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Survival of Seedlings of Native Woody Species in the Rehabilitation of Gold Mined-Site within a Three Years Old *Acacia mangium* Willd Plantation of St. Elizabeth-Mahdia, Guyana

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Abstract: Active restoration is a critical component of biodiversity conservation for degraded tropical forest ecosystems caused by artisanal gold mining, and the success of restoration is dependent on native species selection. However, significant knowledge gaps exist regarding when and where to plant trees. This article reports on a revegetation trial undertaken in St Elizabeth, Mahdia, Guyana, to assess the survival and RGR (Relative Growth Rate) of three native woody trees and shrubs planted within three years old *Acacia mangium* Willd trees pruned and unpruned blocks. ANOVA (Analysis of Variance) for a completely randomized block design with four blocks, two pruned and two unpruned, within *A. mangium* plots. Biochar treatment was added to the plants during transplanting. Thirty-six (36) wildlings of *Humiria balsamifera* (Aublet.) (Tauroniro), *Goupia glabra* Aublet (Kabukalli), and *Vismia guianensis* (Aublet.) Choisy (Bloodwood) were collected and raised in a tree nursery for two weeks. The native plants were transplanted 3 m apart, survival observations and each seedling's initial height and diameter were measured and recorded. After the experiment, 13% of seedlings from a population of 720 had died, with the highest mortality being experienced at the 92 days of the experiment ($t \leq 122$ days). While the overall survival rates were high, emphasizing the importance of field trials on native and exotic species in different environments is essential to fill the knowledge gaps on suitable species for restoration in degraded areas with other land use histories.

Key words: Artisanal gold mining, native species survival, *Acacia mangium*, diameter growth rate.

1. Introduction

ASGM (Artisanal and Small-Scale Gold Mining) has been reported to seriously threaten forest ecosystems in the Guiana Shield, including Guyana [1]. Gold mining is responsible for 41% of deforestation in South America [2]. The most identifiable direct impact of ASGM is mercury contamination and indirect sewage industrial wastes, used oils, lubricants, and other toxic element leaching into rivers through runoff. Forest degradation is an unavoidable event associated with ASGM [3], which is widely defined as a human process that negatively affects the environment and the land to function effectively [4]. In Mahdia, small-scale gold mining operation consists of colluvial and alluvial deposits of gold. Miners remove the overburden using bulldozers, excavators and then high-pressure water flow to loosen the colluvium. The resulting slurry is then pumped to a carpeted sluice box, where water flow and gravity separate the gold and heavy minerals from the gauge. The gold is very fine and is difficult to capture on the sluice bed. Hence the heavy concentrate from the sluice box is collected in large basins, and

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mercury is added to amalgamate the gold. Then the amalgam is separated from the excess mercury using a fine mesh cloth. The resulting amalgam product is burned in the open air using a propane torch and heated in fire [5]. Parrotta et al. [6] reported slow forest recovery on abandoned ASGM. Colonization of vegetation differs at the different proportions of land within the abandoned mine site, where an open pit was located; no regeneration occurred except on a small portion of soils attached to surrounding uprooted trees [7]. Ecological restoration in mining depends on whether or not the topsoil has been lost or retained during open-pit mining [8]. In areas where the soil has been lost, vegetation establishment is difficult due to the substrate's physical and chemical nature, which varies from mine to mine [8, 9]. Understanding plant-species-substrate interaction is fundamental for the successful revegetation of mine lands [8]. As such, management of these lands has either been ignored or revegetated using monoculture plantations with a few numbers of exotic species.

Species selection in the restoration of areas impacted by mining presents serious challenges due to the removal of topsoil and disturbances of the soil profile [6]. The process of species selection for reestablishing forest cover in areas degraded by open-cast mines has been limited to a few exotic and native species [10, 11]. The main reason for selecting the narrow range of species is the availability of documented silvicultural information on a wide range of exotic and native species useful for reforestation programs [11]. Therefore, the basic restoration strategy minimizes the major edaphic constraint to attain plant growth [12]. The introduction of leguminous trees (*Acacia mangium*) as potential species for revegetating abandoned gold mining land in Mahdia started in 2009. Native woody pioneer species can recolonize recently disturbed sites and reproduce faster; therefore, *Acacia mangium* was intercropped with native pioneer plants and shrubs [13].

Hence, this study assessed the survivability and growth performance of three native woody species (two commercial timber trees and one scrub) planted within a three-year-old *A. mangium* plantation using biochar as a soil amendment. The general objective is to assess the growth performance of three native woody species planting within a three-year-old *A. mangium* plantation subjected to biochar treatments. The study focused: (i) on assessing the survival of three native pioneer species planted in pruned and unpruned three-year old *A. mangium* plots; (ii) on comparing the growth of three native pioneer species in pruned and unpruned plots; and (iii) on determining the effect of biochar amendment in the growth performance of the selected species. The research aimed to investigate the growth and survivability of three native pioneer species (tree and shrub) using biochar as a soil amendment in pruned and un-pruned *Acacia mangium* stands previously established in mined land. We address the following questions:

(1) Does biochar amendment in pruned and un-pruned *Acacia mangium* stand have an influence on the survival of three native pioneer species that went planted in a three-years old *Acacia mangium* stand subjected to pruning treatments?

