |
| Sub-total | | 1325.68 | 397.71 | 1723.37 | 787.12 | 369.95 |
| Total | | 1387 | 416 | 1803 | 823 | 387 |

The *Grevillea robusta* was found to have more density compared to other species with 119 ha⁻¹, followed by *Markhamia lutea* 38 ha⁻¹, *Cedrela serrata* 22 ha⁻¹, *Maesopsis eminii* 19 ha⁻¹, *Polyscias fulva* 8 ha⁻¹, *Podocarpus latifolius* 3 ha⁻¹ and *Croton megalocarpus* 1 ha⁻¹. However, *Grevillea robusta* contributed to the highest estimates for both AF species that existed before the Cyamudongo project and were established by the project. In addition to that, it was found to grow faster compared to the other species under this study and the farmers willingly prefer to grow it with a high density among others because it provides them the stakes for climbing beans, firewood, constructing materials, and timbers. The total amount of

AGB, BGB, ABGB and C stock were 1387 t ha⁻¹, 416 t ha⁻¹, 1803 t ha⁻¹, and 823 t ha⁻¹ respectively.

Table 5 summarizes the amount of C sequestration estimated in t ha⁻¹ year⁻¹ of established species varies with species, age, growth rate (both in height and diameter) and planting density as follow: *Grevillea robusta* > *Cedrela serrata* > *Polyscias fulva* > *Maesopsis eminii* > *Markhamia lutea* > *Croton megalocarpus* > *Podocarpus latifolius* with 8.67, 1.92, 0.85, 0.77, 0.76, 0.07 and 0.05 respectively. The total C sequestration rate of AF tree species established by the project is 13.11 t C ha⁻¹ yr⁻¹ corresponding to 47.94 t CO₂ ha⁻¹ yr⁻¹ centered on the young trees of 2 years old.

Table 5. C sequestration rate of AF species established by the Cyamudongo project

| # | Species name | C stock[kg/ha] | C sequestration rate [t/ha/year] | No of individual trees | % |
|-------|------------------------------|----------------|----------------------------------|------------------------|------|
| 1 | <i>Grevillea robusta</i> | 17345.46 | 8.67 | 429 | 63.4 |
| 2 | <i>Cedrela serrata</i> | 3838.44 | 1.92 | 94 | 13.9 |
| 3 | <i>Polyscias fulva</i> | 1709.85 | 0.85 | 24 | 3.5 |
| 4 | <i>Maesopsis eminii</i> | 1548.06 | 0.77 | 79 | 11.6 |
| 5 | <i>Markhamia lutea</i> | 1520.15 | 0.76 | 36 | 5.3 |
| 6 | <i>Croton megalocarpus</i> | 139.03 | 0.07 | 4 | 0.5 |
| 7 | <i>Podocarpus latifolius</i> | 109.66 | 0.05 | 10 | 1.4 |
| Total | | 26210.65 | 13.11 | 676 | 100 |

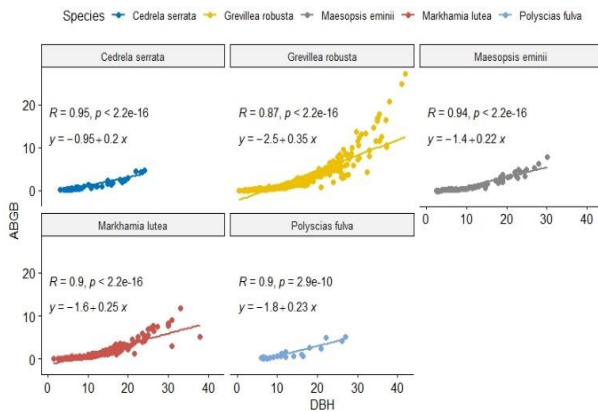
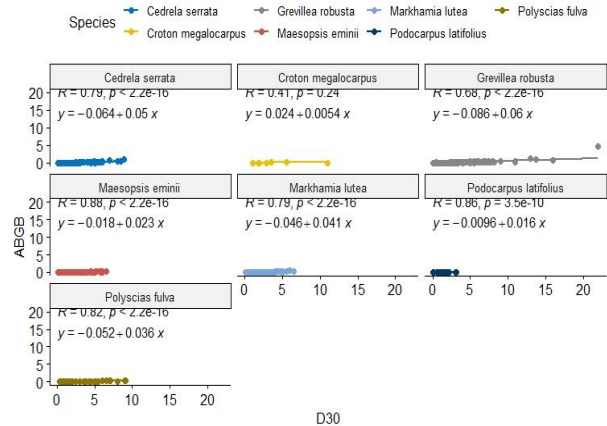


