

## Article

# Biochar rescues native trees in a global biodiversity hotspot

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**Abstract:** Many tropical invasive species have allelopathic effects that contribute to their success in native plant communities. Pyrolyzed biomass (“biochar”) can sorb toxic compounds, including allelochemicals produced by invasive plants, potentially reducing their inhibitory effects on native species. Strawberry guava (*Psidium cattleianum*) is among the most important allelopathic invasive species in tropical islands and recognized as the most serious invasive species threat in the global biodiversity hotspot of Mauritius. We investigated the effects of additions of locally produced biochar on native tree species in a field experiment conducted in areas invaded by strawberry guava within Mauritius’ largest national park. Growth and survivorship of native tree species were monitored over 2 ½ years in plots subjected to four treatments: non-weeded, weeded, weeded + 25 t/ha biochar and weeded + 50 t/ha biochar. Native tree growth and survivorship were strongly suppressed by strawberry guava. Biochar treatments dramatically increased native tree performance, with more than a doubling in growth, and substantially increased native tree survivorship and species diversity, while suppressing strawberry guava regeneration, consistent with growth-promoting properties and sorption of allelochemicals. We conclude that biochars, including “sustainable biochars” produced from locally accessible biomass using low-tech pyrolysis systems, have considerable potential to counteract effects of allelopathic invaders and increase the capacity for native species regeneration in tropical island ecosystems.

**Keywords:** allelopathy; biochar; invasive species; island ecosystems; *Psidium cattleianum*

## 1. Introduction

The global success of non-native invasive plants is typically attributed to the lack of natural enemies that allow them to fully exploit their potential for resource competition and displace native species. Recent studies support an alternative theory that many exotic species may benefit from ecological advantages of “novel chemistry” via allelopathy outside their native range [1-2]. Allelochemical interference can contribute to the dominance of an invader in its introduced habitat [3]; allelopathy is estimated to be present in more than half of invasive plants globally [4], including many of the most notorious cases [1].

The list of the most widespread and destructive invasive plant species in the world is dominated by species such as *Imperata cylindrica*, *Lantana camara* and *Chromolaena odorata* that are copious producers of allelochemicals that have strong inhibitory effects on native plant species [5-7]. *Psidium cattleianum* Sabine is likewise a highly invasive species that forms dense monotypic stands [8] and can suppress native vegetation by releasing allelochemicals into the soil [9-11]. Invasive plant species such as *P. cattleianum* have a high potential for expanding their range in tropical biodiversity hotspots and threatening plant diversity globally [12]. The negative impacts of invasive species on native biota are increasing rapidly [13], particularly in tropical island ecosystems where ecological impacts are most acute due to limited habitat area, poor competitive ability of native species, and putative vacant niches [14].

*Psidium cattleianum* (strawberry guava) is one of the world's 36 most invasive plant species [12] and has been widely introduced to tropical islands on account of its edible fruit [10]. Outside its native range in Brazil, strawberry guava has had serious impacts on tropical forest ecosystems in Mauritius and Hawaii, threatening native flora and fauna, particularly rare and endangered endemic species [8, 15]. The native forests of Mauritius exist only as isolated fragments that are increasingly dominated by strawberry guava [15]. This species' success can be attributed to competitive traits, such as high reproductive capacity, clonal growth, and tolerance of a wide range of light conditions [8, 16]. In addition, strawberry guava has a deep rooting system that can lower the water table, reducing flow in streams and groundwater systems, with negative impacts on native trees [10]. In the lowland wet forests of Mauritius and Hawaii, native plant regeneration is particularly constrained by high densities of strawberry guava [9, 17-19]. Mauritius is part of the globally significant Mascarene biodiversity hotspot [20-21], with almost 700 native angiosperm species, of which 39.5% are endemic to the island. Over 80% of the endemic flora is threatened by the invasion of introduced species such as strawberry guava [22], and there is an urgent need to restore the native forest communities.

Allelopathy has long been suspected to facilitate the invasion of strawberry guava and contribute to its dominance in the upland forests of Mauritius [10, 23]. A recent study showed that strawberry guava releases allelochemicals from its leaves [24] that may contribute to its success by inhibiting germination and growth of native plant species. Practical cost-effective solutions to remove and control the spread of strawberry guava are not available. In Mauritius, the mechanical control of strawberry guava has a positive effect on the native flora, but due to high operational costs, only ~1% of native forest remnants have received this treatment [18]. Alternatives to mechanical control include biological control agents, specifically the release of natural enemies, such as *Tectococcus oyatus* and *Eurytoma* sp. [25]. Although *T. oyatus* and *Eurytoma* sp. have high specificity for strawberry guava, native species in the Myrtaceae family may also be impacted by the non-native biocontrol agents. These risks are especially critical in small, isolated ecosystems such as Mauritius, where only ~5% of native forest remains [26].

One potential but untested mitigation strategy is the addition of biochar, which can be produced from various biomass sources at low cost and does not carry the risks of biocontrol or herbicide use. Biochar - or charcoal used as soil amendment - has been widely hailed for its potential to enhance productivity and sequester carbon [27], with a recent focus on its capacity to sorb soil contaminants [28]. Research on biochar mitigation of allelopathy, however, has been limited to laboratory and greenhouse trials [24, 29-31]. Long-term field studies on allelopathic interactions are scarce [32-33] and none has examined the capacity of biochar to increase native tree growth in forest areas invaded by allelopathic species.