(2) Does biochar amendment in pruned and un-pruned Acacia mangium plots influence the growth performance of three native pioneer species planted within a three-year old *Acacia mangium* stand?

Hence, the hypothesis tested was: Tropical native pioneer woody plants show greater survival in pruned plots with biochar amendments.

2. Material and Methods

The study area is situated in a mining community about 6 h from Georgetown, Guyana's capital city. It is known as the Potaro-Siparuni region (Fig. 1). This region was established by the Africans in 1884 after emancipation. They travelled to this region in search of gold. Inside this region, the St. Elizabeth *A. mangium* plantation is located. The plantation was established with 1,000 trees *A. mangium* trees (5°17′33.2″ N; 59°07′56.0″ W). Our experiment was carried-out within this location.

Annual precipitation is above 1,800 mm, and an average ambient temperature of 37 ℃. In 2012 the study site experienced above 1,000 mm of annual rainfall (Fig. 2). The area has approximately 415 m (1,360 feet) above sea level. The predominant soil type was sandy soil, with clay in some areas within the plantation.

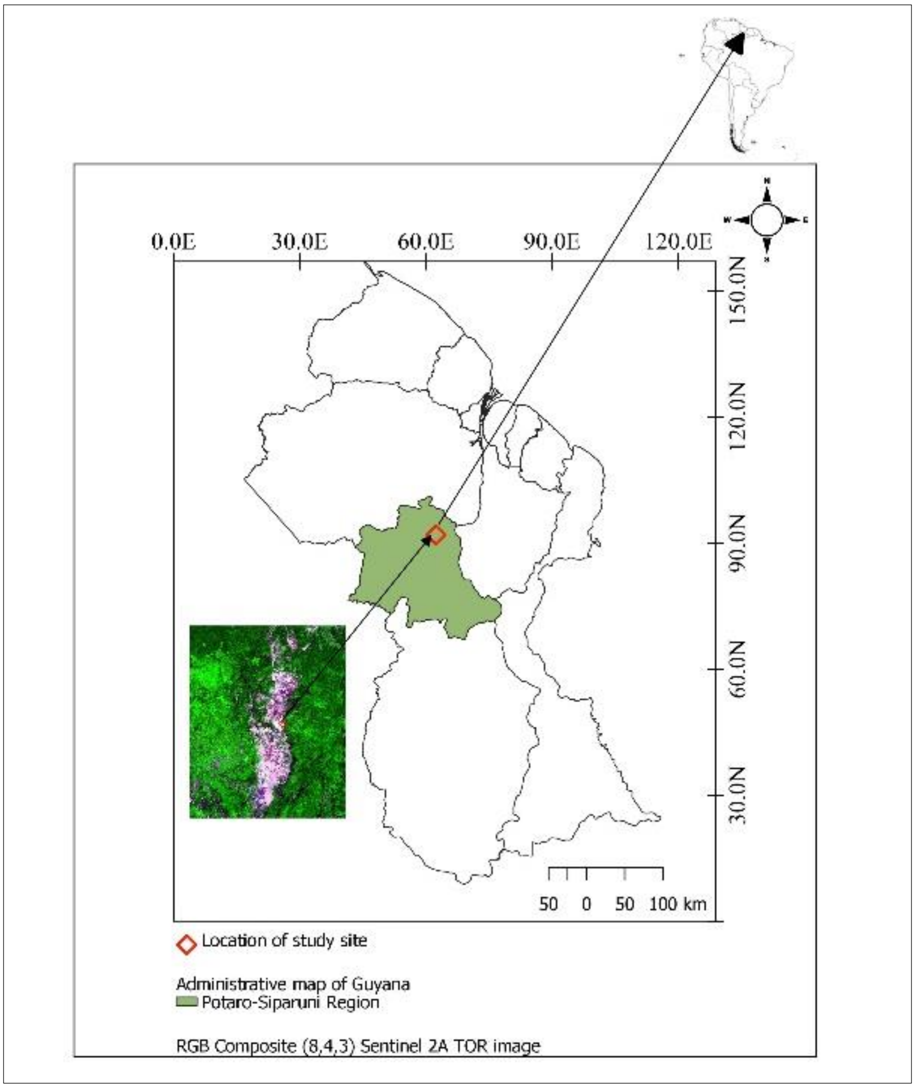


Fig. 1 Geographic location of the study site which is located in the Potaro-Siparuni, Administrative region 8 of Guyana (Source: Google Earth Engine, Sentinel-2A satellite image).