Figure 5. Regression analysis between ABGB and DBH for existing species before the project

Figure 6. Regression analysis between ABGB and D₃₀ for seven AF species established by the project

The tests showed differences in terms of WSD for various tree species. The WSD varied with tree species and age. The mature AF tree species were found to have more density compared to the young tree species of 2 years old. For mature trees, *Grevillea robusta* was found to have the highest density 494 kg m⁻³, followed by *Markhamia lutea* 475 kg m⁻³, *Cedrela serrata* 428 kg m⁻³, *Maesopsis eminii* 385 kg m⁻³ and *Polyscias fulva* 262 kg m⁻³. For the young AF tree species, *Croton megalocarpus* was found to have the highest WSD value (567 kg m⁻³), followed by *Podocarpus latifolius* (487 kg m⁻³), *Grevillea robusta* (442 kg m⁻³), *Markhamia lutea* (431 kg m⁻³), *Cedrela serrata* (412 kg m⁻³), *Maesopsis eminii* (347 kg m⁻³) and *Polyscias fulva* (211 kg m⁻³). The correlation between DBH and ABGB was very strong (R=0.90-1) for all species except for *Polyscias fulva* which was strong (R=0.70-0.89).

The D₃₀ was strongly correlated with ABGB for all species except for *Croton megalocarpus* and *Grevillea robusta* which were moderately correlated (R=0.40-0.69).

Stand Variables, Biomass, and C Stock for Different AF Tree Species of Ruhande

The mixture of AF trees was established on contour lines with large spacing, resulting in a stand density of 426 stems ha⁻¹. *Polyscias fulva* dominate the other species in the stand with 183 stems ha⁻¹, followed by *Grevillea robusta* with 170 stems ha⁻¹, then *Cedrela serrata* with 53 stems ha⁻¹ and finally, *Maesopsis eminii* with 20 stems ha⁻¹. Further, the analysis showed that *Grevillea robusta* has the highest WSD with 555 kg m³ followed by *Cedrela serrata* with 427 kg m³, *Maesopsis eminii* with 419 kg m³ and *Polyscias fulva* with 342 kg m³. This result showed a significant difference among different AF tree species (df = 3, p-value < 2.2e-16). Hereafter, there was a need to check which AF tree species the difference exactly is significant. The analysis did not confirm any significant

differences between *Cedrela serrata* and *Maesopsis eminii*, as far as WSD is concerned. The WSD was affected by tree species, size, and age. The obtained values are barely distinguishable from Kuyah et al. (2012) who found the high value (610 kg m³) on large trees and low values (390 kg m³) on small trees.

The C content was determined and on average, we found values for, *Grevillea robusta*, *Cedrela serrata*, *Maesopsis eminii*, and *Polyscias fulva*, which was 47.4%, 46.4%, 45.8%, and 45.8% respectively. Further analysis showed that the C sequestration rate of the Ruhande AF plot is 6.85 t ha⁻¹ yr⁻¹ corresponding to 25.07 t CO₂ ha⁻¹ yr⁻¹. The contribution of each AF species depended on its growth stage (both H and DBH), stand age, C content, and WSD. These species were ranked chronologically based on their contribution to C sequestration. *Grevillea robusta* > *Polyscias fulva* > *Cedrela serrata* > *Maesopsis eminii* with 5.18 t ha⁻¹ yr⁻¹, 0.84 t ha⁻¹ yr⁻¹, 0.81 t ha⁻¹ yr⁻¹ and 0.01 t ha⁻¹ yr⁻¹ chronologically.

