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Potential of Biochar to Mitigate Allelopathic Effects in Tropical Island Invasive Plants: Evidence From Seed Germination Trials

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Abstract

Many tropical invasive species have strong allelopathic effects. Pyrolyzed waste biomass (“biochar”) has sorptive properties that can reduce the bioavailability of a variety of toxic organic compounds, including pesticides and naturally occurring phenolic acids; however, sorption of allelochemicals has received little attention. Strawberry guava (*Psidium cattleianum*) and lemongrass (*Cymbopogon flexuosa*) are important tropical island invasives thought to be allelopathic. Leaf extracts of both species were treated with two biochars (made from maize stalk and coconut husk feedstocks) and applied to maize (*Zea mays*) and radish (*Raphanus sativus*) seeds in a factorial design involving leaf extract and biochar dosages. Leaf extracts of both species had large inhibitory effects on germination and seedling growth, particularly at higher dosages, consistent with allelopathic effects. Biochar treatments positively affected seed germination and early seedling development consistent with sorption of these allelochemicals; in some cases, “rescue” effects occurred, in which biochar treatments completely counteracted allelopathic effects. Biochar leachates alone also generally had positive effects on seed germination and seedling development. We conclude that biochars have promise as a tool for combatting invasive allelopathic plants in tropical island ecosystems. The relative ease of biochar production using “low-tech” methods, and multiple benefits of biochar in enhancing soil productivity and carbon sequestration, may make such an approach viable in many developing countries.

Keywords

allelopathy, biochar, ecological restoration, invasive species, tropical islands

Introduction

Some of the most serious ecological impacts of invasive plants occur in the tropics, and in particular in tropical island ecosystems. Allelopathy is thought to be an important factor abetting the success of many noxious tropical invasive species that have large negative ecological and economic impacts: Notorious examples of allelopathic tropical invasives include lantana (*Lantana camara*; Sahid & Surgau, 1993), Siam weed (*Chromolaena odoratum*; Sahid & Surgau, 1993), and famine weed (*Parthenium hysterophorus*; Kanchan & Jayachandra, 1980). In many other cases of tropical invasive plants, allelopathy is strongly suspected. Two such putatively allelopathic species that have particularly large impacts in tropical island ecosystems are strawberry guava (*Psidium cattleianum* Sabine) and lemongrass (*Cymbopogon flexuosa* (Nees ex Steud.) W. Watson). Tropical island ecosystems tend to be more vulnerable

to the establishment and invasion of exotic species than mainland ecosystems (Rejmánek, Richardson, & Pyšek, 2013). This vulnerability is attributed to limited and fragmented habitat area, poor competitive ability of native species, poor dispersal of native plants, and the availability of “vacant niches” that are not fully exploited by existing island communities (Denslow, 2003). While the leading theory for the success of invasive species is the lack of natural enemies that allow them to fully utilize their potential for resource competition (Keane & Crawley, 2002), an alternative theory for their success,

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applicable in many cases, is “novel chemistry” via allelopathy (Callaway & Aschehoug, 2000; Cappuccino & Arnason, 2006).

Psidium cattleianum commonly known as strawberry guava (sometimes erroneously called Chinese guava) is one of the most aggressive nonnative woody species introduced to tropical islands. Native to Brazil, it has had particularly serious impacts on upland forests of the islands of Mauritius and the Hawaiian archipelago, threatening the native flora and fauna (Huenneke & Vitousek, 1990; Lorence & Sussman, 1986). In Mauritius, indigenous forest persists only of isolated fragments, and the majority of these are dominated by exotic species, of which strawberry guava is by far the most common (Page & D’Argent, 1997). Feral pigs, frugivorous birds, and humans commonly aid in the seed dispersal of strawberry guava. Traits that likely contribute to this species’ dominance include escape from natural enemies (Dietz, Wirth, & Buschmann, 2004), high reproductive capacity aided by copious fruiting, capacity for suckering to form dense stands (Huenneke & Vitousek, 1990), and high shade tolerance coupled with an ability to recruit under high light conditions (Schumacher et al., 2008). Strawberry guava has a deep rooting system and can lower water level from soils, streams, and groundwater systems, with impacts on water supply to agriculture, native plant species, and local communities (Patel, 2012). Strawberry guava can also be a threat to native forest ecosystems and agricultural systems by supporting large populations of fruit flies (Uowolo & Denslow, 2008). Strawberry guava has been hypothesized to release allelochemicals through its leaves and roots (Patel, 2012), and it is strongly suspected that the allelopathic effect of strawberry guava reduces the ability of slow-growing native species to compete with this faster growing exotic species (Virah-Sawmy, Mauremootoo, Marie, Motala, & Sevathian, 2009). The most abundant chemical constituent of leaf oils from strawberry guava is β -caryophyllene (Patel, 2012), a volatile hydrocarbon that has been shown to inhibit the growth of radish, mungbean, and tomato seedlings (Kong, Hu, Xu, & Lu, 1999). This gives us a strong reason to suspect that allelopathy contributes greatly to the successful invasion of strawberry guava.

Cymbopogon flexuosus, commonly known as lemongrass, is widely recognized for its medicinal and antibacterial properties. For these reasons, it is widely cultivated in many countries around the world. However, lemongrass has become an invasive species in the tropical forests of the Caribbean islands since its introduction in the 1940s for soil conservation purposes (Robbins, Eckelmann, & Quinones, 2008). Lemongrass is adapted to frequent fires and can resprout quickly after wildfires. As uncontrolled wildfires burn through dry and low scrub forests, killing the trees that are not resistant to

fires, lemongrass invades the burned areas. This process has resulted in some 1,200 ha of Caribbean islands being dominated by dense monospecific thickets of lemongrass (Robbins et al., 2008). Although the clonal propagative ability of lemongrass facilitates its invasion, allelopathic properties are thought to enhance its dominance. Poonpaiboonpipat et al. (2013) suggested that lemongrass has the potential to act as a bioherbicide due to its allelopathic effects on plants. Essential oils from lemongrass were found to reduce seed germination, increase wilting, and reduce photosynthesis in the seedlings of *Echinochloa crus-galli* (Poonpaiboonpipat et al., 2013). The potential allelopathic effects of lemongrass on agricultural crops or tropical native plants have not been evaluated.

Several management control strategies have been implemented to reduce the spread of invasive species in tropical island ecosystems, but costs are commonly high and success limited. The mechanical removal of strawberry guava in the native forest remnants of Mauritius has a positive effect on native species (Monty, Florens, & Baider, 2013). However, due to the high cost of mechanical tending, this operation has only been carried out on ~1% of the habitat remnants of native species. Alternatives to mechanical removal have focused on biological control agents, such as release of the natural enemies *Tectococcus oyatus* and *Eurytoma* sp. (Wikler, Smith, & Pedrosa-Macedo, 1996). While both *Tectococcus oyatus* and *Eurytoma* sp. have a high specificity for strawberry guava, there are risks associated with the introduction of any nonnative biological control agents since they may themselves become invasive and attack native species. These risks are particularly acute in small island ecosystems such as Mauritius, where only 5% of the native forests remain (Safford, 1997). In parts of the Caribbean, lemongrass presents a similar threat to both agriculture and native vegetation (e.g., Dominica Ministry of Agriculture, 1996).