Here we report the results of a 30-month experiment on the effects of biochar addition on native tree growth and survivorship in strawberry guava-invaded forests of Mauritius. Physical removal of aboveground biomass (weeding) and biochar treatments were applied to native tree species to test the following hypotheses: (i) strawberry guava inhibits growth and survivorship of native species, (ii) strawberry guava weeding increases native tree growth and (iii) biochar reduces the inhibitory effects of strawberry guava and promotes native tree growth and survivorship.

## 2. Materials and Methods

### 2.1. Study Site

The experiment was established in January 2017 at the Pétrin site in Black River Gorges National Park in Mauritius (20.4264° S, 57.4509° E) at 600 m altitude. The average annual temperature is 26°C, with a maximum monthly mean temperature of 29°C in December and a minimum of 16°C in July. Soils on Mauritius are of volcanic origin and in the study area, they are primarily Oxisols, with patches of relatively unweathered lava [34]. Heath-type vegetation forms a large part of native plant communities in the region

and the canopy rarely exceeds 3 m [35]. Annual precipitation in the region is 3500-4000 mm and the rainy season lasts from December to March. Strawberry guava is present throughout the study area and generally dominates native plant communities where present in the absence of mechanical weeding. In certain areas, it forms almost monospecific stands reaching up to 2 m in height and comprises more than 90% of all non-native woody stems. Weeded areas of the site have a more open structure than strawberry-guava-dominated stands. Allelopathic compounds such as  $\beta$ -caryophyllene,  $\alpha$ -pinene and phenolics have been identified in strawberry guava leaves and fruits [10-11].  $\beta$ -caryophyllene is the primary constituent in strawberry guava leaf oils [36] that has been shown to inhibit the growth of tomato, radish and mungbean seedlings [37]. Inhibitory effects have also been shown for  $\alpha$ -pinene, which reduced early root growth and caused oxidative damage in the root tissue of several target species [38]. Evidence of allelopathic effects in strawberry guava leaves [24, 39] points to likely inhibitory effects on native species in invaded plant communities.

## 2.2. Experimental Design

The field experiment was carried out using native woody plant species because they are strongly impacted by the presence of strawberry guava and are relevant test subjects for restoration projects in Mauritius. Four treatments (control without strawberry guava weeding or biochar addition, and strawberry guava weeding with 0, 25 or 50 t/ha biochar addition) were applied to 2 m x 2 m plots using a randomized complete block design, with 5 replicates per treatment. Within blocks, plots were surrounded by a 1-m buffer zone and blocks were separated from each other by a minimum distance of 30 m. Strawberry guava was manually removed from 15 plots (for three treatments with weeding and 0, 25 or 50 t/ha biochar addition), by cutting the stems at the base. The stems were removed from the study site but fallen leaves and stumps of strawberry guava remained. The plots were not re-weeded for the duration of the experiment. In biochar treatment plots, coconut husk biochar was applied at rates of 25 t/ha and 50 t/ha. Biochar was carefully incorporated into the upper 5 cm of soil, avoiding damage to root systems of native trees on site. In each plot, up to 9 seedlings of native species were planted, with a minimum distance of 50 cm between seedlings. Existing native trees in each plot were also included in the experiment, for a total of 10 individuals per plot. Seedlings of *Pittosporum senacia* Putt. (Pittosporaceae), *Sideroxylon puberulum* C.DC. (Sapotaceae), and *Tambourissa peltata* Baker (Monimiaceae) were obtained from nurseries managed by the National Parks and Conservation Service in Mauritius. Other volunteer plants in each plot were identified to species, and included *S. puberulum*, *T. peltata*, *P. senacia*, *Syzygium coriaceum* Bosser & J. Guého, *Ochna mauritiana* Lam., *Turraea rigida* Vent., and *Erythrospermum monticolum* Thouars var. *monticolum*.

## 2.3. Biochar Synthesis and Characterization

A two-barrel nested retort pyrolysis system was used to make charcoal from coconut husks, which are readily available and typically discarded in large quantities in Mauritius. A 100-L drum of coconut husks was placed inside a larger 200-L drum. The space between the two drums was filled with dry branches and twigs to serve as fuel for the fire. A hole at the top of the 200-L drum allowed gases to escape in small amounts. Each batch of coconut feedstock was pyrolyzed at ~ 400°C for 4 h and the yield was ~30% by volume. Biochar from three batches was homogenized before application. Biochar samples from each batch were collected for chemical characterization at the University of Toronto. Samples were analyzed for pH and EC, % organic matter (OM), and total C and N. Samples were dried at 60°C for 24 h, diluted to 1:20 (v:v) ratio of sample to deionized water that was shaken for 1 h, filtered, and measured for pH and EC using a glass electrode pH meter (IQ Scientific Instruments, USA) and a conductivity meter (Orion Star A112, Thermo Scientific, USA), respectively.. OM was obtained by loss on ignition in a muffle furnace at 500°C for 2 h. Samples were weighed before and after

combustion to determine the amount of OM that had been lost. Samples were dried at 105°C for 1 h and ground to < 0.5 mm for total C and N determination using a CN analyzer (628 Series, LECO Corporation, USA).

