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Biochar mitigation of allelopathic effects in three invasive plants: evidence from seed germination trials

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Abstract: Many invasive species show allelopathic effects that contribute to competitive impacts on native vegetation for which few control measures exist. We investigated the potential for pyrolyzed organic material used as a soil amendment (“biochar”) to sorb allelochemicals and mitigate allelopathic effects on seed germination and early seedling development in three common invasive plants in Canada, garlic mustard (*Alliaria petiolata*), tree of heaven (*Ailanthus altissima*) and yellow sweetgrass (*Melilotus officinalis*). We hypothesized that biochars would mitigate effects on germination and early seedling development (radicle extension and cotyledon development) through sorption of allelochemicals. Laboratory assays of seed germination and early seedling development of two agricultural crops (*Lactuca sativa* and *Raphanus raphanistrum*) and two native grass species (*Andropogon gerardi* and *Poa palustris*) were conducted using water extracts from leaves. Seeds were treated with plant extracts exposed to four different biochars (red oak (*Quercus rubra*), jack pine (*Pinus banksiana*), shipping pallet and construction waste, and, high-carbon wood ash) using a range of extract and biochar dosages. Treatment of allelopathic plant extracts by biochars significantly ($p < 0.05$) mitigated effects on seed germination for *Alliaria* and *Melilotus*. Effects on seed

germination and early seedling development depended on extract concentration, biochar dosage, and the target species assessed. Controls treated with biochar leachate alone showed significantly ($p < 0.05$) increased germination for two of the biochars tested (jack pine biochar and high-carbon wood ash). Results indicated that biochars can mitigate allelopathic effects of invasive species through sorption of allelochemicals, however this will be effective in only some cases.

Key words: *Ailanthus altissima*, allelopathy, *Alliaria petiolata*, *Melilotus officianlis*, soil amendment, invasive species, seedling development

Introduction

Allelopathic interactions involve plants producing chemicals that negatively affect the performance of other plants; such interactions are widespread and influence competitive relationships and patterns of plant community composition (Rice 1983).

Allelopathy has long been thought important as a mechanism for plant invasiveness (Hierro and Callaway 2003). Recent data syntheses suggest that allelopathy is present in more than half of invasive plants globally (Kalisz et al. 2021), including such notorious examples as Siam weed (*Chromolaena odoratum*), spotted knapweed (*Centaurea maculosa*), and parthenium (*Parthenium hysterophorus*). There is evidence that the lack of prior exposure of native plants to those that possess traits that are uncommon or absent in the local ecosystem is important in enhancing allelopathic effects of invasive species, providing a theoretical basis for predicting a general pattern of allelopathy abetting invasiveness (Callaway and Ridenour 2004; Cappuccino and Arnason 2006).

Given the widespread importance of allelopathy in interactions between native and non-native plants, control of allelopathic invasive plants represents a global problem with important conservation, management, and policy implications (Zhang et al. 2021).

Options for control measures that directly address allelopathic interactions, however, remain limited. Removal of aboveground biomass by hand, or through the use of herbicides or biocontrol agents can act to increase production of allelochemicals (Siemens et al. 2002; Thelen et al. 2005). Allelochemicals can also persist in the soil for long periods (e.g., Rice 1972; Grove et al. 2012), and are often produced by belowground structures that are difficult to remove (Bais et al. 2003; Bertin et al. 2003).

Biochar, defined as charcoal intended for use as a soil amendment (Lehman and Joseph 2015), has received increasing attention not only for its potential in carbon sequestration and agricultural use, but also in the context of restoration ecology (Beesley et al. 2011; Thomas and Gale 2015). Biochars in general show physical and chemical properties that result in a high capacity to sorb a broad range of organic and inorganic substances (Mohan et al. 2014). Negatively charged surface groups, a high surface area to mass ratio, and high total pore volume are properties that enable sorption of both polar and non-polar molecules by biochars (Smernik 2009). The capacity of biochars to sorb or otherwise immobilize toxic metals has received considerable recent research attention due to its potential to rehabilitate contaminated soils and mine tailings (Beesley et al.