The term “biochar” has recently been widely used to describe pyrolyzed biomass, or charcoal, intended for use as soil amendment to enhance productivity (Lehmann, Gaunt, & Rondon, 2006), with an additional important motivation being carbon sequestration (Woelf, Amonette, Street-Perrott, Lehmann, & Joseph, 2010). While research has mostly focused on biochar’s applications to agricultural systems, ecological and restoration applications of biochar are increasingly receiving attention due to biochar’s ability to ameliorate soil properties and reduce bioavailability of soil contaminants (Beesley et al., 2011; Macdonald, Farrell, Van Zwieten, & Krull, 2014; Thomas & Gale, 2015). A “charcoal effect” stimulating postfire growth in boreal ecosystems has long been recognized, and studies point to the importance of sorption of growth-inhibiting phenolic compounds as a primary mechanism (Wardle, Zackrisson, & Nilsson, 1998).

Activated carbon has also long been used as an assay for allelopathy: For example, Mahall and Callaway (1992) observed lower inhibitory effects on root elongation of *Ambrosia dumosa* by *Larrea tridentata* in the presence of activated carbon, due to sorption of allelochemicals produced by *Larrea*. Sorption of allelochemicals associated with corn crop residues by certain biochars has also been reported (Rogovska, Laird, Cruse, Trabue, & Heaton, 2012). However, the capacity of biochars to sorb allelochemicals released by tropical invasive species appears to have received no prior research attention.

In the present study, we test for possible allelopathic effects of strawberry guava and lemongrass on seed germination and early seedling development of crop plants, and the potential for biochar treatment of plant extracts to mitigate such allelopathic effects. To enhance applicability, we used biochar feedstocks widely available within tropical island ecosystems, and dosages calibrated to be similar to those used operationally for mitigation of organic soil contaminants (Hale, Cornelissen, & Werner, 2015). Germination experiments on maize (*Zea mays*) and radish (*Raphanus sativus*) seeds were carried out to test the following hypotheses: (a) Leaf extracts of strawberry guava and lemongrass have inhibitory effects on maize and radish germination and early seedling development and (b) biochar reduces these inhibitory effects, consistent with sorption resulting in reduced bioavailability of allelochemicals.

Methods

Leaf Extracts

Leaves of strawberry guava were obtained from dense thickets of the species in Plaine Champagne, Mauritius (20°25' 33.9" S, 57°27' 55.6" E). After the leaves were collected, they were stored at 2.0°C for 3 weeks until they were used for germination trials. Lemongrass was grown from seed in a commercial soil mix in a greenhouse for 10 weeks, after which the leaves were harvested a day prior to making the leaf extract solution. Leaves of strawberry guava and lemongrass were air-dried for 24 h, shredded into approximately 2 cm by 2 cm pieces, and soaked in deionized water at 25°C to prepare 0.1 g/mL and 0.3 g/mL concentrations (fresh weight basis) of leaf extract solutions. After 48 h, the leaf extract solutions were suction filtered (using Whatman #4 filter paper) and the filtrate collected. Different dosages of biochar were added to each concentration of leaf extract filtrate to obtain 0 (control), 0.002 and 0.02 g biochar/mL filtrates. Each treatment solution containing biochar was mixed using an orbital shaker table rotating at 50 rpm for 24 h. Each treatment solution containing a mixture of biochar or leaf extract was then suction filtered and the filtrate collected for germination experiments.

Biochar Preparation

Coconut biochar was obtained from coconut husks pyrolyzed at ~350°C for 3 h in a small-scale batch pyrolysis system (80 L capacity). Maize feedstock for biochar production was obtained from maize plants grown and harvested in a previous greenhouse study. The maize biomass was pyrolyzed at 350°C for 3 h in a lab pyrolysis system consisting of a programmable tube furnace purged with N₂ gas. Maize and coconut biochars were prewashed with a 1:1 (v:v) mixture of deionized water on orbital shaker table rotating at 50 rpm for 24 h to remove leachates that can potentially have negative effects on germination and plant growth (Gale, Sackett, & Thomas, 2016). Biochar-water slurries were suction filtered, and the filtrate collected for germination experiments. The biochar residue was dried at 60°C for 24 h and applied to leaf extract solutions at different dosages. Properties of biochars used (prior to prewashing) are given in Table 1.

Experimental Design

Germination experiments were carried out using two target seed species: *Zea mays* L. (hereafter maize) and *Raphanus sativus* L. (hereafter radish); these species have been extensively utilized in prior studies allelopathy and are widely cultivated in the tropics. Although both strawberry guava and lemongrass have mainly received attention due to impacts on natural ecosystems, both have origins in agricultural introductions and occur as agricultural weeds. The experiments were carried out in two phases, one for strawberry guava and a second for lemongrass. For each of the extract source species, treatments consisted of a three-way factorial combination of two concentrations of leaf extract (0.1 g/mL and 0.3 g/mL), two types of biochar (coconut and maize feedstocks), and three biochar concentrations (0, 0.002, and 0.02 g/mL). In addition, there were three controls: Deionized water and leachates from each biochar (at 0.02 g/mL only) washed

Table 1. Properties of Biochars Used in Experiment. Property Determinations Were Based on 2 to 6 Samples Per Char (With Only One Measurement of a Pooled Sample for C and N); Values Are Listed \pm 1 SE.

Attribute	BC1	BC2
Feedstock	Coconut husk	Maize stalks
Peak pyrolysis temp.	350°C	350°C
Moisture (%)	4.75 \pm 0.58	7.09 \pm 1.61
Ash (%)	11.4 \pm 2.9	24.2 \pm 2.1
pH	7.19 \pm 0.01	7.05 \pm 0.02
EC (μ S/cm)	99.0 \pm 2.2	80.6 \pm 1.5
Carbon (%)	55.4	63.1
Nitrogen (%)	0.97	0.68

with deionized water, thus giving a total of $2 \times 2 \times 3 + 3 = 15$ treatments. This full combination of treatments was assessed for the two target seed species. Replicates consisted of 90-mm diameter Petri dishes containing a Whatman grade #1 filter paper, with 10 ml of each treatment solution added to each replicate. Ten seeds were added per dish for maize, and 25 per dish for radish; there were 10 replicates of each treatment for maize, and 5 for radish for strawberry guava extract trials, and 7 and 4 replicates, respectively, for lemongrass extract trials. The Petri dishes were kept under incandescent light bulbs simulating a 12-h day or night cycle at room temperature ($\sim 25^\circ\text{C}$ day and $\sim 20^\circ\text{C}$ night temperatures). Each phase of the germination experiment ran for 7 days during which daily measurements of germination and cotyledon development status were taken. Radicle length measurements were taken for three seeds per Petri dish on Day 4 of each trial.

