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Biochar Effects on Two Tropical Tree Species and Its Potential as a Tool for Reforestation

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Abstract: Research Highlights: We find that biochar plus fertilizer has synergistic and positive effects on seedling growth and robustness, but slightly lowers early seedling survival. Biochar plus fertilizer has the potential to greatly decrease costs associated with afforestation as compared to traditional fertilization and gives better results. Background and Objectives: Biochar can improve soil fertility and plant yield in crops. However, there is little information regarding the effects of biochar on trees, even though reforestation/afforestation projects are increasing and are often unsuccessful due to soil fertility limitations. This study aims to increase knowledge of biochar use as a reforestation tool. Materials and Methods: We measured survival and growth in an early ((*Guazuma crinita* Mart. [n = 240])) and a late (*Terminalia amazonia* (J.F. Gmel.) Exell. [n = 240]) successional species under 6 different biochar treatments in a 6-month nursery experiment. Results: (i) Survival was highest in the 1 t/ha biochar treatment, while treatments containing fertilizers or biochar at 5 t/ha lowered the survival rate of both species compared to the control; (ii) simultaneous addition of biochar and fertilizer lead to significant increases in height, diameter, total number of leaves, and aboveground and belowground biomass of both species as compared to other treatments; (iii) biochar treatment containing 1 t/ha with and without fertilizer showed significantly better results than applications of 5 t/ha; and (iv) *Guazuma crinita* responded more strongly to treatments containing biochar and fertilizers compared to *Terminalia amazonia*, which is suggestive of greater synergetic effects of biochar and fertilizer addition on early successional tree species. Conclusions: Applying biochar and fertilizer is synergistic and outperforms any single treatment, as well as the control, in terms of plant performance. This case study suggests that biochar can greatly improve reforestation/afforestation projects by increasing plant performance while substantially reducing fertilizer and labor maintenance costs. Field experiments and testing of additional species is needed to generalize the findings.

Keywords: biochar; *Terminalia amazonia*; *Guazuma crinita*; *Bertholletia excelsa*; phenorythm; forest restoration; agroforestry; soil amendments; mining

1. Introduction

More than seven million hectares of forests are lost every year [1], even though they play a central role in soil and water protection and hundreds of millions of people depend on them for their livelihoods [2]. As natural forests are removed, the number of reforestation projects is increasing [3], and many countries have recently pledged massive reforestation/restoration projects [4] to support livelihoods and to sequester CO_2 [5,6]. Nonetheless, reforestation and forest restoration projects are complex, time-demanding, and expensive [7]. Indeed, project failure rates are high, in large part because trees die before reaching maturity due to the high cost of long-term soil and seedling care and insufficient funding [8], putting a focus on methods that can increase seedling survival, growth, and vigor, while reducing fertilizer and labor costs.

Biochar (thermochemically decomposed organic matter) is increasingly being investigated as a potential alternative for enhancing soil fertility [9,10]. Biochar application in soils is known (among other things) to reduce nutrient leaching, increase water retention, increase density and diversity of soil biota, reduce greenhouse gas emissions from soil, and increase soil pH [11–16], though the effect of biochar on plant production is highly dependent on plant and soil type, biochar characteristics, and climate [15,17,18]. However, most studies to date consider herbaceous crop response to biochar addition [15,19], while very few have examined the effect of biochar on trees [14,20,21].

A great majority of crop-based studies report an increase in yield (+10%–25%) after biochar addition [16,22]. Biochar appears to have a stronger positive effect when applied on acidic, nutrient-poor soils such as those widespread in the tropics [23–25]. For trees, the results of the 17 studies analyzed in Thomas and Gale [14] suggests that the positive effect of biochar on tree growth appears higher for angiosperms than conifers and in boreal and tropical systems rather than temperate ones. However, the authors reported a high variability in the results. The positive effect of biochar reported on crop yield and its optimistic preliminary results on tree growth suggest that biochar could play an important role in fulfilling challenging reforestation/restoration projects.

This study aims to explore the effects of pure and nutrient-enriched biochar on two contrasting successional tropical tree species. We measured the survivorship, growth, and biomass accumulation of *Terminalia amazonia* ("terminalia") and *Guazuma crinita* ("bolaina") seedlings in a nursery experiment with nutrient-poor, sandy soil from an abandoned gold mine, under 6 different treatments (three doses of biochar, with and without fertilizer) for a period of 6 months.

2. Materials and Methods

2.1. Study Site

The experiment was carried out in a tree nursery at the scientific station of the "Instituto de Investigaciones de la Amazonia Peruana" (IIAP) (12°39′06.6″ S 69°19′13.5″ W), located in the Tambopata Province, 20 km from the town of Puerto Maldonado in the Madre de Dios region, Peru. The climate is considered to be hot tropical humid [26]. The average temperature during the time of the experiment was 25.3 °C and the average monthly precipitation was 81 mm/month [27].