#### 2.4. Soil Collection and Analysis

Soil samples were taken from the uppermost 10 cm of mineral soil (one per plot for all treatments) at the end of the experiment in July 2019. Soil samples were analyzed for pH, EC, %OM, and total C and N; methods were similar to those used for biochar samples, but soil samples were measured for pH and EC using 1:3 (v:v) ratio of sample to deionized water.

#### 2.5. Plant Measurements

Plant survivorship, height (to the nearest cm), and root collar diameter (to the nearest mm) were measured at the start of the experiment in January 2017, after 6 months in July 2017, and at the end of the experiment in July 2019 (30 months following initiation). Root collar diameter (RCD to the nearest 0.1 mm) was also measured for all trees. The number of strawberry guava individuals in each plot was measured after 6 and 30 months.

We estimated total aboveground plant biomass using diameter<sup>2</sup> x height as a proxy to avoid destructive sampling of trees that are protected for conservation in the study area. Allometric relationships between diameter<sup>2</sup> x height and biomass have been shown to closely approximate a linear relationship for several tropical tree species [40-42]. Relative volume growth rate (RGRvol) was calculated as:

$$\text{RGRvol} = (\ln(D_2^2H_2) - \ln(D_1^2H_1))/\Delta t,$$

where  $D_1^2H_1$  is the product of root collar diameter squared and height at the start of the experiment and  $D_2^2H_2$  is the product of root collar diameter squared and height either 6 or 30 months later, and  $\Delta t$  is the duration between the two measurements in months.

#### 2.6. Statistical Analysis

Linear mixed effects models were fitted to the RGRvol data, and alternative models including block and replicate as random effects were compared using a minimum Akaike information criterion (AIC) approach [43]. Random effect terms were dropped when they were not significant and RGRvol responses were then examined using analysis of variance (ANOVA). Species-specific responses to treatments were analyzed using a 2-way ANOVA after excluding *O. mauritiana*, *T. rigida* and *E. monticolum* from the dataset due to missing replicates in treatment groups. Seedling survivorship was analyzed using generalized linear mixed models with a binomial family and block and replicate were treated as random effects. If random effect terms were not significant, survivorship was analyzed using a 2-way generalized linear model. Treatment differences for soil and biochar properties (pH, CEC, % OM, total C and N) were analyzed using one-way ANOVA. Post-hoc Tukey honest significant difference (HSD) tests were used following ANOVA analyses to test pairwise treatment differences (with  $p < 0.05$  considered significant) for RGRvol, survivorship, and soil and biochar properties. The Shannon diversity index  $H'$  was calculated for each plot and treatment effects analyzed using a one-way ANOVA. Treatment differences for strawberry guava density were analyzed using a one-way ANOVA. Post-hoc Scheffe tests were used to find pairwise treatment differences (with  $p < 0.05$  considered significant) for Shannon diversity and strawberry guava density.

We used structural equation modelling (SEM) with the R package piecewiseSEM [44] to examine drivers of early native tree growth, native seedling survivorship, strawberry guava density, and tree diversity. We made an a priori model for the hypothesized pathways between treatment type and response variables for native tree performance and strawberry guava density (Supplementary Fig. S1). The relationships between treatment type (biochar and strawberry guava removal) and response variables (native tree growth, native seedling survivorship, strawberry guava density and Shannon index) and relationships among response variables were modelled using linear models. The best-fitting model with the lowest AIC value was obtained by removing nonsignificant pathways from poor-fitting models. Tests of directed separation were used to determine which hypothesized pathways were significant, and whether non-hypothesized significant pathways ought to be added to the model. The model was accepted if Fisher's C statistic had an associated p-value >0.05 [44]. The proportional weight of each path was determined using standardized path coefficients [45]. Conditional pseudo-r<sup>2</sup> values were generated for each pathway in the model. Data analysis was conducted in R (version 1.1.463) specifically making use of the lm(), lmer(), glm(), glmer(), and psem() functions for the main analyses.

### 3. Results

#### 3.1. Soil and Biochar Properties

Soil pH and EC of the no-biochar treatments were significantly lower than the biochar treatments ( $p < 0.001$ ). Biochar additions increased the organic matter content of the soil, compared to no-biochar treatments, but not significantly ( $p > 0.05$ ). Percent C was at similar levels across soil treatments. Biochar addition at 25 t/ha significantly decreased soil nitrogen, compared to the non-weeded control ( $p < 0.05$ ). The coconut biochar used in the experiment had a higher pH, EC, %OM, %C and %N, compared to any soil treatment (Table 1).