Statistical Analysis

Germination rate and seedling cotyledon development at the end of 1 week were analyzed using analysis of variance (ANOVA). Since data were expressed as proportions, we transformed variates with a logit function (Warton & Hui, 2011), modified to handle 0 and 1 values: $t = \log(y + e / 1 - y + e)$, where t is the transformed variate, y is the observed proportion, and e is small value set to $1/2$ of the smallest possible value of y in the dataset (thus where $N = 25$, $e = 0.02$, and where $N = 10$, $e = 0.05$; Martín-Fernández & Thió-Henestrosa, 2006). Post hoc Tukey honest significant difference (HSD) tests were used following ANOVA to elucidate pairwise treatment differences (with $p < .05$ considered significant). Data for the time-course of germination and cotyledon development were examined using linear mixed effects models; alternative models including treatment effects, day, and replicate (treated as a random effect) were compared using a minimum Akaike information criterion (AIC) approach (Bates, Maechler, Bolker, & Walker, 2014). In cases where the random effect term was not significant, the term was dropped, and p values are reported based on a conventional ANOVA. Analyses for each combination of target seed species and putatively allelopathic species used were treated separately. Data analysis was conducted in (R Core Team, 2016) specifically making use of the `lm()` and `lmer()` functions for the main analyses.

Results

Leaf Extract Effects on Germination and Cotyledon Development

Leaf extracts of strawberry guava suppressed germination of maize and radish seed relative to controls,

particularly at high extract concentrations (Figure 1(a) and (c)). In all treatments, germination percent leveled off after 1 week; based on these values, germination was significantly suppressed relative to deionized water controls. Effects were significant for both maize and radish seed exposed to strawberry guava extracts, $F(2, 27) = 21.0$, $p < .001$ for maize; $F(2, 27) = 29.1$, $p < .001$ for radish. Contrasts were significant ($p < .05$) for high extract dosages versus control and low extract dosages: Germination rates at 1 week were 96% versus 73% and 97% versus 75% in controls versus high-dosage treatments in maize and radish, respectively (Figure 1). Leaf extracts of strawberry guava had even more dramatic effect on cotyledon development. At 1 week, 50% of maize seeds had formed cotyledons in controls, versus 10% in low-dosage and only 4% in high-dosage treatments ($F(2, 27) = 57.3$, $p < .001$; contrasts significant for both treatments vs. control). In radish, 95% of seeds formed cotyledons in controls at 1 week, versus 91% in low-dosage and 26% in high-dosage treatments ($F(2, 27) = 154.2$, $p < .001$; contrasts significant for high-dosage treatment vs. control).

Leaf extracts of lemongrass also significantly suppressed germination rates and cotyledon development of treated seeds in most cases (Figure 2). Effects were again initially assessed at Day 7. No effect was detected of lemongrass extracts on germination of maize, $F(2, 39) = 0.644$; $p > .05$; however, radish germination was strongly suppressed, $F(2, 21) = 47.5$; $p < .001$, with contrasts significant between both dosages and controls ($p < .05$): 99% germination in controls versus 89% in low-dosage and 70% in high-dosage treatments (Figure 2(c)). Cotyledon development was significantly reduced in both cases. At 1 week, 31% of maize seeds had formed cotyledons in controls, versus 13% in low-dosage and 9% in high-dosage treatments ($F(2, 39) = 9.9$; $p < .001$; contrasts significant for both treatments vs. control). In radish, 99% of seeds formed cotyledons in controls at 1 week, versus 77% in low-dosage and 56% in high-dosage treatments ($F(2, 39) = 10.0$; $p < .001$; contrasts significant for both dosage treatments vs. control).

Linear mixed effects models were used to assess treatment effects on the time-course of germination and cotyledon development (Table 2). The minimum-AIC model included an extract treatment \times day interaction term in every case involving *Psidium* extracts, and for effects of *Cymbopogon* extracts on cotyledon development (Table 2). In the case of *Cymbopogon* extract effects on germination, models excluding the extract treatments were selected (Table 2), though models including an extract treatment \times day interaction term differed by 4 to 5 AIC units. Qualitatively, the extract treatment \times day interaction effects on seed development corresponded to a delay in germination and cotyledon development in

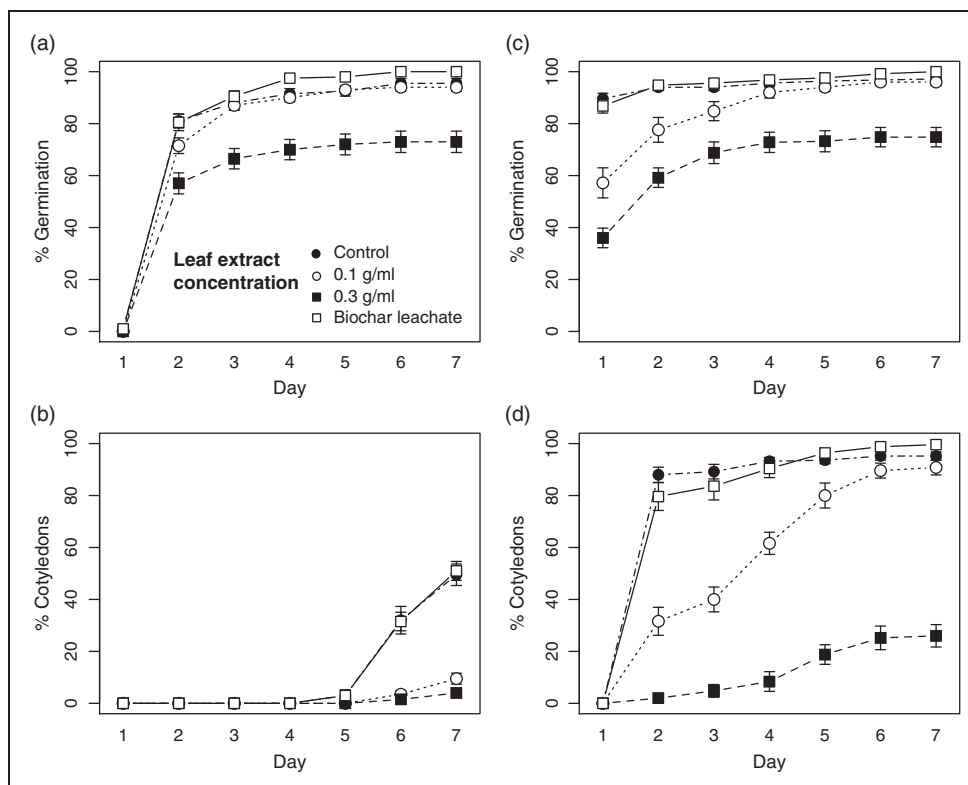


Figure 1. Percent germination and percent cotyledon formation of seeds of maize (a) and (b), and radish (c) and (d), treated with raw leaf extracts of strawberry guava (*Psidium cattleianum*) at two concentrations. Controls include distilled water and leachates of biochars tested (averaged over both feedstock types). Means are plotted \pm 1 SE.

radish seeds treated by either *Psidium* or *Cymbopogon* extracts (Figures 1 and 2(d)).