2.2. Species

Two species were chosen. *Terminalia amazonia*, a typical tree of humid to very humid climate, able to grow on acidic, sandy to clayey soils [28] and commonly used as timber [29], was selected as the late successional species. *Guazuma crinita*, a marketable fast-growing tree [30], was selected as the early successional species. Each species was planted at an equivalent development stage (at appearance of two first leaves) in a 280 mL germination tube and were kept under 40% shade cloth to avoid direct sunlight. During the dry season, the seedlings were watered twice per day (morning and evening) using micro sprinklers.

2.3. Soil, Biochar, and Fertilizer

The soil used as growing media originated from a former artisanal gold mining area in the San Jacinto Native Community, −20 km away from the nursery. It was excavated to 15 cm using a shovel and sieved at 2 mm to exclude gravel. Analysis of 12 soil samples revealed a highly sandy texture with a pH of 5.31 ± 0.35 (average ± standard deviation), an organic matter content of 0.12 ± 0.08% and a CEC of 4.55 ± 0.52 cmol (+)/kg.

Biochar was produced using a top-lit up-draft 55-gallon drum with locally obtained Brazil nut (*Bertholletia excelsa*) husks as the substrate. The biochar was crushed and sieved between 1 and 2 mm for homogeneity of the particle size. The biochar produced in this method was tested and granted a "Premium Quality Grade" according to the European Biochar Certificate standard [31] (Table 1). Fertilizer treatments used a mixture of granulated NPK 20-20-20 (MOLIMAX©, Molinos & Cía fertilizantes, Lima, Peru). The fertilizer was applied once, before transplant, in its granulated form either alone or mixed with biochar depending on the treatment.

Table 1. Biochar properties according to the methodology of the European Biochar Certificate [32].

Biochar Parameter	Unit	Value
Bulk density	kg/m^3	486
Specific surface	m^2/g	147.6
Ash content (550 °C)	% (*w*/*w*)	7.8
Carbon	% (*w*/*w*)	87.6
Total Nitrogen	% (*w*/*w*)	1.07
H/C_{org} ratio (molar)	-	0.19
pH in $CaCl_2$	-	9.6
Calcium (Ca)	% (*w*/*w*)	0.5
Magnesium (Mg)	% (*w*/*w*)	0.3
Potassium (K)	% (*w*/*w*)	2.1
Phosphorus	% (*w*/*w*)	0.3

2.4. Experimental Design

This experiment followed a randomized complete block design with a total of 12 treatments, where two types of soils (unfertilized and fertilized) and three rates of biochar applications (0, 1.1, and 5.5 t/ha) were tested on the two species (early and late successional species). Each treatment was represented by 10 individuals, replicated in 4 blocks, for a total of 480 individuals. Species and treatment locations were fully randomized, with further blocking to control for any unobserved spatial variation in the shade house.

Biochar and fertilizer content were calculated considering the reforestation rate of 1100 seedlings per hectare with a volume of soil for each seedling equivalent to a cube of 30 cm per side. The doses BC1 (1.1 t/ha) and BC5 (5.5 t/ha) represent the local application of 1 and 5 kg of biochar around each seedling and were selected considering their feasibility in the field. These amounts translate to 2.5 and 12.5% *w*/*w* of biochar in the experimental tubes for treatments BC1 and BC5, respectively. The fertilizer treatment mirrors the application rates of 100 kg/ha of NPK (20-20-20), which is within the range of initial fertilizer application for forestry practice in similar soil types [33–35]. The control treatment consisted exclusively of the sieved soil from the former mining area. All treatments were prepared beforehand by mixing the appropriate contents in large containers and leaving to rest for two weeks.

2.5. Data Collection

The seedlings were planted at the first two leaves development stage on the 19 April 2018. The initial measurements were taken on the 23 April 2018. Measurements of the diameter [mm] at the base of the seedling, and height [mm] from the tube-rim to the apex, were taken with a Vernier caliper. Additionally, the total number of fully developed leaves were counted.

Midterm results were taken exactly three months after first measurements. End measurements were taken on the 30 October 2018, 6 months and 8 days after first measurements (or 191 days). After final measurements, the seedlings were thoroughly washed with water, and each seedling was then divided at the root collar and freeze-dried for 72 h to measure the dry weight of the above and below ground biomass.

2.6. Statistical Analysis

Data processing, statistical analysis and figures were produced using R version (3.5.1) [36]. The effect of treatments was calculated through a mixed-model analysis of variance (ANOVA) with species and treatment levels as fixed effects and blocks as random effects. Post-hoc analyses were carried out using Tukey's honestly significance difference (HSD). The normality of data distributions and equal variance between treatments were tested using the Shapiro test and Bartlett's test, respectively [37]. Survivorship analyses were carried out using the generalized linear mixed-effect model "glmer" function from the "lme4" R package [38], and the potential interactions between the variables were assessed using the R package "lmerTest" [39] (Appendices A and B). To model the interaction of biochar and fertilizer with species, and their interactions with each other, we used a factorial design and coded "Fertilizer" as a factor with two levels (presence or absence of fertilizer in the treatment) and "Biochar" as a factor with three levels (0 t/ha, 1.1 t/ha and 5.5 t/ha) for the different amount of biochar present in each treatment, including Species and Blocks factors with Block set as a random factor.