**Table 1.** Properties of soil samples and biochar used in the experiment. Values were based on 3 samples for biochar and 5 samples for each soil treatment (one per plot replicate). Mean values are listed  $\pm$  1 SE; soil treatment differences significant at  $p < 0.05$  (Tukey HSD post hoc tests) are indicated by different letters.

| Attribute        | Biochar (BC)    | Soil Treatment               |                               |                              |                               |
|------------------|-----------------|------------------------------|-------------------------------|------------------------------|-------------------------------|
|                  |                 | Non-weeded                   | Weeded<br>+BC0                | Weeded<br>+BC25              | Weeded<br>+BC50               |
| pH               | 8.33 $\pm$ 0.08 | 6.04 $\pm$ 0.17 <sup>b</sup> | 4.75 $\pm$ 0.08 <sup>c</sup>  | 6.8 $\pm$ 0.22 <sup>a</sup>  | 7.04 $\pm$ 0.08 <sup>a</sup>  |
| EC ( $\mu$ S/cm) | 1194 $\pm$ 110  | 118 $\pm$ 22.1 <sup>a</sup>  | 80.6 $\pm$ 6.86 <sup>a</sup>  | 214 $\pm$ 27.4 <sup>b</sup>  | 249 $\pm$ 12.1 <sup>b</sup>   |
| OM (%)           | 87 $\pm$ 1.52   | 20.6 $\pm$ 4.1 <sup>a</sup>  | 25.6 $\pm$ 4.23 <sup>a</sup>  | 40.6 $\pm$ 8.43 <sup>a</sup> | 35.7 $\pm$ 1.37 <sup>a</sup>  |
| Carbon (%)       | 60.5 $\pm$ 0.79 | 9.53 $\pm$ 0.34 <sup>a</sup> | 7.93 $\pm$ 0.89 <sup>a</sup>  | 8.35 $\pm$ 0.53 <sup>a</sup> | 10.3 $\pm$ 1.78 <sup>a</sup>  |
| Nitrogen (%)     | 0.71 $\pm$ 0.01 | 0.46 $\pm$ 0.01 <sup>a</sup> | 0.40 $\pm$ 0.01 <sup>ab</sup> | 0.32 $\pm$ 0.01 <sup>b</sup> | 0.38 $\pm$ 0.05 <sup>ab</sup> |

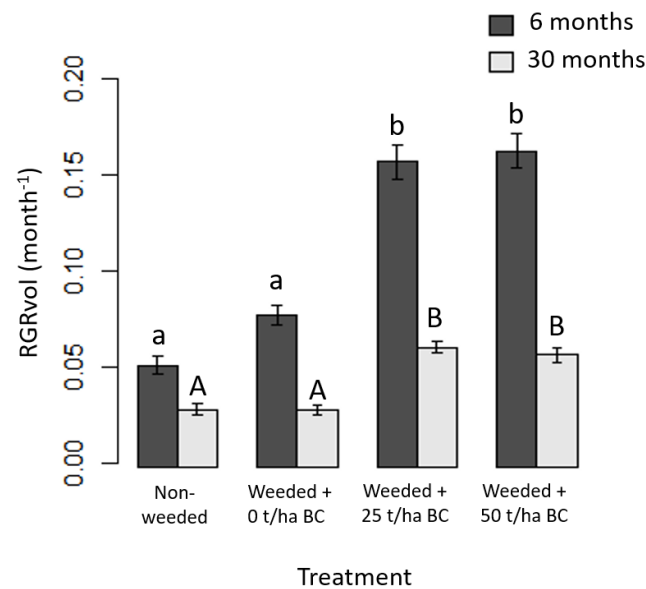
#### 3.2. Native Tree Growth and Survivorship

Strawberry guava weeding increased RGRvol relative to the control, but not significantly (Tukey HSD test:  $p > 0.05$ ) (Figure 1). Biochar addition significantly increased RGRvol ( $p < 0.001$ ) after 6 and 30 months, with much larger differences in comparison to the control. On average, biochar addition resulted in a 205% and 103% increase in RGRvol after 6 and 30 months, respectively.

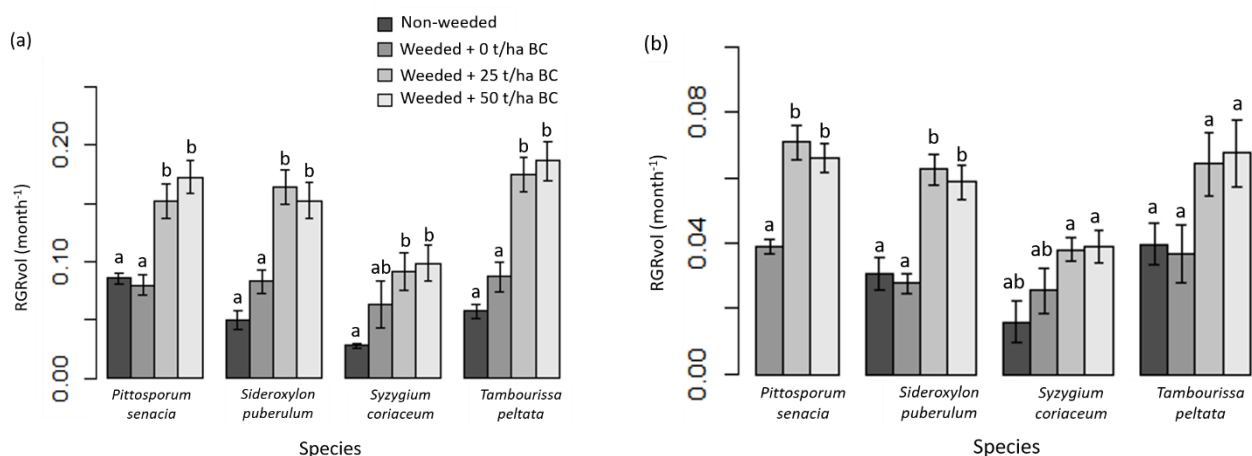
Biochar addition had the most pronounced effects on *S. coriaceum*, *T. peltata* and *S. puberulum* with a threefold increase in RGRvol after 6 months ( $p < 0.05$ ) (Figure 2a). The



effects of biochar on *P. senecio* were also notable, with a 90% increase in RGRvol. After 30 months, RGRvol was still enhanced by biochar, but the effects were not significant for *S. coriaceum* and *T. peltata* (Figure 2b). On average, biochar addition increased RGRvol by 104% in comparison to controls, after 30 months. Species-specific responses to weeding were not significant and there were no significant differences between the low and high doses of biochar addition.