Biochar Leachate Effects on Germination and Cotyledon Development

Treatment with biochar leachate alone generally resulted in small but positive effects on germination or cotyledon development. Germination at 7 days in control treatments for maize averaged 95.5%, compared with 100% germination for biochar-leachate-treated seed (Supplementary Figure 1(a)). This difference was statistically significant, $F(3, 36) = 6.1$, $p = .002$. Germination at 7 days in control treatments for radish averaged 97.2%, compared with 100% germination for biochar-leachate-treated seed (Supplementary Figure 1(a)); this was likewise statistically significant, $F(3, 36) = 5.6$, $p = .008$. No effects of biochar leachates on cotyledon development in maize were detected, $F = 0.83$, $p > .05$. Cotyledon development at 7 days in control treatments for radish averaged 95.2%, compared with 99.6% for biochar-leachate-treated seed (Supplementary Figure 1(d)); this difference was also significant, $F = 8.36$; $p = .001$. In no case were contrasts between leachates of the two biochars different (Tukey HSD test: $p > .05$).

Biochar Mitigation of Allelopathic Effects on Germination and Cotyledon Development

Reductions in germination and cotyledon formation due to strawberry guava extract were ameliorated by biochar treatments in essentially every case examined (Figure 3). Analyses examined the 3×2 factorial component of the experiment (i.e., 2 biochar dosages plus control \times 2 extract dosages). Day 7 results (Table 3) indicate a significant extract term and a significant biochar term in every case (with the exception of the biochar term for maize germination for BC1, for which $p = .08$). In all cases, extract treatments had negative effects, and biochar treatments positive effects, with the higher biochar concentration resulting in larger effect than the lower concentration (Figure 3). The most dramatic effects were found for radish seeds. High extract concentrations reduced germination rates to 65% to 75%, while high-dosage biochar treatments resulted in germination rates of 90% to 95%, similar to distilled water controls (Figure 3(e) and (g)). Cotyledon formation in high extract treatments was reduced to 20% to 30%, with high-dosage biochar treatments increasing cotyledon formation to $\sim 65\%$ for BC1, and $>95\%$ for BC2 (Figure 3(f) and (h)). The latter case was again

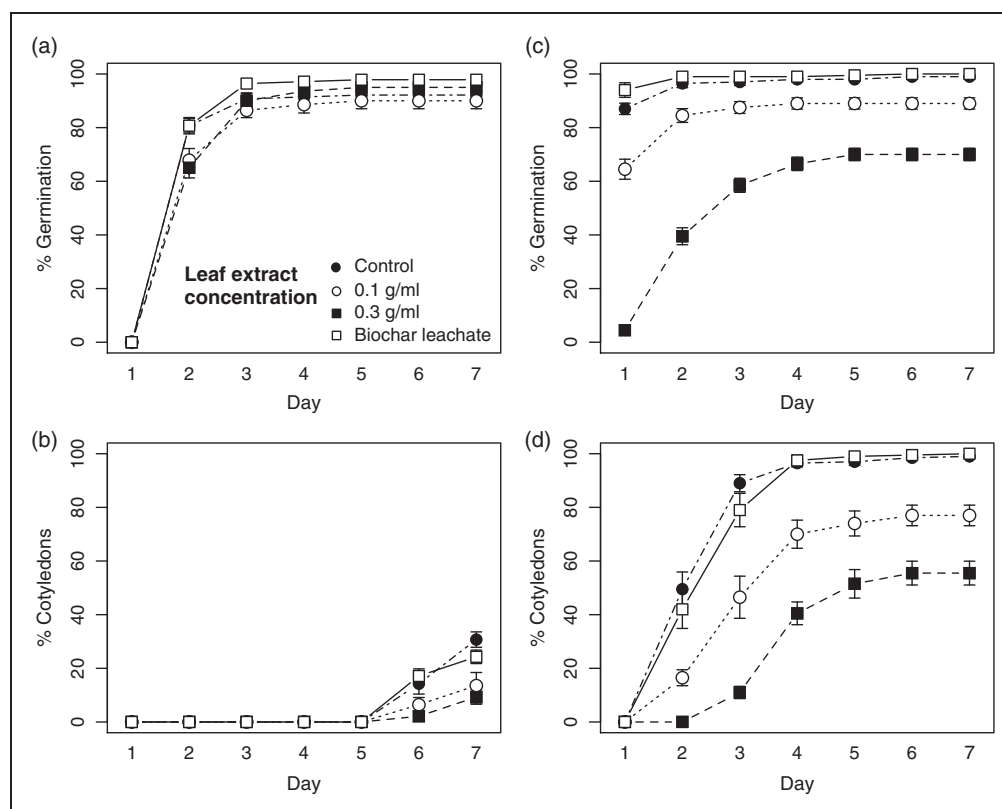


Figure 2. Percent germination and percent cotyledon formation of seeds of maize (a) and (b), and radish (c) and (d), treated with raw leaf extracts of lemongrass (*Cymbopogon flexuosus*) at two concentrations. Controls include distilled water and leachates of biochars tested (averaged over both feedstock types). Means are plotted \pm 1 SE.

Table 2. AIC Values for Alternative Models Describing the Time-Course of Germination and Cotyledon Development in Experiments, Corresponding to Figures 1 and 2. AIC Is Expressed as the Difference Between the Minimum Value Among Models (Indicated in Bold).

	Terms included in model						
	Null	Day	Day + rep	Day + ext	Day + ext + rep	Day \times ext	Day \times ext + rep
Psidium extracts							
Maize germination	385.17	57.68	22.29	5.32	6.63	0.00	7.25
Maize cotyledon dev.	584.43	303.57	39.74	126.81	10.92	106.98	0.00
Radish germination	1272.47	195.17	51.49	129.67	54.68	0.00	5.26
Radish cotyledon dev.	303.68	215.58	77.50	14.43	25.28	0.00	17.56
Cymbopogon extracts							
Maize germination	273.03	0.00	0.01	2.39	4.19	5.00	11.47
Maize cotyledon dev.	363.34	223.21	42.37	20.03	10.75	4.01	0.00
Radish germination	903.32	41.17	0.00	29.69	7.50	5.44	4.32
Radish cotyledon dev.	250.38	130.38	56.75	5.09	20.23	0.00	20.74

indistinguishable from distilled water controls. Interaction terms in analyses were significant for radish but not maize results (Table 3). In all cases, the interaction was due to stronger effects of biochar treatments at the higher extract dosages compared

with the lower extract dosages. Treatment effects on the time-course of germination cotyledon development were analyzed using a linear mixed model approach. These analyses indicate that cases with reduced germination or cotyledon formation at Day 7 generally