Table 6 shows the amount of living biomass and C stock of four different AF tree species of the Ruhande AF plot located in Ruhande Arboretum. The *Grevillea robusta* contributes more than other species in terms of wood biomass (AGB & BGB), C stock, C sequestration, and CO₂e. The second AF tree species that were found to contribute more to the above-mentioned variables are *Polyscias fulva* followed by *Cedrela serrata* and the least was *Maesopsis eminii*. The variability in studied parameters was mainly due to the stand density (number of stems ha⁻¹) and AF tree species.

WSD [kg/m³] for old species 34 years old in Ruhande was studied and the highest value was found on *Grevillea robusta* (555 kg m⁻³) followed by *Cedrela serrata* (427 kg m⁻³), *Maesopsis eminii* (419 kg m⁻³) and *Polyscias fulva* (342 kg m⁻³). The correlation between DBH and ABGB was very strong for all AF trees.

Table 6. The estimated amount of living biomass and C stock of four AF species of Ruhande AF plot

| # | Species name | Density (stems/ha) | AGB (t/ha) | BGB (t/ha) | ABGB (t/ha) | C stock (t/ha) | C sequestration rate (t/ha/year) | CO ₂ e (t/ha) |
|---|--------------------------|--------------------|------------|------------|-------------|----------------|----------------------------------|--------------------------|
| 1 | <i>Cedrela serrata</i> | 53 | 45.79 | 13.74 | 59.53 | 27.66 | 0.81 | 13 |
| 2 | <i>Grevillea robusta</i> | 170 | 284.99 | 85.5 | 370.49 | 176.13 | 5.18 | 82.78 |
| 3 | <i>Maesopsis eminii</i> | 20 | 32.99 | 9.9 | 42.89 | 0.45 | 0.01 | 0.21 |
| 4 | <i>Polyscias fulva</i> | 183 | 48.17 | 14.45 | 62.62 | 28.7 | 0.84 | 13.49 |
| | Total | 426 | 411.94 | 123.58 | 535.52 | 232.94 | 6.85 | 109.48 |

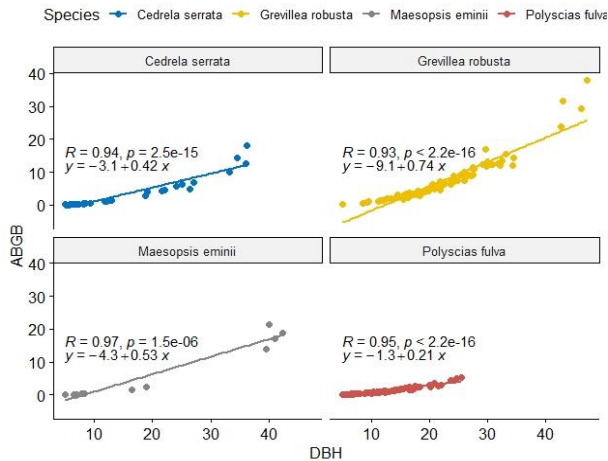


Figure 7. Regression analysis between ABGB and DBH for four AF species of Ruhande AF plot

Discussion

It was found that the amount of C sequestered and stored by various AF tree species and their mixture in various study areas was affected by tree species, stand density, age and tree size, and environmental conditions in terms of rainfall. A lot of rainfall was recorded in Cyamudongo while less intensive rainfall was recorded in the Ruhande study area (Figure 4). Nair et al. (2009) affirmed that the extent of C captured by the AF system depends on various factors including edaphic, climatic, and applied management practices.