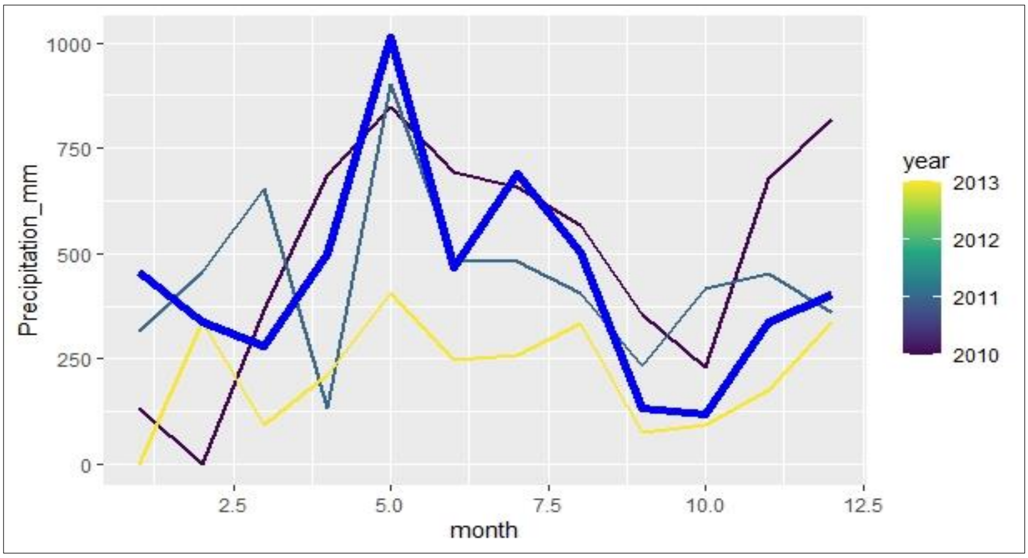


Fig. 2 Precipitation data. The bold blue line indicates the monthly rainfall for the year 2012 (Source: CHIRPS).

3. Species Studied

Kabukalli (*Goupia glabra* Aublet) belongs to the Goupiaceae family, formally included in the Celestraceae family [14]. Large canopy tree, sometimes emergent above the canopy layer Kabukalli is a widely distributed pioneer species [15] that forms dense stands in secondary forests along roads as young trees [16]. It is frequently found in sandy soils and old clearings in the Iwokrama forest [17], and locally abundant in the Guianas [14]. *G. glabra* is long-lived pioneer species. For instance, *G. glabra* was found to be long-lived pioneer taxa of third-phase secondary forests among the dominants of secondary forests in the upper Rio together with *Vochysia* spp. and *Jacaranda copaia* and *G. glabra* shown to be present in the soil seed bank, must contribute to the rapid colonization of recently disturbed sites [18]. In the American tropics, the abundance of pioneer species such as *Goupia glabra*, *Vismia guianensis* and species of *Inga* and *Miconia* indicates that forest is secondary [19, 20]. Copious seed production and vegetative reproduction by suckers from the roots or at the base of the stem are examples of early seral trees in the American tropics, such as *Cecropia*, *Goupia glabra* and *Trema* [20]. The late-successional species that can penetrate deeper soil horizons than other co-occurring species is Kabukalli; it is present early and during the successional process. However, this species is also frequently found emergent in primary forests [21]. The fruit of *G. glabra* is a small berry which is dispersed by birds, howler monkeys and spider monkeys (endozoochorous dispersal mode) [14].

Tauroniro (*Humiria balsamifera* Aublet) belongs to the Humiriaceae family. *Humiria balsamifera* is partial shade-tolerant species [22]. *H. balsamifera* is a canopy tree with a heavy rounded crown; the bole is cylindrical. *H. balsamifera* is dominant species in Marsh forests, occasionally frequent in seasonal forests on brown sand, Wallaba forest on white sand in the near interior and Rupununi district of Guyana; young trees are abundant in secondary vegetation along the road edges [16]. Trees to 35 m in height can be found in wet savanna forests or rainforests on sand and shrub in wet savanna [23]. The species used in this study are large trees belonging to the var. *balsamifera* with ovate, stem-clasping, and sessile leaves alternate arranged. The fruit is an oblong-ellipsoid drupe with an edible, sweet, juicy mesocarp and a bony endocarp.

Bloodwood (*Vismia guianensis* Aublet) belongs to the Guttiferae family. *Vismia guianensis* is a short-lived pioneer and early successional species [6, 15]. The twigs and leaf blades of Vismia species, which are found as trees and shrubs, are covered in dense layers of rusty stellate hairs. Dark orange exudate is visible