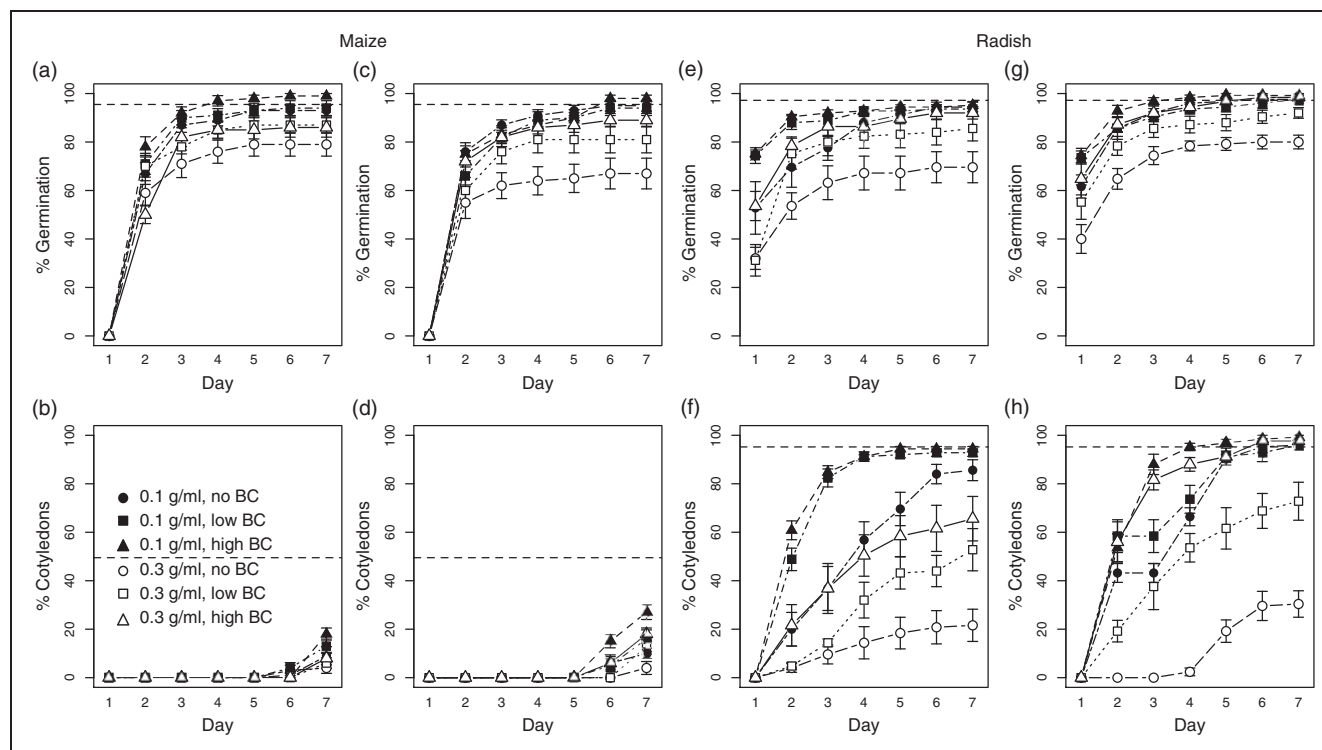


Figure 3. Amelioration of leaf extract effects of strawberry guava (*Psidium cattleianum*) by two biochars. Percent germination and cotyledon formation of seeds of maize (a) to (d), and radish (e) to (h) is plotted over time. BC1 (a), (b), (e), and (f) was derived from coconut feedstock, and BC2 (c), (d), (g), and (h) from rice hull feedstock. Controls include distilled water and leachates of biochars tested (averaged over both feedstock types). Means are plotted \pm 1 SE.

also showed a delay in seedling development (e.g., Figure 3(h); statistical results not shown).

Lemongrass results for germination and cotyledon formation were broadly similar to those for strawberry guava but with less-pronounced effects of both leaf extract and biochar treatments (Figure 4; Table 2). Day 7 results (Table 3) indicate a significant extract term and a significant biochar term in every case for BC2, with extract treatments showing negative effects, and biochar treatments positive effects (Figure 3). For BC1, effects were only significant for cotyledon development: A significant negative effect of extracts was found for maize, with a corresponding positive effect for biochar (the latter marginally significant: $p = .053$); in the case of radish, only the positive effect of biochar on cotyledon development was significant. In no case was the extract \times biochar interaction significant for lemongrass analyses (Table 3).

Treatment Effects on Radicle Extension

Leachates alone from both biochars acted to strongly enhance radicle extension growth in both plant species examined (Figures 5 and 6). Contrasts between distilled water controls and biochar leachate treatments were

significant in 5 of 8 cases. This effect was most pronounced in the case of radish seed in the strawberry guava experiment, where a nearly threefold effect was detected (Figure 5(d)); averaged across biochars, biochar leachates resulted in a 60% increase in radicle extension in maize seeds, and a 119% increase in radicle extension in radish seeds.

ANOVA indicated pronounced interactive effects of treatment effects on radicle extension growth in the case of strawberry guava extracts (Figure 5): Extract \times biochar interaction terms were significant for both biochars in the case of radish seeds, and for BC2 in the case of maize seeds (Table 4). In each of these cases, higher dosages of biochar resulted in larger increases in radicle extension growth, with a more pronounced effect observed at the low (0.1 g/ml) extract dosage (Figure 5(b) to (d)). In the case of BC2, the high biochar dosage and low (0.1 g/ml) extract dosage resulted in a *rescue effect* on radicle extension growth, with radicle extension similar to that observed in control treatments (Figure 5(b) and (d)). In the case of BC1 and maize seeds, strawberry guava extracts did not result in significant decreases in radicle extension growth in comparison to controls at any combination of biochar and extract dosages (Figure 5(a)).

Table 3. *P* Values for Linear Models Describing Effects of Leaf Extracts (E) and Biochars (B) and Their Interaction (E × B) on Germination Rate and Cotyledon Development Rate in Maize and Radish Seed, Corresponding to Data Shown in Figures 3 and 4.

Seed species	Trait	Char	Figure	E	B	E × B
<i>Psidium</i> extracts				ANOVA term		
Maize	germination	BC1	3(a)	<0.001	0.080	0.605
Maize	cotyledon dev.	BC1	3(b)	0.004	0.028	0.618
Maize	germination	BC2	3(c)	<0.001	0.001	0.010
Maize	cotyledon dev.	BC2	3(d)	<0.001	<0.001	0.001
Radish	germination	BC1	3(e)	<0.001	0.032	0.232
Radish	cotyledon dev.	BC1	3(f)	0.022	<0.001	0.287
Radish	germination	BC2	3(g)	<0.001	0.001	0.010
Radish	cotyledon dev.	BC2	3(h)	<0.001	<0.001	0.001
<i>Cymbopogon</i> extracts						
Maize	germination	BC1	4(a)	0.754	0.971	0.489
Maize	cotyledon dev.	BC1	4(b)	0.030	0.053	0.869
Maize	germination	BC2	4(c)	<0.001	0.048	0.468
Maize	cotyledon dev.	BC2	4(d)	<0.001	0.017	0.203
Radish	germination	BC1	4(e)	0.731	0.772	0.184
Radish	cotyledon dev.	BC1	4(f)	0.301	0.001	0.480
Radish	germination	BC2	4(g)	<0.001	0.048	0.468
Radish	cotyledon dev.	BC2	4(h)	<0.001	0.017	0.203

Note. ANOVA = analysis of variance.