3. Results

3.1. Survivorship

68% of *Guazuma crinita* and 75% of *Terminalia amazonia* individuals survived until the end of the experiment. Treatments had large effects, with within-species survival nearly doubling depending on the treatment (e.g., F vs. BC1; Figure 1). In both species studied, treatment BC1 and control reported a significantly higher survival rate than treatments BC5, BC5 + F and F ($p \leq 0.001$). The results from our midterm mortality count show that 97% of the mortality occurred during the first 3 months, with only 4 additional plants succumbing during the second half of the experiment.

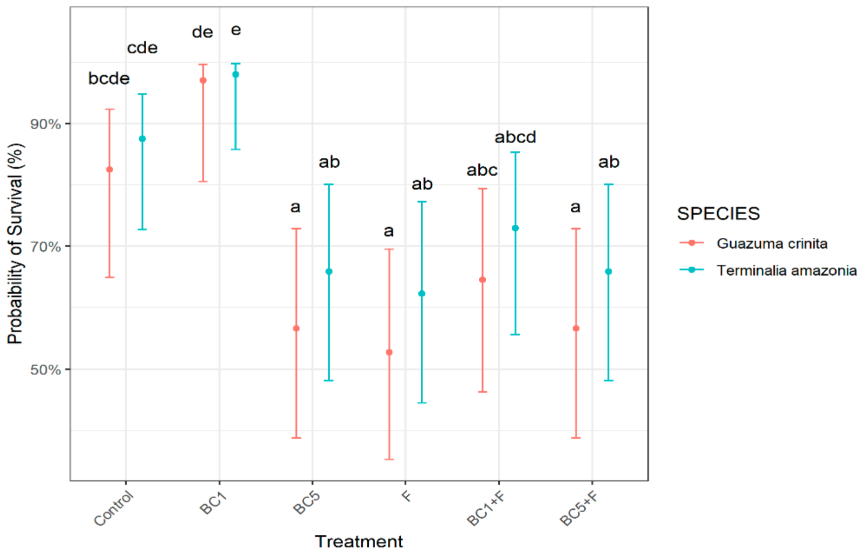

Figure 1. Probability of survival expressed as percentages among two tropical tree species in response to biochar and fertilization treatments (BC1 = Biochar 1 kg/plant; BC1 + F = Biochar 1 kg/plant + Fertilizers; BC5 = Biochar 5 kg/plant; BC5 + F = Biochar 5 kg/plant + Fertilizers; Ctrl = Control; F = Fertilizers). Final number of plants alive is reported in Figure 2 for each treatment and species. Different letters above each treatment level represent statistically significant differences (Tukey-adjusted comparisons, $p < 0.05$). Error bars indicate the 95% confidence interval of the mean.

3.2. Growth and Biomass

Treatment BC1 + F significantly improved height and diameter monthly growth, as well as the final number of leaves for both species compared to the control and all other treatments except for BC5 + F, BC1 and F for *Terminalia amazonia* height growth and F for *Guazuma crinita* diameter growth (Figure 2).

Biochar alone (BC1 and BC5) did not have a significant effect compared to the control, except as BC1 on the monthly height growth of both species and the final number of leaves of *Terminalia amazonia*.