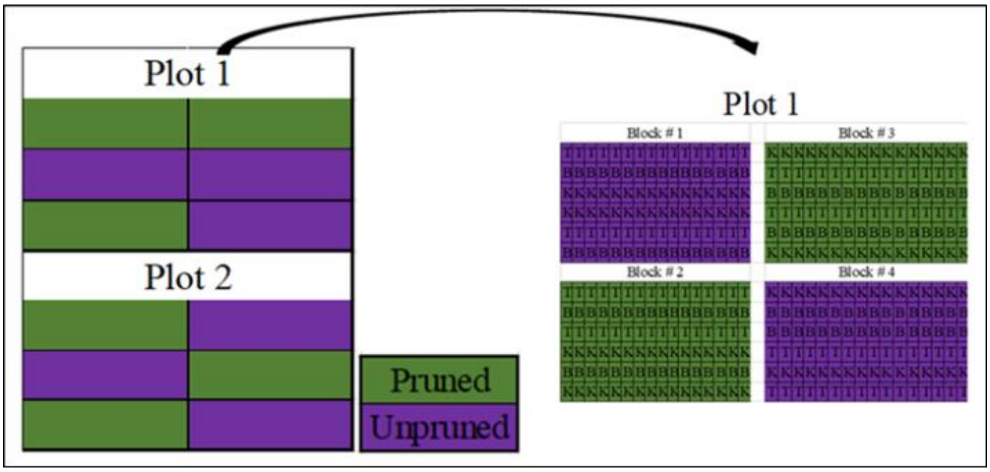


Fig. 3 Left: Layout of the pruned and unpruned *A. mangium* subplots for Plot 1 and Plot 2. Right: Experimental layout for the planting of native woody species within *A. mangium plantation* Plot 1.

when the bark is sliced, or the twigs are broken [24]. For example, *V. guianensis* is a short tree of approximately 8-11 m tall with slower diameter and height growth, higher wood density, and higher height: crown ratios, which dominated forest regrowth after 4-5 years of pasture abandonment in forest fragments in Central Amazonia [25]. Bloodwood is an abundant secondary species [23]. Brazil is home to 30 of the 60 neotropical *Vismia* species. The essential characteristics for identification are "leaf indumenta, presence and absence of glands and/or canals in the petals and sepals, number of stamens per fascicles, fruit shape and sepal arrangement in mature fruits" [26].

4. Experimental Design

Two pruned subplots, namely block one and two unpruned subplots, namely Block 2 were randomly selected within Plot 1 (Fig. 3). The experimental design was a CRBD (Completely Randomized Block Design). Three native woody species (two commercial timber trees and one scrub) were collected from the mine road edges nearby the planting sites. Thirty-six (36) wildlings of each species: *Humiria balsamifera* (Tauroniro), *Goupia glabra* (Kabukalli) and *Vismia* spp. (Bloodwood) were collected and raised in a tree nursery for two weeks. Four planting areas were then chosen, two pruned and two unpruned within *A. mangium* plots (Fig. 3). The native plants were transplanted 3 m apart, and each seedling's initial height and diameter were measured and recorded.

5. Seedling Measurement

Differences in survival related to three covariates (species, pruned vs. unpruned treatments, time, biochar amendments vs. without biochar amendment) were assessed by two native woody commercial timber species (*Humiria balsamifera* and *Goupia glabra*) which were planted in between the *Acacia mangium* plantation. One hundred forty-four native woody species and scrub were planted within the pruned and unpruned rows of the A. *mangium* trees, 72 per unpruned and 72 per pruned plots. All tree and scrub seedlings within each row were marked with 2 × 3.5 cm aluminum tags loosely attached by copper wire to the stem base. The study was conducted for a short period of eight (8) months. We planted the seedlings during the rainy season in January 2012; the initial height and stem base diameter measurements were taken for all the seedlings before field planting. Plant height measurement was taken from the root collar of the seedling to the terminal shoot or apical meristem using a measuring tape; if the apical meristem was damaged, height was taken from the lateral branch, and these seedlings were categorized as resprouting. The diameter was measured using a digital caliper and was taken 10 cm from the root collar. The second measurement was collected during the dry season in August of the same year. The data set included: (i) 3 woody plant seedlings (trees and scrub), (ii) 720 diameter and height increments and (iii) 4 survival observations.

Seedlings were considered dead only if all of the stems were obviously desiccated, with no apparent chance for resprouting. If a stem was missing in a row, it was declared dead as of the first date of "missing" status. On some occasions (most commonly in Bloodwood plants within the unpruned trees/plots) and on one occasion (*Humiria balsamifera* and *Goupia glabra* in pruned and unpruned plots), seedlings that were leaflets but the root collar of the stem was green were categorized as alive, similarly for stems were resprouted after the epical meristem being declared either broken or dried. Resprouted stems were reclassified as alive and height measurement was taken from the base to the new terminal shoot. On two occasions, two Bloodwood plants were completely removed by people. Most of these cases occurred within the unpruned *A. mangium* trees.

6. Statistical Analysis

Statistical significance of the planting treatment/condition was analyzed using Statistic 10 software for ANOVA (Analysis of variance) for a completely randomized complete block design with four blocks. Post hoc analysis was performed using Fisher's LSD (Least Significance Difference) test ($p < 0.05$) [27]. For *Acacia mangium* plantation survival percentages, comparison data were arcsine transformed [28], though data are reported as original means with standard errors.

Species survivorship was calculated in a plot basis, as the percentage of initially planted seedlings still alive 122 days after planting. Diameter and height RGR (Relative Growth Rates) were calculated for all surviving plants as follows: $RGR_{diameter}$ = [ln(final diameter) - ln(initial diameter)]/122 days [29].