Dependent variables were logit-transformed prior to analysis.

p < .05 are shown in bold.

Lemongrass extracts generally showed a trend toward reducing radicle extension growth only in the case of high extract dosages without biochar (Figure 6(c) and (d)), and these negative effects were only marginally statistically significant ($0.1 < p < .05$). For maize seeds, the overall trend was toward increased radicle extension growth in seeds exposed to higher dosages of lemongrass extracts (Figure 6(a) and (b)), with a significant (positive) effect of extracts in the case of BC2 (Table 4). For the combination of BC1 and maize seeds, the biochar main effect term was significant, with higher biochar dosages resulting in increased radicle extension (Figure 6(a), Table 4). There were more complicated interactive effects in the case of radish seed, with opposite effects of biochar treatments on radicle length extension at low versus high extract dosages (Figure 6(c)). Both of these cases are consistent with sorption of the allelochemical agent: At the low (0.1 g/ml) dosage, the lemongrass extract had a positive effect on radicle extension that was reduced in biochar-treated extracts. At high dosages, the effect of lemongrass extracts was negative, and this negative effect was alleviated at higher biochar dosages (Figure 6(c)). BC2 did not show this complex effect but rather simply a negative effect of high extract dosages and

positive effect of increased biochar dosages (Figure 6(d), Table 4).

Discussion

Our results support the hypothesis that water-soluble allelochemicals released by both strawberry guava and lemongrass have pronounced inhibitory effects on seed germination and seedling development. Strawberry guava extracts had especially strong inhibitory effects at the higher concentration examined (0.3 g leaf extract/ml), consistent with prior suggestions that this species releases allelopathic agents that inhibit the germination and growth of native plants (Patel, 2012; Virah-Sawmy et al., 2009). Stronger inhibitory effects were seen for radish seed germination and development than for maize. This is also consistent with prior studies that have found radish to be particularly sensitive to allelopathic effects (e.g., McCarthy & Hanson, 1998). Large-seeded species are generally buffered from allelopathic effects (Liebman & Sundberg, 2006), so it is notable that inhibitory effects were pronounced for both radish (seed mass ~12.3 mg) and maize (seed mass ~0.26 g, or ~20-fold the mass of radish).

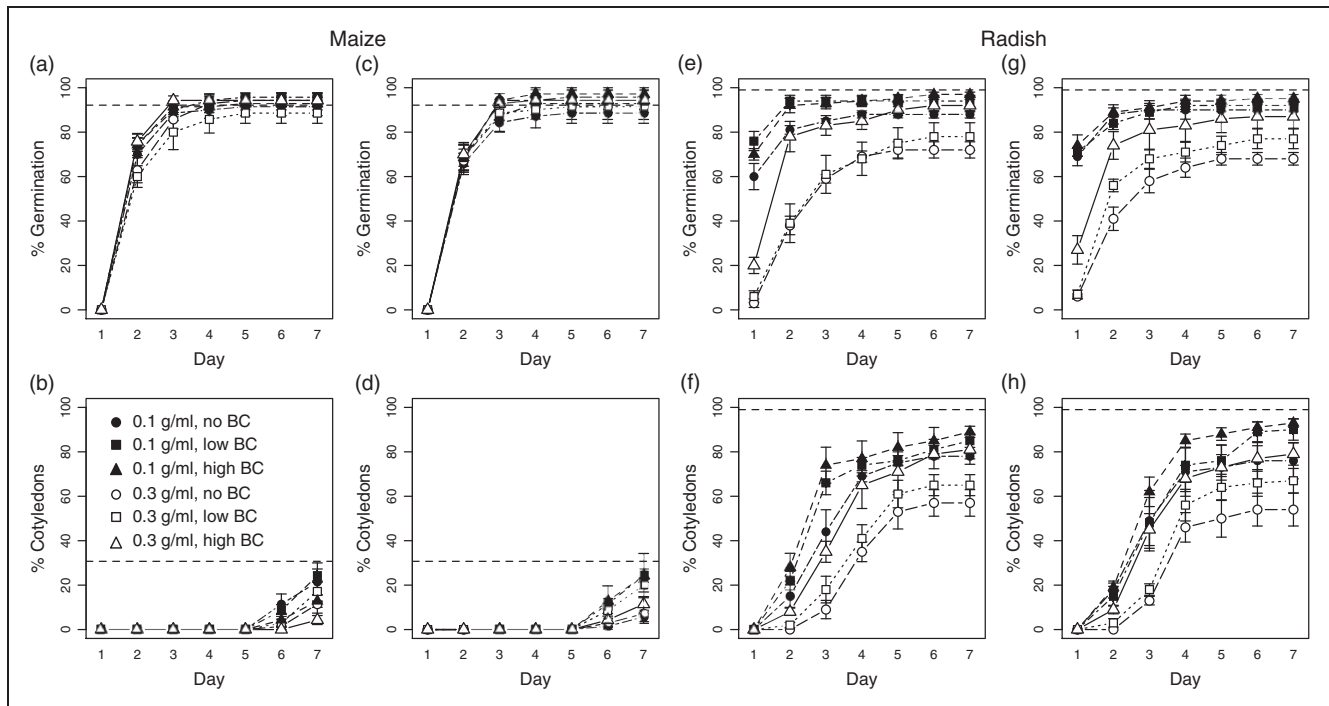


Figure 4. Biochar treatment amelioration of leaf extract effects in lemongrass (*Cymbopogon flexuosus*). Percent germination and cotyledon formation of seeds of maize (a) to (d), and radish (e) and (h), treated with raw leaf extracts of strawberry guava (*Psidium cattleianum*) at two concentrations. Controls include distilled water and leachates of biochars tested (averaged over both feedstock types). Means are plotted \pm I SE.