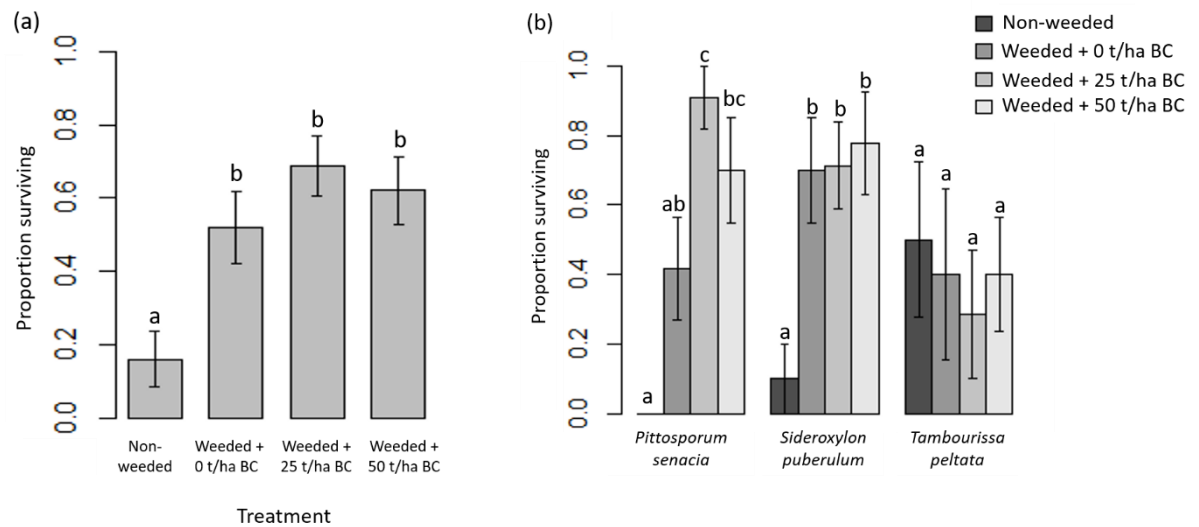
**Figure 1.** Relative growth rate (RGRvol) in all native seedlings and trees examined after 6 and 30 months. Weeding and biochar treatments at rates of 0, 25 and 50 t/ha are compared to the control (no weeding). Means are plotted  $\pm 1$  SE; differences significant at  $p < 0.05$  (Tukey HSD post hoc tests) are indicated by different letters.



**Figure 2.** Relative growth rate (RGRvol) by treatment of *P. senecio*, *S. puberulum*, *S. coriaceum*, and *T. peltata* after 6 months (a) and 30 months (b). Weeding and biochar treatments at rates of 0, 25 and 50 t/ha are compared to the control. Means are plotted  $\pm 1$  SE; differences significant at  $p < 0.05$  (Tukey HSD post hoc tests) are indicated by different letters.

Biochar addition significantly increased the survivorship of planted native seedlings, particularly *P. senecio* and *S. puberulum* seedlings, after 30 months ( $p < 0.05$ ) (Figure 3). More than 70% of *P. senecio* and *S. puberulum* seedlings survived under

biochar treatments, in comparison to 0% and 10% survivorship in control plots, respectively. Weeding also had large positive effects on the survivorship of *S. puberulum* seedlings, with a 6-fold increase compared to the control ( $p < 0.01$ ). The survivorship of *T. peltata* did not differ among treatments.



**Figure 3.** Proportion of native seedling survivorship after 30 months by treatment: (a) pooled data; (b) by species for *P. senacia*, *S. puberulum*, and *T. peltata*. Weeding and biochar treatments at rates of 0, 25 and 50 t/ha are compared to the control (no weeding). Means are plotted  $\pm 1$  SE; differences significant at  $p < 0.05$  (Tukey HSD post hoc tests) are indicated by different letters.