2011; Rees et al. 2014). Likewise, biochars are able to sorb salts (Thomas et al. 2013; Hammer et al. 2015), toxic organic compounds (Hale et al. 2015), and soil-active herbicides and pesticides (Kookana 2010, Graber and Kookana 2015).

Activated carbons have been used in experimental protocols to remove putative allelochemicals from extracts and from the soil solution (Callaway 2003, Lau et al. 2008). Activated carbons differ from biochars as a consequence of activation step(s) that enhance porosity and remove impurities; in addition, commercial activated carbons are usually made from anthracitic coal or nutshells (Downie et al. 2009), while biochars are mostly derived from forestry or agricultural residues. Biochars have recently been used as cost-effective, low-tech substitutes for activated carbon in applications such as water purification (Mohan et al. 2014) and remediation of organic soil and sediment contaminants (Hale et al. 2015). In addition, prior literature from boreal forest responses to biochars has indicated strong sorption of phenolic compounds that contribute to large plant growth responses to biochars (Zackrisson et al. 1996, Wardle et al. 1998, Keech et al. 2005).

The potential for biochar to mitigate allelopathic effects has received limited prior research attention. Elmer and Pignatello (2011) assessed the ability of a fast-pyrolysis biochar to mitigate effects of crop-residue allelopathic chemicals on asparagus (*Asparagus officinalis*), finding evidence that biochar additions reduced effects of additions of cinnamic, coumaric, and ferulic acids on crop performance. Sujeeun and Thomas (2017) documented biochar sorption of allelochemicals produced by two tropical invasive species (*Psidium cattleianum* and *Cymbopogon flexuosa*) using seed germination trials as a bioassay. Similar results were obtained in a study examining the allelopathic tree *Eucalyptus camaldulensis* (Alshahrani and Suansa 2020), and the widespread tropical invasive *Imperata cylindrica* (Shen et al. 2020). There is increasing evidence that biochars sorb a wide range of allelochemicals, including those produced by invasive species. However, biochars show high variability, and typically have lower surface areas and sorptive capacity than does activated carbon (Hale et al. 2015). In addition, allelochemicals vary greatly in terms of polarity, water solubility, molecular size, and other characteristics (Kong et al. 2019). The efficacy of biochars to sorb allelopathic agents may not, therefore, be assumed.

In the present study we test for biochar sorption of allelochemicals produced by garlic mustard (*Alliaria petiolata* (M.Bieb.) Cavara & Grande), tree of heaven (*Ailanthus altissima* (Mill.) Swingle), and yellow sweet clover (*Melilotus officinalis* (L.) Pall.), three important invasive species in Eastern North America for which evidence of allelopathic effects and characterization of allelochemicals involved is relatively well developed. Water-soluble organics and inorganics present on biochars may have either positive or negative effects on germination and early plant performance (Solaiman et al. 2012, Gezahegn et al. 2021); trials therefore included controls to assess and account for these potential confounding effects. We specifically address the following hypotheses: (1) treatment of plant extracts of allelopathic species with biochars will reduce their negative effects on germination and early seedling performance; (2) such responses will predictably vary with the concentration of plant extracts used, and with biochar dosage; (3) biochar leachates themselves will affect seed germination and early seedling performance, but such effects will not offset reductions in allelopathic effects consistent with sorption of allelochemicals.