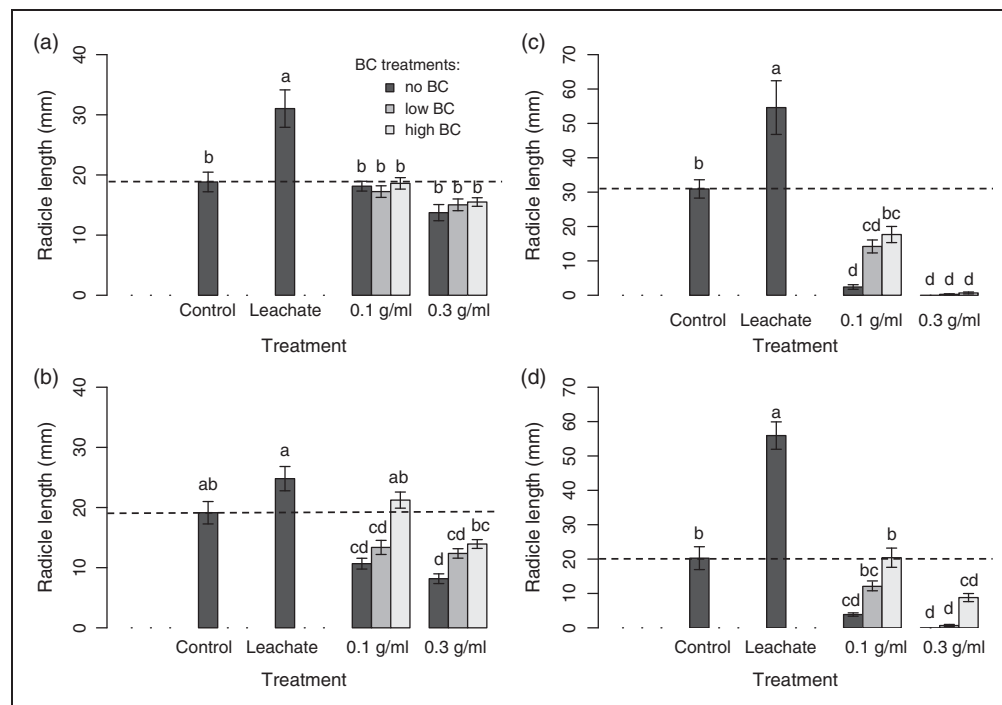


Figure 5. Seedling radicle length by treatment in seeds of two agricultural species exposed to *Psidium* leaf extracts. Distilled water and biochar leachate controls are compared with a factorial combination of two concentrations of *Psidium* extract (0.1 and 0.3 g/ml), and three biochar treatments. Means are plotted \pm I SE; differences significant at $p < .05$ (Tukey HSD post hoc tests) are indicated by different letters. Control mean is indicated by dotted line. (a) *Psidium* extract, BC1: maize, (b) *Psidium* extract, BC2: maize, (c) *Psidium* extract, BC1: radish, (d) *Psidium* extract, BC2: radish.

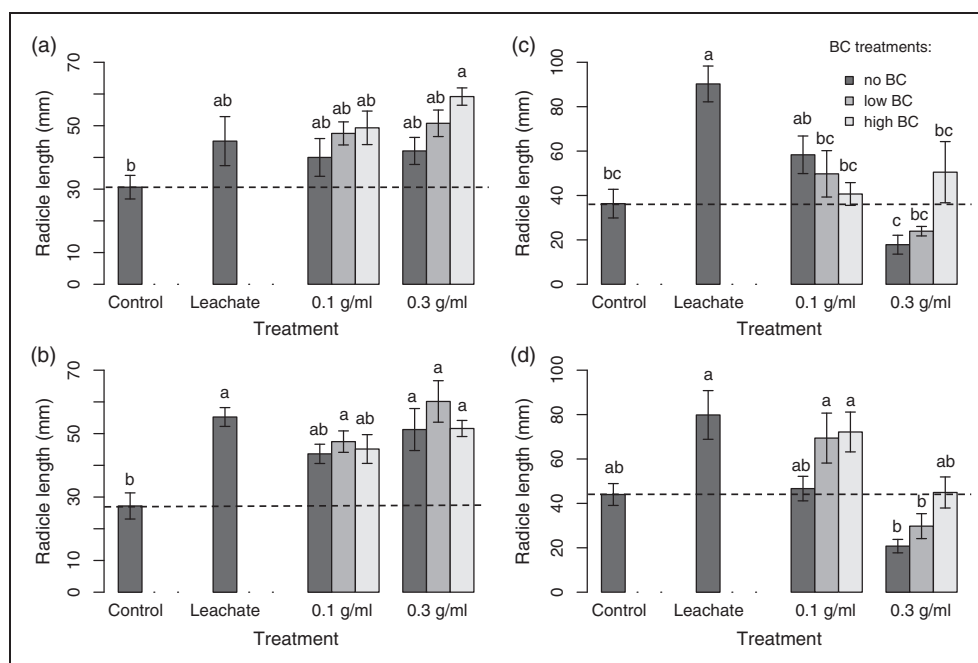


Figure 6. Seedling radicle length by treatment in seeds of two agricultural species exposed to *Cymbopogon* leaf extracts. Distilled water and biochar leachate controls are compared with a factorial combination of two concentrations of *Cymbopogon* extract (0.1 and 0.3 g/ml), and three biochar treatments. Means are plotted \pm 1 SE; differences significant at $p < .05$ (Tukey HSD post hoc tests) are indicated by different letters. Control mean is indicated by dotted line. (a) *Cymbopogon* extract, BC1: maize, (b) *Cymbopogon* extract, BC2: maize (c) *Cymbopogon* extract, (d) *Cymbopogon* extract, BC2: radish BC1: radish.

Table 4. P Values for Linear Models Describing Effects of Leaf Extracts (E) and Biochars (B) and Their Interaction ($E \times B$) on Radicle Extension Growth in Maize and Radish Seed, Corresponding to Data Shown in Figures 5 and 6.

Seed species	Char	Figure	E	B	$E \times B$
<i>Psidium</i> extracts			ANOVA term		
Maize	BC1	5a	<0.001	0.483	0.533
Maize	BC2	5b	<0.001	<0.001	0.006
Radish	BC1	5c	<0.001	<0.001	<0.001
Radish	BC2	5d	<0.001	<0.001	0.013
<i>Cymbopogon</i> extracts					
Maize	BC1	6a	0.176	0.018	0.644
Maize	BC2	6b	0.027	0.359	0.787
Radish	BC1	6c	0.013	0.539	0.021
Radish	BC2	6d	<0.001	0.011	0.599

Note. ANOVA = analysis of variance.
 $p < .05$ are shown in bold.

In addition, our results strongly support the hypothesis that biochars acted to sorb or otherwise immobilize allelochemicals present in aqueous extracts of both plants examined. Both biochars examined (derived from maize

and coconut feedstocks) acted to reduce the inhibitory effects of strawberry guava on germination, cotyledon formation, and radicle growth in radish and maize seeds. In some cases, biochar treatments resulted in

“rescue effects”—that is, effects in which performance metrics for biochar plus extract treatments were similar to controls (Figure 3(b) and (d); Figure 5(e) to (h); Figure 6(e) and (g)). Our results are thus consistent with physiochemical sorption of allelopathic compounds by biochar, though we can only speculate on the detailed processes involved. Sorption processes of organic molecules to biochar likely include ionic hydrogen bonding by peripheral carboxyl or phenoxyl groups present on biochars (Teixidó et al., 2013), in addition to the physical occlusion of molecules within biochars’ pore structure (Hale et al., 2015). However, precipitation or altered mobility of allelochemicals related to pH changes could also possibly be a mechanism for immobilization (as shown in heavy metals: Rees, Simonnot, & Morel, 2014); in this case, sorption per se would not occur.