### 3.3. Strawberry Guava Density

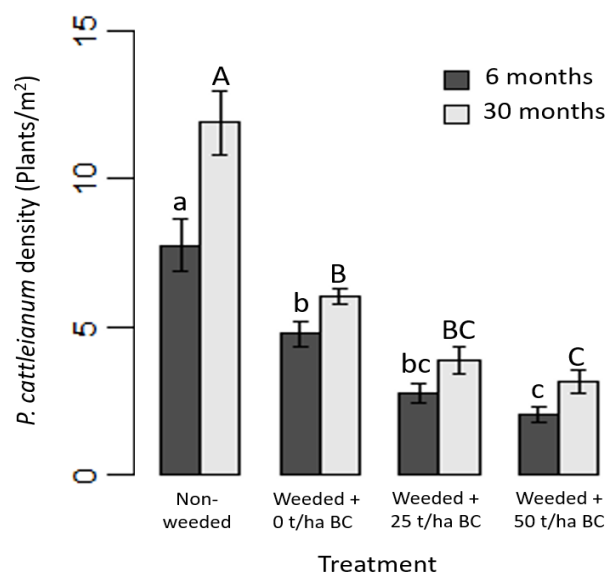
Strawberry guava regeneration was significantly reduced in response to weeding and biochar addition after 6 and 30 months ( $p < 0.01$ ) (Figure 4). Strawberry guava density was significantly higher for the control, compared with all other treatments, but strawberry guava density between biochar-addition treatments were not significantly different ( $p > 0.05$ ). Strawberry guava density in the no-biochar weeded treatment was ~50% lower than the control after 30 months. Biochar addition dramatically reduced strawberry guava density which was four times lower than the control treatment.

### 3.4. Diversity

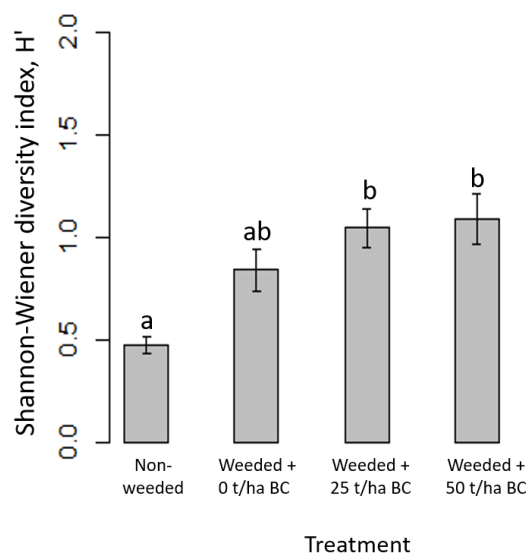
Weeding increased the Shannon diversity index for all trees detected within plots after 30 months, compared to the control treatment, but not significantly ( $p > 0.05$ ) (Figure 5). Biochar addition significantly increased Shannon diversity ( $p < 0.01$ ) after 30 months, with larger differences in comparison to the control. On average, Shannon diversity doubled with biochar addition, compared to the control treatment, but it was not significantly different between the two biochar-addition dosages.

### 3.5. Structural Equation Model

Our structural equation model (SEM) showed that strawberry guava density decreased native tree growth ( $p < 0.01$ ) while biochar additions increased growth ( $p < 0.01$ ). Strawberry guava density was significantly reduced by strawberry guava removal ( $p < 0.001$ ) and biochar additions ( $p = 0.002$ ), with stronger effects of weeding compared to biochar. Native seedling survivorship increased significantly in response to early tree growth ( $p < 0.01$ ). Native tree diversity increased with survivorship ( $p < 0.001$ ) and was negatively affected by strawberry guava density ( $p < 0.01$ ).

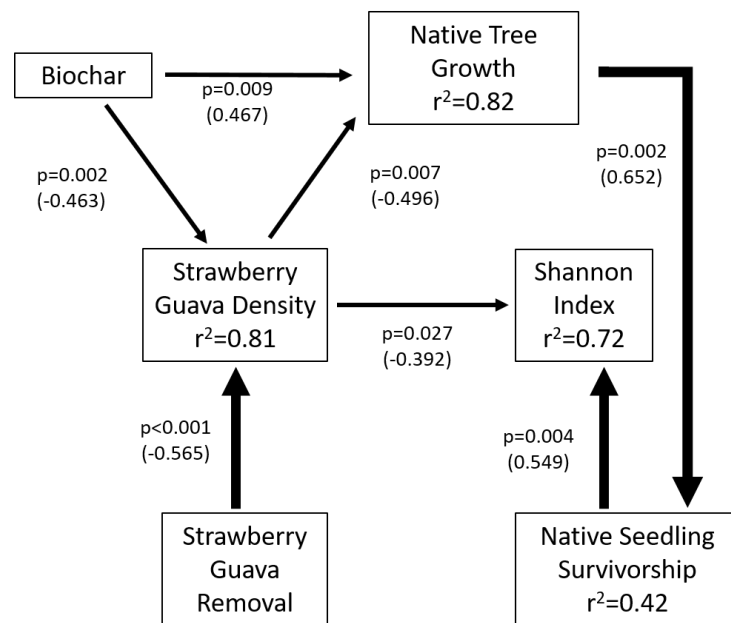


**Figure 4.** Density of strawberry guava individuals by treatment after 6 and 30 months. Weeding and biochar treatments at rates of 0, 25 and 50 t/ha are compared to the control (no weeding). Means are plotted  $\pm 1$  SE; differences significant at  $p < 0.05$  (Scheffe post hoc tests) are indicated by different letters.



**Figure 5.** Shannon diversity by treatment after 30 months. Weeding and biochar treatments at rates of 0, 25 and 50 t/ha are compared to the control (no weeding). Means are plotted  $\pm 1$  SE; differences significant at  $p < 0.05$  (Scheffe post hoc tests) are indicated by different letters.





**Figure 6.** Structural equation model illustrating drivers of native tree growth, native seedling survivorship, strawberry guava density and tree diversity in the experiment. Arrow thickness is proportional to path coefficients. Values listed by arrows are standardized path coefficients with p-values. An  $r^2$  value is shown in the box of each response variable.