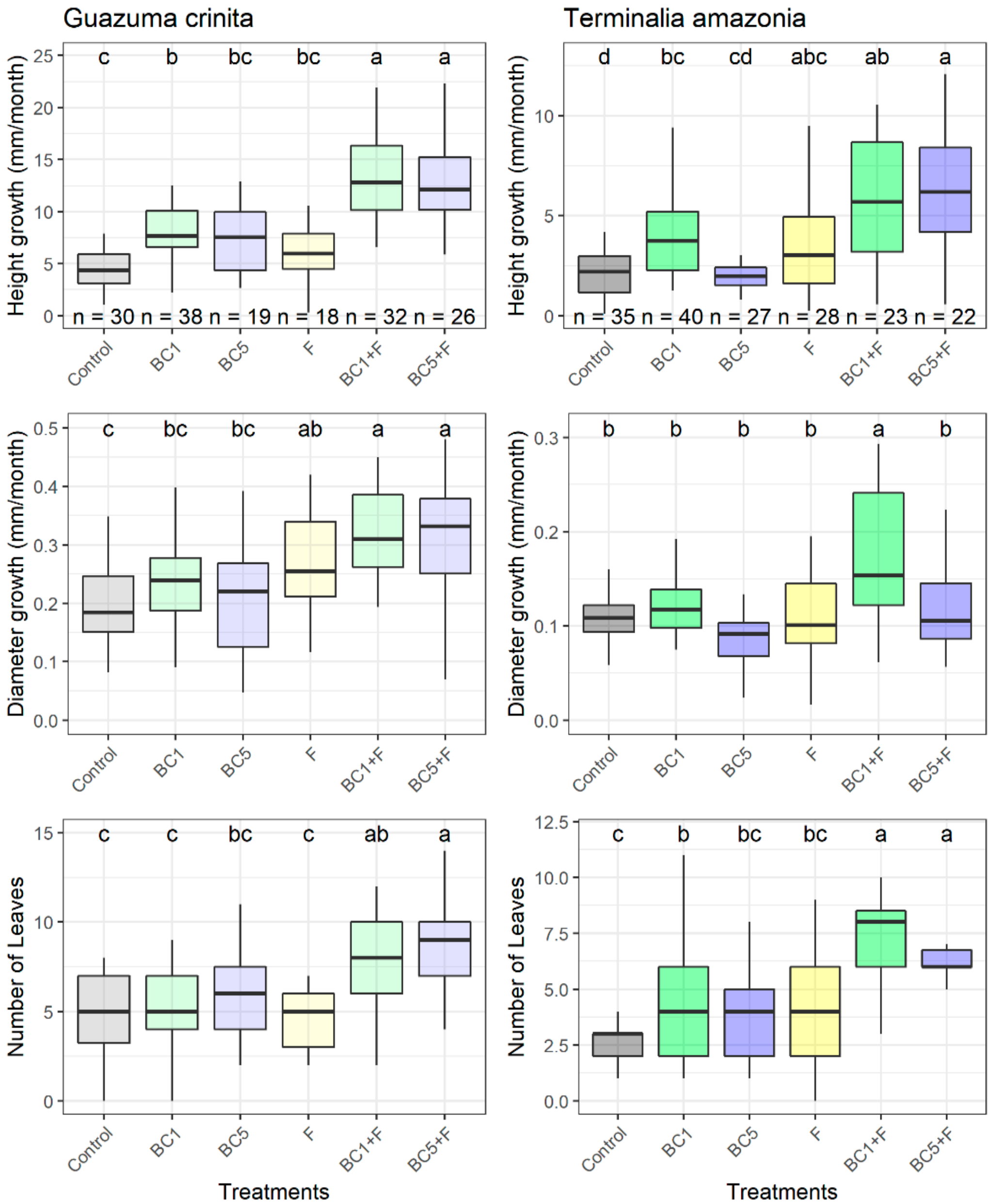

Figure 2. Height and diameter species growth, and final number of leaves across six different biochar treatments (BC1 = Biochar 1 kg/plant; BC1 + F = Biochar 1 kg/plant + Fertilizers; BC5 = Biochar 5 kg/plant; BC5 + F = Biochar 5 kg/plant + Fertilizers; Ctrl = Control; F = Fertilizers). Different letters above each plot represent significant statistical differences, ANOVA + TUKEY post hoc ($p < 0.05$). The end of the upper (lower) whisker represent the largest (smallest) value within 1.5 times interquartile range above (below) 75th (25th) percentile, as defined by the ggplot2 package of R software version (3.5.1) [36].

Treatments BC1 + F and BC5 + F increased monthly height growth of *Guazuma crinita* by a factor of 3 and 2.9 compared to the control, respectively. For *Terminalia amazonia*, seedlings in treatments BC1 + F and BC5 + F grew in height on average 2.7 and 2.8 times faster than the control, respectively. *Guazuma crinita* growing in treatment BC1 + F and BC5 + F gained in diameter 1.6 times faster than plants in control treatment. Meanwhile, the diameter of *Terminalia amazonia* increased 1.7 and 1.1 times faster with treatment BC1 + F and BC5 + F, respectively, compared to the control treatment (Figure 2).

Apart from monthly diameter growth of *Terminalia amazonia*, the overall results are consistent: the best performance is in treatments combining fertilizers and biochar, followed by treatments containing biochar only, treatment with fertilizer only, and, finally, the control. Regarding biomass, Treatments with BC1 + F showed significantly higher below- and above-ground biomass for both species compared to other treatments with only biochar, only fertilizers, and the control, except for below-ground biomass at BC1 (Figure 3).