Materials and methods

Species

Alliaria petiolata (hereafter *Alliaria*), *Ailanthus altissima* (hereafter *Ailanthus*), and *Melilotus officinalis* (hereafter *Melilotus*), common invasive species in Eastern North America, were used to prepare plant tissue extracts. All species have been well characterized in the literature in terms of both chemical composition (Barto and Cipollini 2009; Cipollini and Gruner 2007; Demasi et al. 2019; Zhang et al. 2018) and allelopathic effects (Lawrence et al. 1991; Heisey 1996; Shnute 1984; Blackshaw et al. 2001). *Alliaria* is among the most important invasive species in Eastern North America and tends to form nearly pure stands in forest understories and edge sites, with allelopathy being commonly implicated as an important mechanism for competitive dominance (Prati and Bossdorf 2004, Stinson et al. 2006, Wixted and McGraw 2010). A variety of allelochemicals have been isolated from *Alliaria*, including cyanide, glucosinolates, flavonoid glycosides, and the cyanoallyl glycoside alliarinoside (Barto and Cipollini 2009). *Ailanthus* also shows strong allelopathic effects (Prati and Bossdorf 2004); a set of quassinoid compounds (including ailanthone, ailanthinoine, chaparrine, and ailanthinol B) have been identified as the primary allelochemical agents (De Feo et al. 2003). The active compound

responsible for the well-documented allelopathic effects of *Melilotus* is coumarin (Zhang et al 2018). The significant inhibitory effects of *Melilotus*-derived coumarin on seed germination and seedling growth of several species is so effective that it has promoted investigation of the use of *Melilotus* as a natural herbicide (Wu et al. 2016).

The seed species on which extracts were tested were *Lactuca sativa* L. (hereafter lettuce), *Raphanus sativus* L. (hereafter radish), *Poa palustris* L. (hereafter fowl bluegrass) and *Andropogon gerardi* Vitman (hereafter big bluestem). We chose lettuce and radish as target species as they were readily available, show rapid germination and seedling development, have been used in many prior allelopathy bioassay studies (e.g., McCarthy and Hanson 1998, Pisula and Meiners 2010, Cipollini et al. 2012), represent different plant families, and are generally sensitive to allelochemicals. Fowl bluegrass and big bluestem were utilized as target species in a related field study examining the impacts of *Melilotus* invasion on mine tailings reclamation and were investigated as a part of this experiment in order to understand the mechanisms driving the results of that study. Seeds were obtained from OSC Seeds (Ontario, Canada).

Biochars

Four biochars were used in the experiment, the attributes of which are described as follows and summarized in Table 1. The first biochar (BC1) was generated using a batch pyrolysis system with red oak (*Quercus rubra*) wood chip feedstock harvested and processed at Haliburton Forest and Wild Life Reserve, Ltd., Haliburton, Ontario. The system was run for ~2 h to completion of the pyrolysis reaction; the peak temperature obtained during pyrolysis was ~400°C. The second biochar (BC2) was derived from a mixed feedstock of shipping pallets and construction wastes, pyrolyzed at ~700°C, for ~30 min., and was obtained from Burt's Greenhouses in Odessa, Ontario. BC2 was previously found to reduce bioavailability of PCBs in a laboratory experiment (Deynes et al. 2012). The third biochar (BC3) is charcoal harvested from a wildfire that occurred in a forest dominated by jack pine (*Pinus banksiana*) in northern Ontario, Canada in 2011. Average temperatures at 7-9 m height during flaming for experimental crowning fires in jack pine in this general region range from 486-729°C (mean $613 \pm 90^\circ\text{C}$ SD), with peak temperatures of 760-1202°C (mean $1021 \pm 146^\circ\text{C}$ SD) (de Groot et al. 2003). These values thus give a likely indication of pyrolysis temperatures of the jack pine charcoal utilized in the experiment. The fourth biochar (BC4) is a high-carbon wood ash biochar that was

provided by Wood Ash Industries, a privately-owned wood ash disposal site in Kirkland Lake, Ontario, Canada, that obtains wood ash as a waste product from Kirkland Lake Power, a wood-fired co-generation power plant. This material is considered a biochar under Canadian Food Inspection Agency standards and has been used in previous experimental field trials (Bieser and Thomas 2019). The feedstocks utilized by Kirkland Lake power include mixed wood wastes (sawdust and bark) from local softwood forestry operations of which the dominant species include *Picea glauca* and *Picea mariana*. The wood-fired gasification system operates at a temperature of approximately 1100°C. The biochars were pre-treated by washing with deionized water for 24 hours intermittently on a shaker table at 50 rpm to remove mobile organic compounds that can be produced during pyrolysis and cause negative plant growth responses (Gale, et al. 2016).