7. Results

The results of the Shapiro-Wilk Normality Test approach one for normally distributed data (W = 0.8855), the p-value = 0.0107, suggested that the survival data were not normally distributed. Since the data showed no normal distribution, the survivorship and growth performance of seedlings' statistical analysis used a non-parametric and post-hoc test after the statistical test.

8. Seedling of Native Species Survival

At the conclusion of the experiment, 13% of seedlings from a population of 720 had died, with the highest mortality being experienced at the 92 days of the experiment ($t \leq 122$ days). The woody shrub Bloodwood (*Vismia* spp.) was the species with the highest mortality throughout the experiment, whilst Kabukalli (*Goupia glabra*) experienced high mortality only at 92 days of the experiment (Fig. 4).

A significant difference between both treatments (with biochar and without biochar) was present during the experiment (p = 0.0123) (Figs. 5a and 5b).

Seedling (tree and shrub) survival decreased with biochar and without biochar amendments from the initial day of observation, and differences were non-significant throughout the experiment (p = 0.3152). Biochar amendment significantly affects seedling survival in pruned and unpruned plots (Fig. 5a). Native seedlings within the *A. mangium* Willd pruned plots with biochar amendment had higher survivability when compared to the unpruned plots without biochar amendment (Figs. 6a and 6b). The seedling mortality rate was higher in the unpruned plots without biochar amendments. Above 95% of survival occurs in all the species in the pruned plots when compared to the unpruned plots. *H. balsamifera* showed the highest survival (86%) when compared to the other two species.

The comparison of the means of the native species under the *A. mangium* Wild plantation is illustrated in Figs. 7a and 7b. The ANOVA for the CRBD showed no significant pairwise differences in the survival means of the three species in the pruned and unpruned plots (CV = 95%) within *A. mangium* three-year old plantation (p = 0.3152), but there was a significant

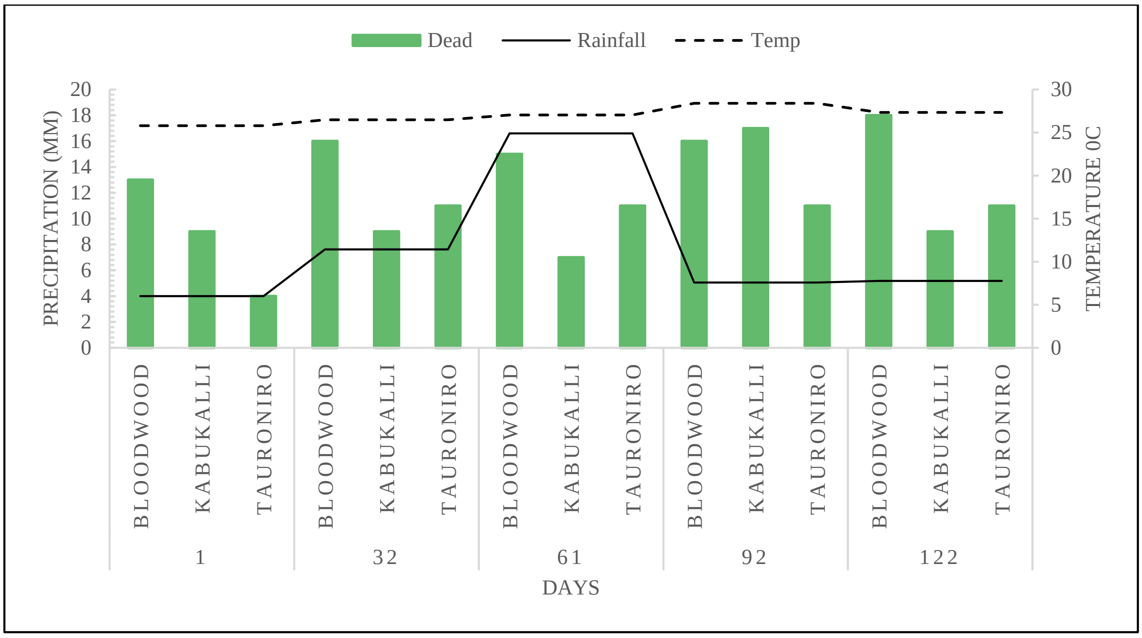
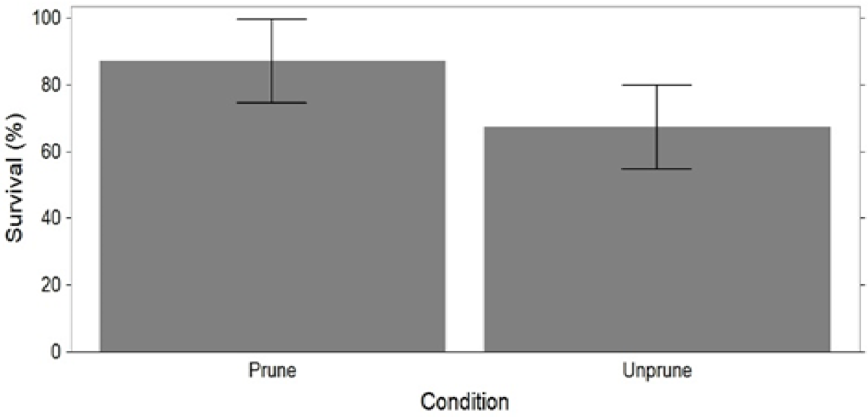


Fig. 4 A comparison of the number of seedlings and average rainfall by days per species.