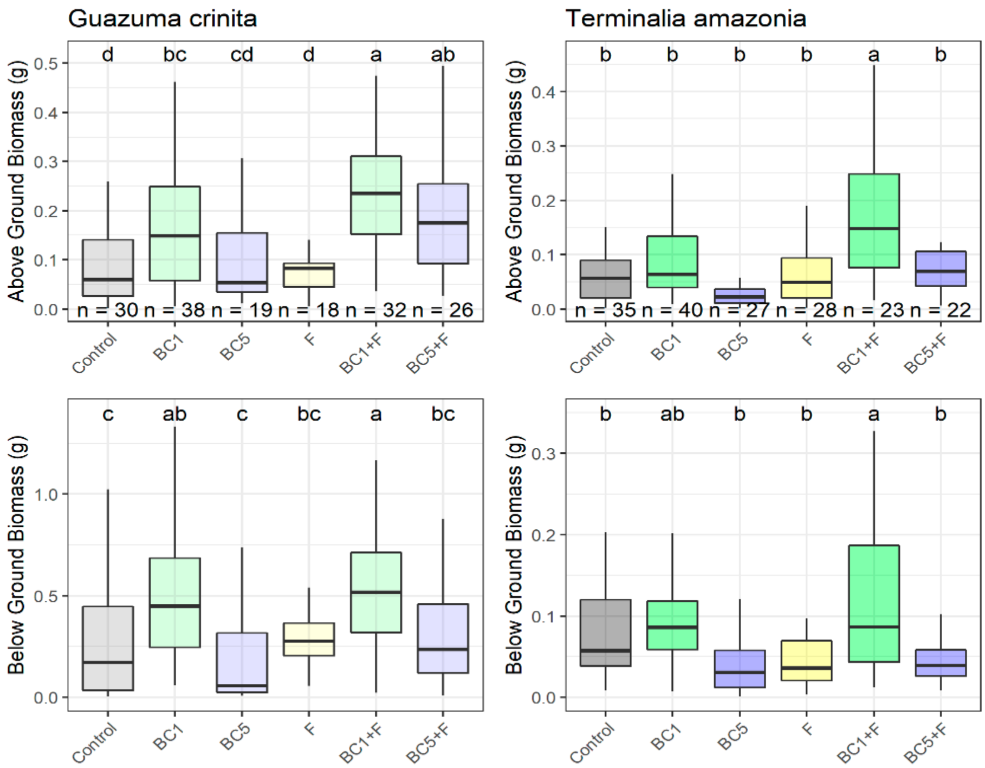

Figure 3. Species dry above- and below-ground biomass across six different biochar and fertilization treatments (BC1 = Biochar 1 kg/plant; BC1 + F = Biochar 1 kg/plant + Fertilizers; BC5 = Biochar 5 kg/plant; BC5 + F = Biochar 5 kg/plant + Fertilizers; Ctrl = Control; F = Fertilizers). Different letters above each plot represent significant statistical differences, ANOVA + TUKEY post hoc ($p < 0.05$). The end of the upper (lower) whisker represent the largest (smallest) value within 1.5 times interquartile range above (below) 75th (25th) percentile, as defined by the ggplot2 package of R software version (3.5.1) [36].

The results of our variable interaction analysis show a significant species by treatment interaction for all of the response parameter studied, except for number of leaves, indicating that the species responded differently to the treatments (Appendix A), although this was most often an exaggeration of a pattern with respect to species responses, rather than a reversal of the order of outcomes (e.g., Figure 3).

Treatments containing biochar and fertilizers (i.e., BC1 + F and BC5 + F) significantly increased monthly growth height response of *Guazuma crinita* compared to *Terminalia amazonia*, with a significant biochar by fertilizer interaction for most of the treatment combinations studied, indicating a synergistic effect of biochar addition greater than the sum of biochar and fertilizer effects independently (Appendix B). Similarly, monthly diameter growth positive response of *Guazuma crinita* to BC5 + F and F treatments was stronger than the response of *Terminalia amazonia*. However, the below-ground biomass result of *Guazuma crinita* was strongly and negatively affected by the BC5 treatment, as compared with the result of *Terminalia amazonia*, as indicated by the significant species × treatment interactions for most variables (see Appendices A and B). An interesting pattern was revealed, in that species differences in their morphometric responses (a significant species × treatment interaction) was due to the effect of biochar (significant species × biochar interaction) for height growth, biomass, and number of leaves, with a nonsignificant species × fertilizer interaction, while for diameter growth, the species × fertilizer interaction was important. In no cases was the three-way interaction (species × biochar × fertilizer) found to be significant (Appendix B).

4. Discussion

Low rates of biochar and fertilizer addition show a synergetic effect, outperforming other treatments and the control on nearly all performance parameters studied, and for both species. Additionally, BC1 addition promotes the survival of both species when compared to all other treatments save for the control.

4.1. Survivorship

Our results showed that survivorship was highest in the biochar addition at the BC1 rate (1.1 t/ha), though these results are not significantly different from the control treatment (Figure 1). A similar observation was made by Farias et al. [40]. More strikingly, survival varied by a factor of two among the different treatments, with BC5 (5 t/ha), BC5 + F, and fertilizer alone, significantly reducing seedling survival as compared to the control and BC1 treatments. Indeed, the addition of NPK fertilizer had the lowest rate of survival of any treatment. This pattern contrasts with the pattern of growth response to the treatment (see below), which showed that biochar plus fertilizer treatments were superior to the control, fertilizer alone, or biochar alone. What this shows is that practitioners will need to assess the relative importance of post-planting survival and growth performance in project management objectives and manage this trade-off accordingly. Additionally, the biochar and fertilizer may be added in two phases, which has the potential to give the combination of the highest survival (BC1) and highest growth (BC1 + F) treatments, though at a small reduction to the overall savings in labor costs from the methodology outlined here.

A study on seedling germination and growth of temperate tree species on biochar enriched and control soil reports similar results [41], with an increased growth in diameter and height but a lower survival rate for the seedlings in the biochar enriched treatment. This negative response to higher biochar application rate may be due to the nutrient immobilization effect of biochar, its indirect effect on the microbial community which could reduce plant growth (e.g., presence of volatile organic compounds in biochar that may affect microbial reactions or their interactions with plants), or to its high alkalinity, increasing the soil pH above optimal level of the native species tested [42,43].