Experimental design and growth conditions

Soilless Petri dish bioassays examining seed germination and early seedling development were conducted at the University of Toronto, St. George campus. We used an augmented factorial experimental design (i.e., fully factorial but including additional control treatments). The treatment factors included allelopathic extract type (*Alliaria*,

Ailanthus or *Melilotus*), seed species (lettuce, radish, big bluestem or fowl bluegrass), extract concentration (0, 0.1 or 0.3 g/ml), biochar type (red oak, shipping pallet and construction waste, jack pine charcoal or high-carbon wood ash), and biochar dosage (0, 0.002, or 0.02 g/ml), expressed as mass of biochar per liquid added). Biochar dosages used correspond to 0%, 0.5% and 5% by mass (assuming 40% gravimetric soil moisture content for a loam soil at field capacity), which are within the range of char additions found to be effective in remediating organic contaminants in soil (Hale et al. 2015). Controls for the experiment consisted of pure deionized water, leachate from biochar washed with deionized water using the same methods as for leaf extracts, and pure untreated leaf extracts. These experimental protocols closely follow those of a prior study examining biochar sorption of allelochemical in two tropical invasive species (Sujeun and Thomas 2017).

Leaf Extracts

Alliaria plants were obtained from Civic Park in Scarborough, Ontario, and the University of Toronto, St. George campus in July 2015. *Ailanthus* samples were obtained

from the University of Toronto, St. George campus in July 2015. *Melilotus* samples were obtained from Tommy Thompson Park, Toronto, Ontario, in October 2016. After the leaves were collected, they were stored at 2.0°C until they were used for the germination trials the following day. *Melilotus*, *Ailanthus* and *Allairia* leaf extracts were prepared by drying leaves for 24 h and then shredding them into 1 cm by 1 cm pieces. The shredded leaves were then soaked in distilled water for 48 hours to create a 0.3 g ml⁻¹ concentration (fresh weight basis). A portion of this extract was then diluted with deionized water to 0.1 g ml⁻¹. After 48 h, the leaf extract solutions were suction filtered using Whatman #4 filter paper and the filtrate collected. Both low and high concentrations (0.1 and 0.3 g ml⁻¹) used were similar to previous studies (Cipollini et al. 2012; Sujeeun and Thomas 2017).

Experiment

Germination tests were conducted in 100-mm diameter covered Petri dishes, with 25 seeds/dish, with 5 replicates for each treatment by species combination. The appropriate treatment solution was allowed to mix with biochar with intermittent shaking on a shaker table at 50 rpm for 24 hours for all biochar treatments. The filtrate was collected and applied as the treatment solution on the Petri dishes. All treatments received 5 ml of

solution per Petri dish on Whatman grade #1 filter paper and were placed under a 12-hour day/night cycle under incandescent light bulbs at ~25°C day and ~20°C night temperatures. The overall experiment was run in four sequential batches, the first consisting of only the *Melilotus* extract and biochar dosages at 0 g/ml (untreated control), 0.002 g/ml, and 0.02 g/ml, the second consisting of only the *Alliaria* extract and biochar dosages at 0 g/ml (untreated control), 0.002 g/ml, and 0.02 g/ml, the third consisting of the *Ailanthus* extract at all dosages, and the fourth repeating the lettuce germination with *Ailanthus* extract at all dosages. Several treatments from the second experimental run were omitted and re-run in the third experiment due to an apparent error in labeling.

We conducted each experiment batch for 7 days. The proportion of seeds germinated, measured as the emergence of the radicle from the seed coat, was recorded daily. Radicle extension was measured on the third day (to the nearest 1 mm) for a subset of 3 germinated seedlings per Petri dish. The proportion of seeds producing cotyledons was recorded on the seventh day.