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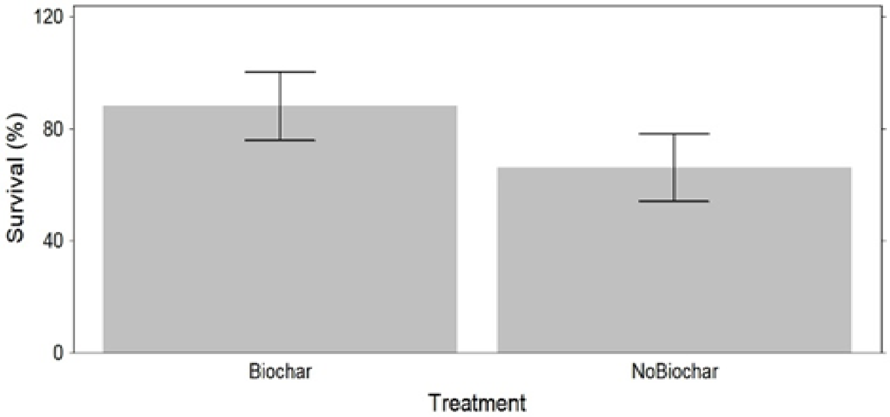
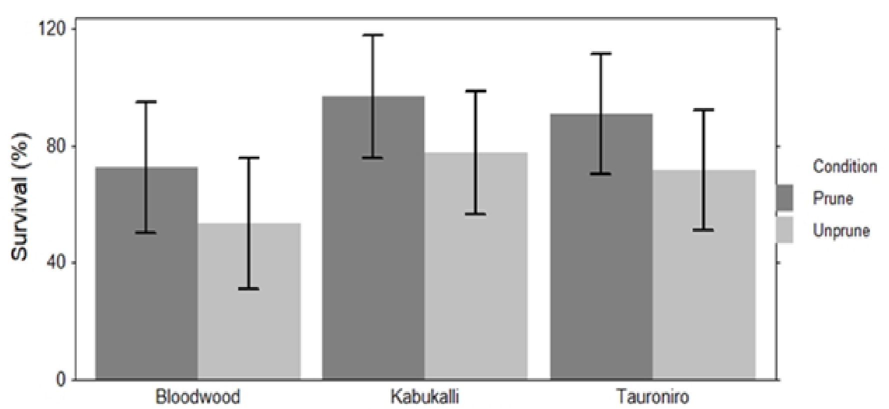


(b)

Fig. 5 (a) Pruned and unpruned plots; (b) plots with biochar and without biochar amendments.



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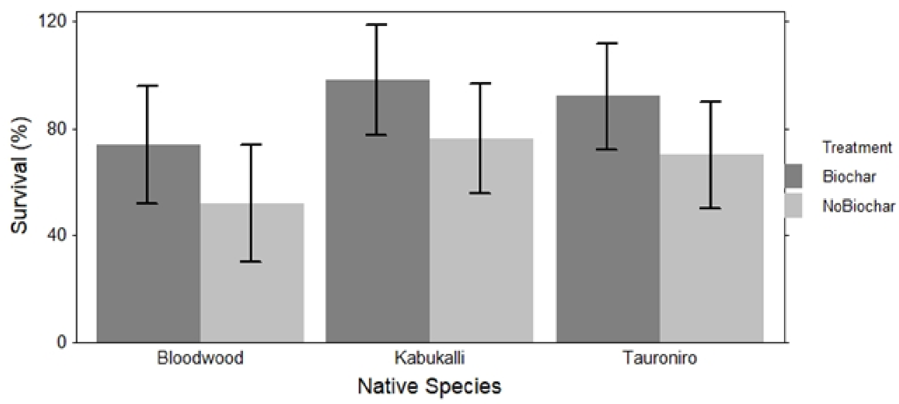


(b)

Fig. 6 Native species survival percentage in pruned: Biochar treatments and environmental effects on the relative height growth rate of Seedlings.

Table 1 Height and ground-basal stem diameter of three native seedlings after 122 days as affected by biochar amendment treatments within *A. mangium* plantation.

Treatment	Biochar	Without biochar	Pruned	Unpruned
Height (cm)	3.5[a]	2.4[b]	3.4[a]	1.4[a]
Diameter (cm)	1.4[a]	1.0[b]	2.5[b]	1.0[b]

Within a row, means with different letters (a and b) indicate significant differences (n = 142).

difference between the survival means of the three native species between the pruned and unpruned plots (Fig. 7). The ANOVA showed higher percentage survival under pruned plots with biochar amendment *Goupia glabra* (Kabukalli) and *H. balsamifera* (Tauroniro) whilst lower percentage survival in *Vismia spp* (Bloodwood) plots with biochar (p = 0.2178). All native species survival percentages were greater in pruned plots with biochar amendment and lower in the unpruned plots without biochar amendment (p = 0.0091) (Figs. 7a and 7b).