4.2. Plant Growth and Development

Considering both species, treatment BC1 + F had the most positive impact on seedlings' developmental indicators in comparison to treatment with biochar or fertilizer alone and to the control, and in most cases exceeded or was equal to higher application rates of biochar + fertilizer (BC5 + F). Similarly, Ghosh et al. [44] reported that the concomitant addition of biochar and compost had a stronger effect than biochar or compost alone on growth of two native tropical species in equivalent soil type (pH: 5.5 and density: 1.6 g/cm^3).

The slightly lower results of treatments containing BC5 compared to BC1 treatments suggest that a high application dose of biochar may impede seedling development. Meng et al. [45] reported a significant increase of seedlings height and biomass in treatments containing activated microorganisms, fertilizers and low levels of biochar amendment (up to 20% in volume) compared to treatment with activated microorganisms, fertilizers and high levels of biochar amendment (20 to 40% in volume). These results coincide with ours, as best results are found at BC1 (8% volume) as compared to BC5 (41% volume). Similarly, Sarauer and Coleman [42] observed that large doses (25% and 50% volume) of biochar in containerized growing media (peat) reduced the height and diameter growth rates of Douglas fir. According to them, this may have been due to an increase of soil pH to harmful levels for seedling development [42].

Another significant result from this study is that biochar plus fertilizer showed a large and significant increase in plant performance, even though the fertilizer and biochar was only applied once at the beginning of the experiment. In their yearly assessment of native species growth in Peruvian abandoned artisanal gold mining areas, Román-Dañobeytia et al. [46] found that the addition of biofertilizer (a macro- and micronutrient-rich solution resulting from the fermentation of organic material and minerals [46]) at a rate of 55 and 555 L/ha significantly increased seedling monthly growth in height and diameter. However, biofertilizer application took place every 15 days for the first 6 months, while the growth increase witnessed in this study relied on a single application of fertilizers at the time of transplant. Over the lifetime of a project, the methodology from the present study would save substantial costs compared to traditional forestry techniques, as fertilizers and labor are regarded as significant expenses in reforestation projects [47,48]. This may be due to the capacity of biochar to retain fertilizers, thus avoiding the leakage of nutrients [49]. The nutrient retention effect of biochar could have large and positive repercussions for reforestation/restoration projects, substantially lowering the maintenance costs and increasing the success chance of a plantation [50]. Additionally, this nutrient-capture effect has been suggested to increase as the biochar ages in soils [51].

Below ground biomass results for *Guazuma crinita* show an 85% and 83% increase for BC1 and BC1 + F treatments when compared to control and F treatments, respectively. Results for *Terminalia amazonia* show a 22% and 88% increase for BC1 and BC1 + F treatments when compared to control and F treatments, respectively. These results are in accordance with the meta-analysis from Xiang et al. [52], who observed an overall increase in root biomass of 32% after biochar application. Plants with a more robust root system are expected to perform better over time and to show higher resistance to drought in comparison to plants with a less robust root system. On the other hand, and for both species, BC5 and BC5 + F treatments showed a decrease or no response in root biomass when compared to control and F treatments, respectively, here again corroborating our hypothesis that BC5 application rate (i.e., 5 kg per seedling in field application) is excessive and impedes the growth of the species under study.

Our variable interaction assessment (see Appendix A) indicates that the early successional species (*Guazuma crinita*) showed a stronger positive response to BC1 + F and BC5 + F treatments for height growth and to BC5 + F for diameter growth compared to *Terminalia amazonia*. This observation suggests that the synergetic beneficial effect of biochar and fertilizer addition may favor early successional species as compared to late ones, though this study does not have the variety of species needed to make this a robust conclusion. The current observation contrasts with Sovu et al. [53], who noted a stronger positive effect of biochar on slow-growing tree species. The preferential response to biochar of either early or late successional species could have important impacts on the resource management plan of reforestation/restoration projects willing to use biochar and understanding the responses of a broader variety of tree species to biochar and fertilizer treatments should be a central goal of future research.

5. Conclusions

This study showed that low applications of biochar resulted in the highest seedling survival rate, and that biochar plus fertilizer greatly increased all measures of plant performance, even when fertilizer was limited to only one application at the beginning of the study. This study adds to a small but growing body of literature revealing the benefits of biochar as a tool for reforestation and forest restoration. It highlights the benefits of joint application of biochar and fertilizer, which shows significant potential for seedling development, as well as providing a caution against high biochar application doses that could impede plant growth. The synergistic effects of biochar and fertilizer were found in both species assayed, though the response of *Guazuma crinita* is greater. : If applied to restoration and forestry, the techniques outlined in this paper may greatly reduce the costs of seedling propagation and plantation management.

Author Contributions: M.S., F.R.-D., J.S. and D.L. designed and set up the experiment. R.C. provided the site and tools to conduct the experiment. M.S., F.R.-D. and D.L. analyzed and discussed the data. The general conception: M.S., F.R.-D., L.E.F., F.C., C.A. The manuscript was written by D.L., F.R.-D., and M.S. accounting from inputs from all other co-authors.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A Assessing the Species by Treatment Interaction

Methodology

We modeled data using a mixed effect model from the "Tests in linear mixed effect models" (lmerTest" in Ref. [39]) package with a focus on understanding interactions, particularly those including species.