Statistical analysis

ANOVA was used to analyze treatment effects on germination rate, radicle extension and proportion of seedlings developing cotyledons, with proportions or means calculated by replicate Petri dish prior to analysis. A 2-way ANOVA was run in each case based on the subset of data that was fully factorial (i.e., the 2 x 3 factorial combination of two extract treatments by 3 biochar treatments (none, low, and high dosages)). A second (1-way) ANOVA was then performed including the distilled water and biochar leachate controls, with multiple comparisons presented based on this ANOVA using the Tukey HSD procedure. In the case of proportion data (germination and cotyledon development) we transformed variates with a logit function (Warton and Hui 2011). The function was modified to handle zero and one 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 $\frac{1}{2}$ of the smallest possible value of y in the dataset ($= 0.02$) (Martín-Fernández and Thió-Henestrosa 2006).

Analyses for the four target seed species used were treated separately. Agreement of model residuals with parametric statistical assumptions of normality and equal variance were assessed using graphical diagnostic plots. Agreement with assumptions was deemed good in most cases, except where there was complete or nearly complete suppression of

seedling development. Global p-values should be interpreted conservatively in these cases (but are generally < 0.001). Data analysis was conducted in R (version 1.1.456), specifically making use of the `lm()` and `TukeyHSD()` functions.

Results

Alliaria

Lettuce germination showed significant effects of *Alliaria* extracts, biochars, and their interaction (Table 2). Germination rates for lettuce were not suppressed at the low extract dosage (0.1 g/ml) but were significantly suppressed at the high dosage (0.3 g/ml) (Fig. 1 ab). At this high dosage biochar treatments resulted in a germination rate which was not distinguishable from controls; this was the case for both biochars tested (BC1 and BC2) at both low and high dosages. Radish germination showed a similar pattern, though germination rates in biochar-addition treatments remained slightly lower than controls (Fig. 1 cd). Radish germination showed significant effects of extract, biochar, and the extract x biochar interaction for both biochars (Table 2).

Cotyledon development showed little response to the low extract dosage but was significantly suppressed by high dosages of *Alliaria* extracts in both lettuce and radish seedlings (Table S1; Fig. S1). Biochar did not have a consistent positive effect on cotyledon development: in lettuce seedlings there was even a significant decline in cotyledon development at the highest dosage of BC1 relative to controls (Fig. S1a). However, among the high extract dosage treatments (0.3 g/ml) in three of four cases high biochar dosages showed higher rates of cotyledon development than the no biochar or low biochar treatments (Fig. S1). These patterns are reflected in significant ANOVA terms for extract effects in most cases (Table S1).

Radicle development responses showed dramatic inhibition responses to *Alliaria* extracts with no significant positive effect of biochar treatments (Fig. S4). Biochar leachates alone did not significantly affect germination, cotyledon formation, or radicle extension in the *Alliaria* experiments (Fig. 1 and Figs. S1 and S4).

Ailanthus

Lettuce and radish germination showed significant negative effects of *Ailanthus* extracts (Table 2). Germination rates for lettuce (Fig. 2 ab) and radish (Fig. 2 cd) were substantially suppressed at both the low (0.1 g/ml) and high (0.3 g/ml) extract dosages, and in no case did biochar treatments alleviate this effect.

Cotyledon development showed a significant negative response to the low extract dosage compared to the controls and was strongly suppressed by high dosages of *Ailanthus* extracts in lettuce seedlings (Table S2; Fig. S2 ab). As with the germination response, biochar did not have a consistent positive effect on cotyledon development (Fig. S2 b). Cotyledon development was strongly suppressed at both dosages of *Ailanthus* extracts in radish seedlings, resulting in a complete lack of cotyledon development in most cases (Table S2; Fig. S2 cd). Biochar did not have an effect on cotyledon development of radish seedlings other than a significant increase in cotyledon development for low doses of BC1 and BC2 at the low extract dose (0.1 g/ml) (Fig. S2 b). These patterns are reflected in significant ANOVA terms for extract effects in most cases (Table S2).