Height ranged from 3.5 cm (with biochar) to 2.4 cm (without biochar) (Table 1). Seedling height responses with and without biochar amendment were significantly different. Among biochar amendment treatment, all species' response was higher than those without biochar amendment. Ground-based diameter ranged from 1.4 cm (with biochar amendment) to 1.0 cm (without biochar amendment). Seedlings' height and ground-based diameter means were significantly statistically different. The response to biochar amendment was higher in pruned plots with biochar amendment compared to unpruned plots without biochar amendment (Table 1).

9. Biochar Treatments and Environmental Effects on the Relative Diameter Growth Rate of Seedlings

ANOVA for CRBD for the three native species mean revealed no significant differences (p = 0.2523). The effect of biochar amendment as a treatment on RGRs in diameter did not differ significantly for the three native species. However, the RGRs in diameter means for biochar amendments treatments and pruning effect were significantly pairwise different. The Fisher's LSD posthoc comparison test for pruned and unpruned plots showed α = 0.05; SD ± 0.12; p = 0.0010. Similarly, results for biochar and without biochar treatment were significantly different from one another (α = 0.05; SD ± 0.12; p = 0.0059).

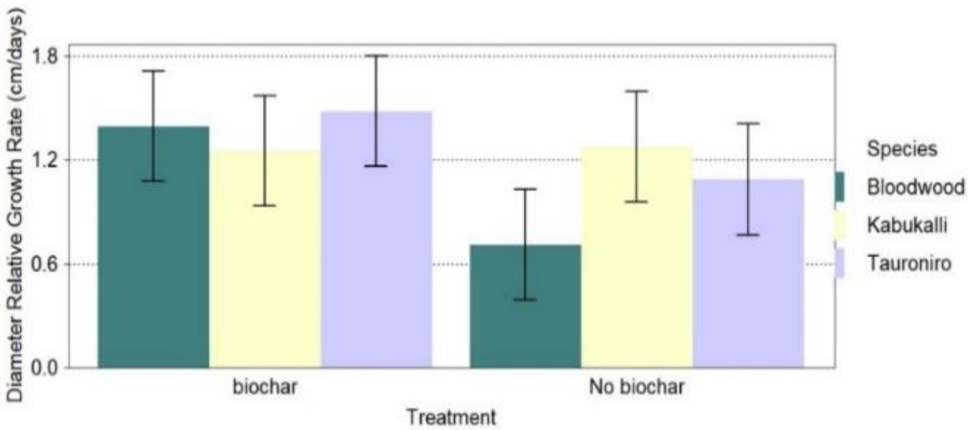
10. Discussion

The first months of planting is when the seedlings were adapted to the harsh edaphic and environmental conditions at the site where they were transplanted. Seedlings that were planted in less canopy cover survived less. These findings were supported by Fagundes Alencar et al. [30], who found that seedlings transplanted in non-vegetated degraded sites are more likely to present higher mortality. Based on the results (Fig. 6) there was a significant difference in the survivability of all species in the pruned and unpruned plots. The unpruned plots had the highest mortality when compared to the pruned plots. The decrease in survival rates over the last period of the experiment could be attributed to the climatic factors in the study location, as rainfall significantly decreased within 61-91 days of the experiment. 2012 was the warmest La Niña year recorded by the National Centers for Environmental Information [31].

The seedlings were transplanted during the rainy season (December-January); however, the monthly average precipitation was extremely low (6 mm) for this region; therefore, the low humidity of the soil must have been one of the limiting factors for the growth performance of some species in some plots [32]. Despite the adverse effects of the La Niña event, native species survival was high in the plantation. The low survivability of seedlings in the unpruned plots may be due to species demand for light in open areas than semi-shade conditions in the case of *Vismia* spp. and *Humiria balsamifera* [25] as well as *Goupia glabra* which is categorized as long-lived pioneer species capable to survive under 37% of canopy opening in the natural environment [15]. The abundance of *H. balsamifera* was reported for a disturbed area by multiple land uses in the state of Amapá in the northern region of Brazil and, together with *G. glabra*, which were classified as late secondary successional species [33].