The model interactions:

BLOCK + SPECIES + TREATMENT
SPECIES × TREATMENT
SPECIES × BLOCK
BLOCK × TREATMENT
With BLOCK set as a random effect.

The model was applied to all response parameters, i.e., Number of Leaves, Monthly Growth in Height, Monthly Growth in Diameter, Above-Ground Biomass, and Below-Ground Biomass.

The results of the analyses are provided below:

Number of Leaves

Table A1. Type III Analysis of Variance Table with Satterthwaite's method.

	Sum Sq	Mean Sq	NumDF	DenDF	*F* Value	Pr (>*F*)
TREATMENT	155.748	31.1496	5	317.02	6.0399	2.338×10^{-5} ***
BLOCK	0.004	0.0041	1	2.01	0.0008	0.98007
SPECIES	6.563	6.5630	1	316.98	1.2726	0.26014
TREATMENT: SPECIES	52.462	10.4924	5	317.33	2.0345	0.07356
BLOCK: SPECIES	13.433	13.4333	1	316.97	2.6047	0.10754
TREATMENT: BLOCK	54.763	10.9526	5	317.02	2.1237	0.06239

Signif. codes: 0 '***'

Monthly Growth in Height

Table A2. Type III Analysis of Variance Table with Satterthwaite's method.

	Sum Sq	Mean Sq	NumDF	DenDF	*F* Value	Pr (>*F*)
TREATMENT	548.17	109.633	5	317.06	11.3993	3.951×10^{-10} ***
BLOCK	16.23	16.231	1	2.05	1.6876	0.3206
SPECIES	242.90	242.902	1	317.02	25.2563	8.406×10^{-7} ***
TREATMENT: SPECIES	379.29	75.858	5	317.39	7.8875	5.104×10^{-7} ***
BLOCK: SPECIES	3.37	3.373	1	317.01	0.3508	0.5541
TREATMENT: BLOCK	76.64	15.329	5	317.06	1.5938	0.1614

Signif. codes: 0 '***'

Monthly Growth in Diameter

Table A3. Type III Analysis of Variance Table with Satterthwaite's method.

	Sum Sq	Mean Sq	NumDF	DenDF	*F* Value	Pr (>*F*)
TREATMENT	0.224286	0.044857	5	317.04	9.6375	1.405×10^{-8} ***
BLOCK	0.004222	0.004222	1	2.03	0.9072	0.439940
SPECIES	0.244849	0.244849	1	317.01	52.6053	3.147×10^{-12} ***
TREATMENT: SPECIES	0.088208	0.017642	5	317.28	3.7903	0.002388 **
BLOCK: SPECIES	0.000036	0.000036	1	317.01	0.0077	0.930126
TREATMENT: BLOCK	0.070144	0.014029	5	317.04	3.0141	0.011287 *

Signif. codes: 0 '***' 0.001 '**' 0.01 '*'

Above-Ground Biomass

Table A4. Type III Analysis of Variance Table with Satterthwaite's method.

	Sum Sq	Mean Sq	NumDF	DenDF	*F* Value	Pr (>*F*)
TREATMENT	0.44429	0.088858	5	317.04	7.9573	4.423×10^{-7} ***
BLOCK	0.02669	0.026692	1	2.04	2.3903	0.259964
SPECIES	0.06797	0.067971	1	317.00	6.0869	0.014147 *
TREATMENT: SPECIES	0.17724	0.035449	5	317.33	3.1745	0.008216 **
BLOCK: SPECIES	0.01270	0.012701	1	317.00	1.1374	0.287022
TREATMENT: BLOCK	0.11145	0.022290	5	317.05	1.9961	0.078916

Signif. codes: 0 '***' 0.001 '**' 0.01 '*'

Below-Ground Biomass

Table A5. Type III Analysis of Variance Table with Satterthwaite's method.

	Sum Sq	Mean Sq	NumDF	DenDF	*F* Value	Pr (>*F*)
TREATMENT	0.49280	0.09856	5	317.18	2.9033	0.0140340 *
BLOCK	0.40934	0.40934	1	2.17	12.0581	0.0656557
SPECIES	2.23639	2.23639	1	317.06	65.8784	1.07×10^{-14} ***
TREATMENT: SPECIES	0.85797	0.17159	5	317.89	5.0547	0.0001792 ***
BLOCK: SPECIES	0.33539	0.33539	1	317.05	9.8796	0.0018292 **
TREATMENT: BLOCK	0.13594	0.02719	5	317.19	0.8009	0.5496668

Signif. codes: 0 '***' 0.001 '**' 0.01 '*'

Appendix B Assessing the Biochar by Fertilizer Interaction

Methodology

We modeled data using a mixed effect model from the "Tests in linear mixed effect models" ("lmerTest") package in Ref. [39] with a focus on understanding whether our experimental results for

BC1+F and BC5+F treatment could be controlled by the fertilizer, the biochar, or both. To do so, we added a factor Fertilizer ("Ferti") with two levels (presence or absence of fertilizer in the treatment) and a factor ("Bioch") with three levels (0, 1 and 5) for the different amount of biochar present in each treatment. We then ran an ANOVA on the model, including the Species and Blocks, with Block set as a random factor.