Ailanthus extracts likewise significantly reduced radicle development in both lettuce and radish, resulting in nearly complete inhibition (Fig. S5). Biochar did not have a consistent positive effect on cotyledon development (Fig. S5), though the ANOVAs indicated significant effects of biochars, extracts, and biochar and extract interaction, for both BC1 and BC2 (Table S2). Biochar leachates alone did not significantly affect germination, cotyledon formation, or radicle extension in the *Ailanthus* experiments (Fig. 2 and Figs. S2 and S5).

Melilotus

Lettuce germination showed significant responses to *Melilotus* extracts (Table 2). Germination for lettuce was completely suppressed at both the low extract dosage (0.1 g/ml) and high dosage (0.3 g/ml) (Fig. 3 ab) with no significant impacts of biochars (BC3 and BC4) (Table 2). Radish germination was likewise significantly suppressed at both the low extract dosages for both biochars (BC3 and B4) (Fig. 3 cd). At the low (0.1 g/ml) extract dosage, the BC3 treatment resulted in a significant negative effect on radish germination compared to the control indicating a potential interaction (Fig. 3 c). At the low (0.1 g/ml) extract dosage, the BC4 treatment resulted in a significant positive effect

on radish germination compared to the control also indicating a potential interaction (Fig. 3 d). Big bluestem germination showed significant effects of *Melilotus* extracts, biochars, and their interaction (Table 2). Germination rates for big bluestem were significantly suppressed at the low extract dosage (0.1 g/ml) and the high dosage (0.3 g/ml) (Fig. 3 ef).

At the low extract dosage, both low and high doses of BC4 treatment resulted in germination rates which were not distinguishable from controls. BC4 leachate also had a significant positive effect on big bluestem germination compared to the control.

Germination rates for fowl bluegrass were significantly suppressed at both the low extract dosage (0.1 g/ml) and high dosage (0.3 g/ml) (Fig. 3 ab) with no significant impacts of biochars (BC3 and BC4) except for the positive effect of the BC4 leachate which significantly increased fowl bluegrass germination compared to the control (Fig 3 h) (Table 2).

Cotyledon development was significantly suppressed by both low and high dosages of *Melilotus* extracts in lettuce seedlings though cotyledon development was significantly enhanced by both BC3 and BC4 leachates compared to the deionized water controls (Table S3; Fig. S3 ab). Cotyledon development was likewise significantly suppressed by

high (0.3 g/ml) dosages of *Melilotus* extracts in radish seedlings. BC4 treatment of radish seedlings treated with low (0.1 g/ml) extract dosages resulted in a germination rate that was not distinguishable from controls (Fig. S3 d). Treatment with BC4 leachate resulted in significantly higher cotyledon development compared to the deionized water control (Fig. S3 d). Cotyledon development was completely suppressed by high (0.3 g/ml) dosages of *Melilotus* extracts in big bluestem seedlings. BC4 treatment of big bluestem seedlings treated with low (0.1 g/ml) extract dosages resulted in a germination rate not distinguishable from controls (Fig. S3 f). BC3 treatment did not have the same effect at the low (0.1 g/ml) extract dosage. Cotyledon development rates for fowl bluegrass were completely suppressed at both the low extract dosage (0.1 g/ml) and high dosage (0.3 g/ml) (Fig. S3 gh) with no significant impacts of biochars (BC3 and BC4) except for the positive effect of the BC4 leachate which significantly increased fowl bluegrass cotyledon development compared to the deionized water control (Fig. S3 h and Table S3). Radicle development responses likewise showed significant inhibition responses to *Melilotus* extracts on lettuce, radish, big bluestem and fowl bluegrass seedlings (Fig. S6).

The BC4 leachate treatment resulted in significantly greater radical extension of radish seedlings compared to the deionized water control.

Discussion

Our results support the general hypothesis that wood-derived biochars can sorb allelochemicals produced by invasive plant species and alleviate allelopathic effects on early plant development; however, such effects varied considerably among allelochemicals and biochars. Leaf extracts of *Alliaria*, *Ailanthus*, and *Melilotus* suppressed germination and seedling development, consistent with prior studies demonstrating allelopathic effects in these species (Heisey 1990ab, Prati and Bossdorf 2004, Wu et al. 2016). In some cases, but not others, these effects were partially or completely alleviated when leaf extracts of allelopathic species were exposed to biochar.