#### 4. Discussion

Our results support the hypotheses that strawberry guava suppresses the survivorship of native trees, that removal of aboveground parts partially alleviates this suppressive effect, and that persistent effects of allelochemicals suppress native trees to a greater extent but can be largely overcome by biochar applications to the upper soil horizons. Large biochar effects were moreover observed for all native tree species examined, and overall tree diversity in plots was strongly enhanced by biochar treatments. Our structural equation model (SEM) indicates that native tree growth was greatly reduced in the presence of high strawberry guava density and increased significantly in response to biochar additions. Direct effects of the predictor variables, strawberry guava density and biochar dosage, explained 82% variation in native tree growth (Figure 6). Our most unexpected result was that strawberry guava removal alone did not significantly increase native tree growth. However, in non-weeded plots, strawberry guava strongly suppressed the survivorship of native seedlings; there were no *P. senecio* survivors in the control plots after 30 months (Figure 3). This result is consistent with prior studies that have found that native seedling survival decreased in the presence of invasive species such as *Ligustrum sinense* [46] and *Lonicera maackii* [47]. Native tree species in Hawaii's lowland wet forests are severely suppressed by high densities of strawberry guava which dominates invaded forest stands [9, 19]. Our findings are also consistent with prior studies that have found removal of strawberry guava to be beneficial for native trees. In lowland wet forests of Mauritius, the reproductive output [18] and regeneration of native tree species have been found to be greater in weeded areas, with effects including resprouting of endemic species that were presumed extinct [48].

The positive survivorship responses of native seedlings to weeding indicate that strawberry guava has negative effects on the native plant community, likely due to a combination of both resource competition and allelopathy [24, 47, 49]. Light limitation under the dense stands of invasive plants has been found to be the most important factor affecting the performance of native species [46, 50]. Increased light availability in our weeded plots was likely an important mechanism for higher survivorship of native seedlings because strawberry guava grows in dense thickets such that very little light reaches the forest floor. Although strawberry guava density was significantly higher in the control plots than the

no-biochar weeded plots (Figure 4), native tree growth was not significantly different between the two treatments (Figure 1), which suggests that resource competition was not the only mechanism affecting tree growth. Allelochemicals released by strawberry guava [24, 51] likely suppressed native tree growth in the no-biochar weeded plots, in addition to the effects from competition for resources such as light, nutrients, and water [46-47]. Strawberry guava releases several allelopathic compounds, including  $\beta$ -caryophyllene,  $\alpha$ -pinene and phenolics [10-11], all of which have been shown to inhibit plant growth [37-38, 52]. Copious fruiting of strawberry guava [8] could substantially increase the inhibitory effects of the allelochemicals because the fruits contain high levels of phenolics and other allelopathic compounds [53]. Similarly, Siemens and Blossey [54] found that higher light availability increased the survivorship but not growth of *Eupatorium perfoliatum* in response to the removal of the invasive plant, *Fallopia x bohemica*. Similar to the present study, they found that activated carbon had greater positive effects on *E. perfoliatum* growth than nutrient additions, consistent with activated carbon acting to alleviate the allelopathic effects of *F. x bohemica*.

Biochar strongly increased the growth of all the native species examined, and effects of biochar additions on tree growth were much greater than the effects of strawberry guava weeding alone (Figures 1 and 2). The primary mechanism for this effect is almost certainly sorption of allelochemicals, which was previously demonstrated in laboratory experiments [24]. Biochar has specifically been shown to sorb some of the major allelochemicals released by strawberry guava, such as phenolic compounds and  $\alpha$ -pinene [55-56], and the large positive responses of native tree growth to biochar addition are consistent with the sorption of these allelochemicals *in situ*. Biochar additions may also positively affect tree growth by other mechanisms, including increased nutrient availability [57-58], increased soil pH, and increased soil water retention [59]. The coconut biochar used in this experiment had a high pH and significantly increased the pH of soil treatments amended with biochar (Table 1). Coconut biochar is very basic [60-61] and can increase the availability of nutrients by increasing the pH of acidic tropical soils. P availability is strongly influenced by soil pH and is bound up in insoluble iron and aluminum phosphates in acidic soils [59], but basic biochars can increase P availability. The solubility of N, Mg, and Ca in acidic soils is also commonly enhanced in the presence of biochars [62-63]. Higher EC in biochar-amended soils (Table 1) indicates the presence of mineral ions [64] which likely contributed to increased tree growth.

Biochar additions resulted in particularly large positive responses on the growth and survivorship of *P. senecioidea* and *S. puberulum* after 30 months. Several studies have found strong species-specific responses to biochar addition [65-66], including negative responses in some cases [67]. Some species have a superior capacity to exploit resources and may benefit more from enrichment of specific nutrients [68]. For example, P addition favored the growth of a dominant pioneer tree *Rollinia exsacca* over coexisting species [69]. Species that are more sensitive to allelopathic effects may also benefit more from sorption of allelochemicals by biochar. Differential responses of native tree growth and survivorship to biochar may have long-term effects on species richness and diversity. According to our structural equation model, strawberry guava density also contributed significantly to tree diversity (Figure 6). We found that tree diversity was lowest in the control plots but increased substantially after the removal of strawberry guava and biochar addition (Figure 5). Prior studies indicate that biochar addition can have strong effects on plant community composition [70-71] and has been found to promote species richness in a reforested site, with potential impacts on overall biodiversity [65]. The present study is the first to demonstrate positive diversity effects of biochar in tropical forest restoration.