The model interactions:

BLOCK+SPECIES × Bioch × Ferti
With BLOCK set as a random effect.

The model was applied to all response parameters, i.e., Number of Leaves, Monthly Growth in Height, Monthly Growth in Diameter, Above-Ground Biomass, and Below-Ground Biomass.

The results of the analysis are provided below:

Table A6. Monthly Growth in Height.

	Sum Sq	Mean Sq	NumDF	DenDF	*F* Value	Pr (>*F*)
BLOCK	34.68	11.56	3	0	1.1943	1.000000
SPECIES	1773.76	1773.76	1	323	183.2302	$<2.2 \times 10^{-16}$ ***
Bioch	899.85	449.92	2	323	46.4773	$<2.2 \times 10^{-16}$ ***
Ferti	870.48	870.48	1	323	89.9209	$<2.2 \times 10^{-16}$ ***
SPECIES: Bioch	325.72	162.86	2	323	16.8233	1.121×10^{-7} ***
SPECIES: Ferti	43.96	43.96	1	323	4.5414	0.033838 *
Bioch: Ferti	108.03	54.02	2	323	5.5798	0.004146 **
SPECIES: Bioch:Ferti	42.32	21.16	2	323	2.1858	0.114044

Signif. codes: 0 '***' 0.001 '**' 0.01 '*'

Table A7. Monthly Growth in Diameter.

	Sum Sq	Mean Sq	NumDF	DenDF	*F* Value	Pr (>*F*)
BLOCK	0.02616	0.00872	3	323	1.8215	0.1430648
SPECIES	1.43954	1.43954	1	323	300.7423	$<2.2 \times 10^{-16}$ ***
Bioch	0.10187	0.05093	2	323	10.6406	3.347×10^{-5} ***
Ferti	0.32738	0.32738	1	323	68.3953	3.536×10^{-15} ***
SPECIES: Bioch	0.02669	0.01334	2	323	2.7878	0.0630377
SPECIES: Ferti	0.05361	0.05361	1	323	11.1993	0.0009148 ***
Bioch: Ferti	0.01621	0.00811	2	323	1.6936	0.1854894
SPECIES: Bioch:Ferti	0.01101	0.00550	2	323	1.1498	0.3179925

Signif. codes: 0 '***'

Table A8. End Plant Number of Leaves.

	Sum Sq	Mean Sq	NumDF	DenDF	*F* Value	Pr (>*F*)
BLOCK	22.34	7.45	3	0	1.4150	1.0000000
SPECIES	210.83	210.83	1	323	40.0596	8.227×10^{-10} ***
Bioch	275.51	137.76	2	323	26.1753	2.915×10^{-11} ***
Ferti	328.21	328.21	1	323	62.3644	4.505×10^{-14} ***
SPECIES: Bioch	34.02	17.01	2	323	3.2325	0.0407378 *
SPECIES: Ferti	4.98	4.98	1	323	0.9454	0.3316131
Bioch: Ferti	93.90	46.95	2	323	8.9208	0.0001694 ***
SPECIES: Bioch:Ferti	16.90	8.45	2	323	1.6058	0.2023362

Signif. codes: 0 '***' 0.01 '*'

Table A9. Below-Ground Biomass.

	Sum Sq	Mean Sq	NumDF	DenDF	*F* Value	Pr (>*F*)
BLOCK	0.1913	0.0638	3	0	1.8318	1.0000
SPECIES	5.6024	5.6024	1	323	160.9417	$<2.2 \times 10^{-16}$ ***
Bioch	1.7347	0.8674	2	323	24.9168	8.640×10^{-11} ***
Ferti	0.1741	0.1741	1	323	5.0018	0.0260 *
SPECIES: Bioch	0.7602	0.3801	2	323	10.9195	2.577×10^{-5} ***
SPECIES: Ferti	0.0628	0.0628	1	323	1.8031	0.1803
Bioch: Ferti	0.0201	0.0101	2	323	0.2889	0.7493
SPECIES: Bioch:Ferti	0.0414	0.0207	2	323	0.5946	0.5524

Signif. codes: 0 '***' 0.01 '*'

Table A10. Above-Ground Biomass.

	Sum Sq	Mean Sq	NumDF	DenDF	*F* Value	Pr (>*F*)
BLOCK	0.01477	0.00492	3	0	0.4332	1.000000
SPECIES	0.14328	0.14328	1	323	12.6089	0.000441 ***
Bioch	0.61228	0.30614	2	323	26.9415	1.51×10^{-11} ***
Ferti	0.36876	0.36876	1	323	32.4524	2.75×10^{-8} ***
SPECIES: Bioch	0.13810	0.06905	2	323	6.0767	0.002567 **
SPECIES: Ferti	0.00609	0.00609	1	323	0.5359	0.464668
Bioch: Ferti	0.07185	0.03592	2	323	3.1615	0.043676 *
SPECIES: Bioch:Ferti	0.04963	0.02481	2	323	2.1837	0.114280

Signif. codes: 0 '***' 0.001 '**' 0.01 '*'

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