These results are broadly consistent with two prior studies that have used similar methods to assess biochar sorption of allelochemicals. Two agricultural feedstock biochars were found to be effective in alleviating allelochemical effects of *Psidium cattleianum* and *Cymbopogon flexuosa*, plant species that are locally important invasives in tropical island ecosystems (Sujeen and Thomas 2017). However, as in the present

study, complete “rescue” effects (where biochar completely alleviated effects of allelochemicals) were found only in some combinations of allelochemicals, biochars, allelopathic species, and target plant species. Alleviation of allelochemical effects was similarly found in a study of *Eucalyptus camaldulensis* leaf extracts (Alshahrani and Suansa 2020). These studies also all document pronounced differences in responses dependent on seedling performance metrics examined, with radicle extension growth commonly being a more sensitive indicator of allelopathic effects than germination or other responses.

Biochar leachates can also have direct effects on seed germination and early seedling development, either positive (e.g., Sujeeun and Thomas 2017), or negative (e.g., Buss and Mašek 2014). These are important to consider in the context of the present experiment since positive leachate effects might be confounded with sorption of allelochemicals; conversely negative leachate effects might mask sorption. Positive direct effects of leachates were found for one of the biochars tested (BC4), though there were also weak (non-significant) apparent effects in other cases. However, these cases were mostly not associated with observed “rescue” effects consistent with high biochar sorption of

allelochemicals. The single exception was *Melilotus* extract effects on big bluestem with BC4 (Fig. 7f) where a strong direct positive effect of biochar leachate on germination, rather than biochar sorption of allelochemicals, may explain observed patterns. Positive effects of biochar leachates may be due to seed “priming” by specific ions or direct pH effects (Gezahegn et al. 2021; Thomas 2021). We did not observe any cases of significant negative effects of biochar leachates on seed germination or early seedling development, which may occur due to condensed water-soluble organic molecules, in particular wood vinegar components such as acetic acid, benzoic acid, and phenol, that are generated during pyrolysis and sorbed by chars (Buss and Mašek 2014, Koltowski and Oleszczuk 2015, Gale et al. 2016, Ruzickova et al. 2021). The absence of negative direct effects of biochar leachates is consistent with the observations that phytotoxic condensed organic compounds are not common in slow pyrolysis biochars (Gezahegn et al. 2021).

We did not conduct direct chemical analyses on biochar sorption of specific allelochemicals; however, prior studies offer some insights into the specific chemistry likely involved. Secondary chemicals produced by *Alliaria* and implicated in allelopathic effects include phytotoxic hydrolysis products of glucosinolates, in particular allyl

isothiocyanate and benzyl isothiocyanate (Vaughn and Berhow 1999), though other unidentified compounds are almost certainly involved (Barto and Cipollini 2009). The main allelochemicals produced by *Ailanthus* are much larger multiple-ring secondary compounds (including aianthone, aianthinoine, chaparrine, and aianthinol B: De Feo et al. 2003). Coumarins (1, 2-benzopyrones), a group of lactone derivatives of cinnamic acid with a heterocyclic two-ring structure, are responsible for the allelopathic effects of *Melilotus* (Niro et al. 2016). It is interesting to note that biochar sorption appeared to be less effective in the case of the larger molecular weight allelochemicals, such as those produced by *Ailanthus*. This is consistent with prior observations of steric effects that inhibit penetration of large molecules into the porous structure of biochars, which have been documented in sorption studies of herbicides (Xiao and Pignatello 2015).

Activated carbon has a long history of use as a means of removing putative allelochemicals from aqueous solutions in both the lab (Schreiner and Reed 1907, Lau et al. 2008), and in field experiments (e.g., Cipollini et al. 2008). The key properties of both commercial activated carbons and biochars involved in sorption of organic molecules are essentially identical: high surface area and total pore volume, heterogeneous pore

structure, and negatively charged surface groups (Chia et al. 2015, Hale et al. 2015).