The obtained C stock values are in good agreement with Hu et al. (2015) who reported more C stock on big size trees than on small trees. They found differences in biomass C that varied with the composition of tree species and their arrangement within the stand. The young trees were found to grow fast than mature ones and this has an impact on C sequestration. The tests highlighted that the total C sequestration rate recorded for young AF tree species established by the project in the surroundings of Cyamudongo isolated rain forest is $13.11 \text{ t C ha}^{-1} \text{ yr}^{-1}$ corresponding to $47.94 \text{ t CO}_2 \text{ ha}^{-1} \text{ yr}^{-1}$ centered on the young trees of 2 years old whereas the analysis further showed that the C sequestration rate of the Ruhande AF plot of 34 years old is $6.85 \text{ t ha}^{-1} \text{ yr}^{-1}$ corresponding to $25.07 \text{ t CO}_2 \text{ ha}^{-1} \text{ yr}^{-1}$. This indicates the capacity of young tree species to mitigate climate change-related issues through the removal of CO_2 from the atmosphere. This is in the line with Hairiah et al. (2010) who reported more C sequestration rate for young species compared to mature trees. The growth stage was found to contribute to the C stored by various AF tree species. By comparing the C stored by the young tree species to the quantity of C stored

by mature or old AF tree species, the tests revealed a high amount of C in mature trees than that of young AF tree species across all study areas. This result is following Agevi et al. (2017) who found that the tree biomass held in trees varies from one region to the other and is dependent on species density, age, and both climatic soil factors.

In Cyamudongo, the existing tree species stored 787.12 t ha^{-1} while the tree species that were established by the Project stored 35.83 t ha^{-1} . The old AF tree species of Ruhande stored 232.94 t ha^{-1} . The high amount of C stored by mature species found in Cyamudongo may be attributed to the favorable environmental conditions in terms of rainfall and other factors such as tree age, growth stage, WSD, and applied management practices including the number of trees per hectare (density). This concurs well with Arora et al. (2014) who reported more wood biomass and C stock on trees with more ages than young species. They further stated that the tree biomass and C stock increased with age. This fits also with Hairiah et al. (2010) who stated that mature AF trees have the potential to have more C stock than newly established tree species and also confirms with previous findings of Slik et al. (2010) and Wassihun et al. (2019) who stated that the AGB is affected by stand density. They further clarified that the AGB and stand density are strongly correlated with each other. Concerning the WSD tested for various AF species, it is clear to conclude that it varies with tree species, age, and environmental conditions. This result lends support to Mukuralinda et al. (2021) who reported various wood densities for different tree species under their study conducted in Ruhande arboretum, Rwanda.

The correlation between DBH and ABGB was very strong for all mature AF trees. This lends support to Chambers et al. (2001) who reported a similar relationship between various tree species as far as DBH and biomass are concerned for mature trees. As reported by Slik et al. (2013) the evidence we found points to the correlations between DBH and biomass which was strongly positive and is an indicator of the contribution of big diameter to the biomass allocation in wood. By comparing the correlation coefficients for various tree variables for young and mature AF tree species, the results showed a high correlation variability for young species than mature or old species recorded in different environmental conditions of Cyamudongo and Ruhande. The young tree species have a high growth rate, which rapidly affects some of the tree variables such as tree height and diameter. This substantiates previous findings in the literature where the wood biomass was found to expressively increased with the size in terms of diameter and age of trees (Healey et al., 2016). These variables and the WSD affect the overall tree biomass. For mature trees, the change in terms of height and diameter is not remarkable. These were found to be the main reasons for

the low variability of correlation coefficients for mature tree species compared to young tree species (Nsengumuremyi, 2021). Returning to the hypothesis posed at the beginning of this study, it is now possible to state that there is a significant difference between the amount of the fixed C in the biomass of AF trees species established by the project and the existing AF trees species of the study areas. In all study areas, it was found that various AF tree species contribute differently to C stock and C sequestration and the amount of C stored and removed from the atmosphere depends on different factors such as tree species, plantation density, growth stage, or age of establishment, applied management practices, WSD, wood C concentration, and climatic conditions in terms of rainfall.

Conclusion

It can be concluded that various agroforestry tree species should be adopted in different agro ecological conditions of Rwanda not only for the purpose of improving the soil productivity through erosion control and providing multiple products such as fuelwood, stakes, fodder, timber but also as an effort to mitigate against the climate change through C sequestration. This work could be the basis for the future researches on C stock and C sequestration of different agroforestry tree species growing in Rwandan landscapes. The environmental policy might promote more species contributing more to C stock like *Grevillea robusta*.

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