The results in Table 1 showed no significant difference for the species Kabukalli and Tauroniro without biochar treatment; however, there was a significant difference between these two species when compared to Bloodwood. A study by Ippolito et al. [34] suggested that biochar can be used to reduce soil pH and heavy metal contamination by acting as a liming source, allowing bioavailable metal concentrations to reduce through interactions with rainfall. Furthermore, biochar has very low N content and, through ammonium sorption, and may bind available N so strongly that it becomes less available to the plant, as such biochar should be used as part of a soil amendment mixture, especially on mine-lands with high levels of soil degradation in forest restoration practices [11]. However, the three-year-old *A. mangium* trees modified the microclimate by providing a thick leaf litter that aided the native seedlings in the adaptation process. According to Ewel and Putz [35], exotic species can provide the initial condition without causing harm, and their introduction should be considered a site-specific decision. Acacia species are thought to be "nurse plants" that helped native species adapt in heavily disturbed, eroded areas of South China [36]. These authors also contend that, in addition to the plantation's climatic, soil, and light conditions, flooding from nearby mining operations was a factor that influenced the mortality of both native species and *A. mangium* trees in some plots located in low flat areas of the plantation. Studies to compare this finding are scares in the Guianas and are regarded as country-specific; therefore, studies that provide information on forest recovery using native species and exotic species are in much needed. Besides those mentioned earlier, long-term studies involving edaphic conditions and environmental parameters are critical to better understanding native seedling survival and growth performance in different disturbed areas at a large scale.

Seedling survivorship, and RGR in height and diameter, varied significantly across the three native species in the pruned and unpruned plots. However, there was a significant difference among the means for height for each species in the pruned plot compared to the unpruned (Fig. 8). However, there was no significant difference among the means for diameter across and among the three species in the pruned and unpruned plots (Fig. 7). Compared to Bloodwood, Kabukalli and Tauroniro, they exhibited rapid relative height and diameter growth in pruned areas with biochar. Whitmore [37] states that such late successional species have superficial root systems, reproduce early, and colonize young areas and natural forest clearings. For example, the regeneration of fast-growing pioneer species. Large canopy gaps are the preferred light environment for Kabukalli, which is expected in the first years of succession. In the juvenile stage, Kabukalli forms high stocking [38], and has been found in soil seed banks. All of these characteristics contribute to the rapid colonization of recently disturbed areas.



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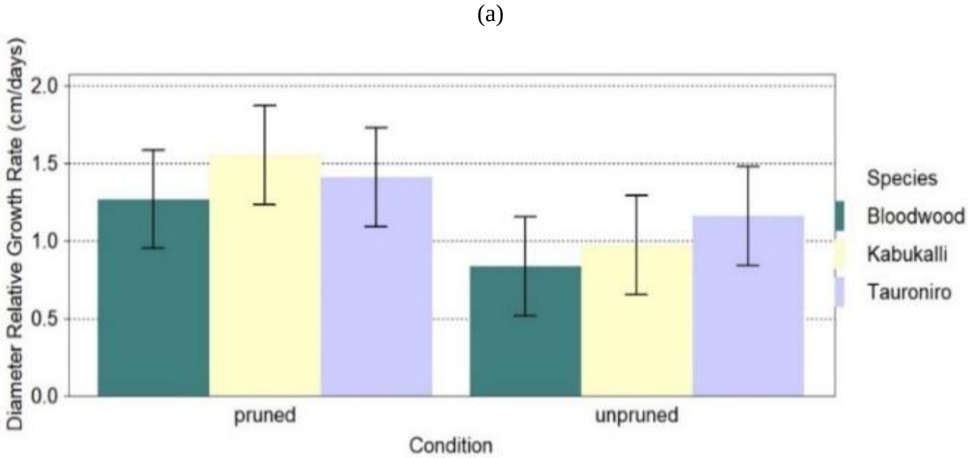

(b)

Fig. 7 The LSD all-pairwise comparisons test showed a significant difference on relative diameter growth among the native species in pruned and unpruned plots.

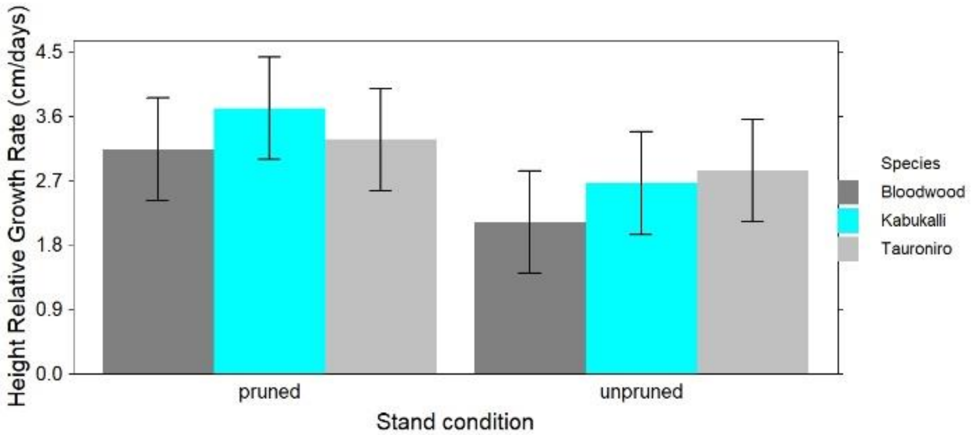

Fig. 8 The LSD all-pairwise comparisons test showed a significant difference on relative height growth among the native species in pruned and unpruned plots.

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