There are only very limited data from prior studies assessing interactions of allelochemicals with biochars. Positive effects of chars on the growth of boreal tree seedlings have been interpreted as indicating sorption of phenolic compounds produced by Ericaceous species (Wardle et al., 1998; Zackrisson, Nilsson, & Wardle, 1996). Rogovska et al. (2012) tested for sorption of allelochemicals associated with corn crop residues by a set of six biochars, finding that all of the biochars substantially enhanced radicle and shoot extension of corn seedlings. A recent study examining temperate invasive species we found strong sorption allelochemicals by biochar in one case (garlic mustard: *Alliaria petiolata*), but only marginal effects in another case (tree of heaven: *Ailanthus altissima*; Thomas, Al-Zayat, & Murtada, unpublished data). In the present study, the two chars examined showed pronounced differences in sorption of allelochemicals, with the maize-derived char showing stronger effects than the coconut-derived char (Figures 3 and 4). In sum, the results to date suggest that sorption of allelochemicals by biochars is common, but point to the importance of matching chars to specific allelochemicals, and of lab trials in advance of large-scale applications. It should also be noted that the capacity of biochars to sorb specific organic molecules can be substantially reduced in complex solution mixtures with high dissolved organic matter relative to single-solute conditions (Shimabuku et al., 2016), also emphasizing the importance of field trials.

There is a long history of use of activated carbons as a means of sorbing allelochemicals as a test for allelopathy (Lau et al., 2008). It is important to note that activated carbons are distinct from biochars, consisting of pyrolyzed material that is secondarily treated to enhance porosity and remove noncarbon material (activation treatments generally use steam or CO₂, or sometimes strong acids or bases: Chia, Downie, & Munroe, 2015). Activated carbons are also commonly made from coals in addition to high-density nonfossil organic materials such

as nutshells. Although both activated carbons and biochars are variable, activated carbons commonly have a higher surface area and carbon content but lower ash and lower concentrations of plant mineral nutrients than biochars (Chia et al., 2015; Hale et al., 2015). Properties other than sorption, such as nutrient retention, have complicated the interpretation of activated carbon additions as a definitive test for allelopathic interactions (Lau et al., 2008). In the context of applied use of biochars to mitigate allelopathic effects, the properties of biochars other than allelochemical sorption (such as direct provision of nutrients and high water holding capacity) may be regarded in most cases as additional benefits. Biochars are expected to have lower sorption capacity for organic molecules than activated carbons (e.g., Hale et al., 2015) but can potentially be produced at much lower cost. Typical prices for activated carbon are ~US\$2000/t (Hale et al., 2015), while recent commercial biochar prices range from ~US\$350–900/t (Shackley, Clare, Joseph, McCarl, & Schmidt, 2015). Prices for biochar at target restoration sites may be reduced substantially by using mobile pyrolysis systems that make use of thinned material for feedstock (Page-Dumroese, Coleman, & Thomas, 2016), or by employing low-tech systems that may be especially practical in the context of developing countries (Joseph, Anh, Clare, & Shackley, 2015).

In addition to the positive effects of biochars on early seedling development due to sorption of allelochemicals, our results provide evidence for direct positive effects of biochar leachates. Prior studies indicate that the biochars can contain hormetic compounds such as phenols, carboxylic and fatty acids, and aromatic compounds that can be beneficial to plant growth at low concentrations but toxic at higher concentrations (Graber et al., 2010). Karrikins, molecules found in wood smoke with pronounced hormone-like effects at trace levels, have also recently been identified in biochars (Kochanek, Long, Lisle, & Flematti, 2016). We found that biochar leachates did generally have positive effects on seedling development, including enhanced germination, cotyledon development, and particularly radicle extension growth. These effects occurred in both seed species and for both biochars tested. In addition to effects of trace levels of organics, it is possible that biochar leachate effects could arise from release of plant nutrients, the most likely being ionic forms of K, P, Ca, and Mg (e.g., Sackett et al., 2015). The effects of biochar leachates are sufficiently strong that we cannot entirely discount some role of hormetic effects or nutrient release, in addition to allelochemical sorption, in mitigating allelochemical effects.

Although extracts of both strawberry guava and lemongrass had consistently inhibitory effects on germination and cotyledon development, effects on radicle extension were mixed. Maize germination was not

inhibited by lemongrass extracts, and radicle growth was in fact enhanced, even at the higher extract concentration used (Figure 4(a) and (b)). Although not statistically significant (by the conservative post hoc tests used), there was some evidence for a similar effect in radish (Figure 4(c) and (d)). A potential explanation for this response is that allelochemicals produced by lemongrass are themselves hormetic. Catechin, an allelochemical found in the invasive weed *Centaurea maculosa*, was found to enhance plant growth at low dosages but inhibit growth at higher dosages (Prithiviraj, Perry, Badri, & Vivanco, 2007). The allelopathic effects of lemongrass also appear to be more species specific than strawberry guava, with much stronger effects seen in radish than maize (Figure 2). Stronger allelopathic effects affecting a broader range of species may help explain the greater invasive success of strawberry guava in many tropical regions. In contrast, lemongrass invasion has been a pronounced problem only in the Caribbean (Huenneke & Vitousek, 1990; Lorence & Sussman, 1986; Robbins et al., 2008).

Implications for Conservation

We conclude that biochars have promise in combatting allelopathic invasive species in island ecosystems. New tools and approaches to this problem are needed, as allelopathy is suspected to contribute greatly to the success of numerous invasive species in the tropics (Ahmed, Uddin, Khan, Mukul, & Hossain, 2007; Prati & Bossdorf, 2004). Manual removal is commonly impractical, and some allelochemicals are long lived (e.g., Grove, Haubensak, & Parker, 2012); moreover, aboveground tissue removal can enhance production in some allelopathic species (Thelen et al., 2005), emphasizing the importance of measures that reduce the bioavailability of allelochemicals. Field studies are essential to test the efficacy of biochar in mitigating these allelopathic effects, as there are complex interactions between biochars and soils that depend on specific soil characteristics, vegetation, and climate. It seems likely that by suppressing the inhibitory effects of strawberry guava and lemongrass, biochar applications could reduce the competitive advantage of these invasive species over native plants, allowing native plants to better regenerate and reduce dominance by invasives. Important questions arising are what dosages are necessary, and how long such an effect would continue. Some studies have found declines in crop productivity at biochar application rates above 25 t/ha (e.g., Rajkovich et al., 2012; Van Zwieten et al., 2010); 25 t/ha has thus been suggested as a dosage that will result in beneficial responses in most cases (Filiberto & Gaunt, 2013). However, higher rates might be necessary in soils with high concentrations of allelochemicals. Regarding duration, Wardle et al. (1998) observed that biochar maintained sorption properties even a century after a

wildfire, such that ancient chars were still capable of reducing negative effects of phenolics in boreal systems. There is thus the possibility that biochar additions may provide a means for dramatic, long-term amelioration of a widespread ecological disaster.

Declaration of Conflicting Interests

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