Strawberry guava density was greatly reduced even 30 months after weeding and biochar addition (Figure 4). The structural equation model showed that direct effects of strawberry guava removal and biochar dosage explained 81% variation in strawberry guava density (Figure 6). Lower strawberry guava density in weeded plots suggests that native trees were able to resist re-invasion by strawberry guava to some extent. The survivorship of native seedlings was higher in weeded plots (Figure 3a) and it seems likely

that strawberry guava was suppressed by a higher density of native species in these plots, compared to the control. Prior studies have found that some native species have great potential to suppress the growth of invasive species in re-vegetation field experiments [72-73]. Replacement of invasive plants with strong native competitors can thus increase the success of native forest restoration projects.

Invasive species commonly have a superior ability to acquire nutrients, and nutrient additions, particularly of N, can often be more beneficial to invasive species than to native species [68, 74-75]. In contrast, low N levels have been found to reduce competitive pressure from invasive species and even promote dominance of native species [76-78]. Strawberry guava performance has been also found to be strongly affected by soil N levels; leaf N uptake and flower production increased rapidly after nitrogen fertilization [79]. Although biochar can increase nutrient availability, in many cases it has been found to reduce available soil N [80]. In the present study, it seems likely that lower N levels in biochar-amended soils (Table 1) helped suppress the regeneration of strawberry guava. Adams et al. [81] reported strong negative effects of biochar addition on the invasive species *Lespedeza cuneata* when N was limiting, but positive effects on the native species *Andropogon gerardii* [81]. Another potential mechanism for reduced strawberry guava regeneration in biochar treatments is sorption of allelochemicals that may be beneficial to the invasive plant. Callaway and Aschehoug [1] found that activated carbon reduced the growth of the invasive Eurasian plant *Centaurea diffusa* in the presence of native North American competitors after controlling for spatial root niche partitioning, suggesting that its competitive advantage is at least partially mediated by allelopathic effects and that sorption of allelochemicals can allow native species to reclaim dominance of the native plant communities.  $\beta$ -caryophyllene, the main allelochemical found in strawberry guava leaves, is also produced by maize plants and has been found to protect maize roots and leaves from herbivore damage by attracting natural enemies of the herbivores [82-83]. Sorption of such allelochemicals by biochar might thus make strawberry guava more vulnerable to attacks by pathogens and herbivores.

Biochar addition at rates of 25 t/ha and 50 t/ha resulted in similar increases in tree growth (Figures 1 and 2). Prior studies suggest that plant growth responses to biochar application peaked at moderate doses of ~20–30 t/ha [84-85], though a higher optimum (of 50-60 t/ha) has been found in a recent study examining growth responses to natural post-fire pyrogenic carbon deposition [86]. In the present study we did not observe large differences between growth responses of native species between the 25 and 50 t/ha treatments. A dose of 25 t/ha may thus be sufficient for an optimal increase in tree growth through biochar treatment and is more practical for application to larger areas of forest soils. In the present study, strawberry guava was only removed once but we recommend repeated weeding to augment the positive effects of biochar on native tree growth. Repeated biochar applications may also be beneficial. Our results suggest that biochar application can reduce the frequency of weeding by suppressing the regeneration of strawberry guava. Further research is needed to investigate the effects of biochar on native fauna prior to large-scale applications to forest soils.

## 5. Conclusions

We conclude that biochar has large positive effects on native tree growth and survivorship in native forest communities invaded by strawberry guava. Our results are consistent with biochar reducing allelopathic effects, and thus altering the competitive balance of native and invasive species, thereby favoring the former. Mechanical removal of invasive species in Mauritius is labor-intensive and costly, but biochar addition can enhance the beneficial effects on native species and reduce encroachment of strawberry guava after initial weeding. Biochar can be produced from readily available plant biomass waste at relatively low cost; in Mauritius, weeded strawberry guava stems are an obvious feedstock option, though further research is needed to test the effects of strawberry guava biochar. Sorption or immobilization of allelochemicals seem to be particularly important for reducing invasions of allelopathic introduced species into native

plant communities. However, the capacity of biochar to mitigate the inhibitory effects of certain invasive species may be less pronounced when larger allelopathic compounds are involved. For example, biochar was only able to alleviate the allelopathic effects of *Ailanthus altissima* marginally, possibly due to steric effects that reduce sorption of large polyaromatic molecules [30]. Biochar appears to be a viable tool for combatting several tropical allelopathic invasive species, but a combination of laboratory and field studies is necessary to examine the properties and dosages of biochar that will optimize sorption of allelochemicals and native tree growth in invaded ecosystems. *Imperata cylindrica*, *Lantana camara* and *Chromolaena odorata* are strongly allelopathic invasive species that have particularly serious impacts on tropical biodiversity: biochar trials targeting these species are thus a priority for further work in this area.

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**Supplementary Materials:** Figure S1. A priori structural equation model of hypothesized pathways between treatment type and measured response variables.

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