Specific surface area (SSA) of activated carbons (as determined by gas sorption) is generally in the range of 500-2000 m²/g; biochars are more variable, but commonly show SSA values up to ~600 m²/g (Chia et al. 2015). Advantages to biochars for applied use in the context of allelopathic invasive species include lower costs and the potential for on-site pyrolysis, possibly making use of invasive species biomass (Sujeen and Thomas 2017).

Allelopathic interactions are challenging to characterize in general, in that a large number of potential interacting allelochemicals with species-specific effects can be involved (Zhang et al. 2021). Indirect effects such as those mediated by soil microflora and mycorrhizae may be common (Stinson et al. 2006), including either microbial degradation of allelochemicals and “indirect allelopathy” where microbial metabolic products show increased allelopathic effects (Romeo 2000; Scavo et al. 2019).

Accordingly, it has been emphasized that laboratory studies of allelopathic effects can only give a qualitative idea of possible interactions, and that field experiments are essential (Inderjit and Callaway 2003). The same points would seem to pertain to any

study aimed at mitigation of allelopathic effects. The bioassay results reported here, in conjunction with prior studies, suggest that biochars may be broadly applicable to the mitigation of allelopathic effects of invasive species, in that many combinations of allelochemicals, biochars, and target species indicated at least some amelioration of allelopathic effects (Zhang et al. 2021). However, biochars show variable effects even in in-vitro assays, and field trials involving soil applications of biochar are necessary to test this approach.

Conclusion

Our results confirmed our hypothesis that biochars can sorb allelopathic compounds indicating that biochar application could be an effective form of invasive species management. While the results of this study are promising, the varied responses of both allelopathic extract and seedling species and biochars indicate a need for additional greenhouse and field trials in order to optimize biochar treatments based on the specific field conditions in which they are ultimately intended. The long-term allelochemical sorption potential of biochars and the potential fate of the allelochemicals in the environment following biochar applications should also be investigated.

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Competing interest statement

The authors declare there are no competing interests.

Contributors' statement

Conceptualization, J.M.H.B. and S.C.T.; methodology, S.C.T.; formal analysis, J.M.H.B.; investigation, J.M.H.B., M.A., J.M.; data curation J.M.H.B., M.A., J.M.; writing—original draft preparation, J.M.H.B. and S.C.T.; writing—review and editing, J.M.H.B. and S.C.T.; supervision, S.C.T.; funding acquisition, S.C.T. All authors have read and agreed to the published version of the manuscript.

Data availability statement

Data are available in the Dryad Digital Repository (DOI will be supplied on acceptance).

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Table 1: Properties of red oak biochar (BC1), shipping pallet and construction waste biochar (BC2), jack pine charcoal biochar (BC3) and high-carbon wood ash (BC4) used in experiments. Analyses for BC3 and BC4 were conducted in triplicate; values for these are listed $\pm$ standard error (SE). “NA” indicates values not available.

Attribute	BC1	BC2	BC3	BC4
Feedstock	Red Oak	Mixed	Jack Pine	Mixed
Peak Pyrolysis Temperature	400°C	700°C	486-729°C	1100°C
Ash (%)	8	20	11.44 $\pm$ 3.32	78.3 $\pm$ 0.46
Moisture (%)	10	2.5	48.03 $\pm$ 2.31	5.56 $\pm$ 0.63
Volatile matter (%)	NA	14.3	19.77 $\pm$ 0.42	6.07 $\pm$ 0.10
Electrical Conductivity (μ S)	NA	NA	44.9 $\pm$ 0.37	231.4 $\pm$ 0.4
pH	7.8	9.0	6.54 $\pm$ 0.11	8.6 $\pm$ 0.01

Carbon (%)	NA	63.2	65.45 ± 1.29	22.0 ± 0.3
Nitrogen (%)	NA	0.3	0.43 ± 0.01	0.25 ± 0.01

Table 2: P values for linear models describing effects of leaf extracts (E) and biochars (B) and their interaction (E x B) on percent germination, cotyledon development, and radicle extension growth, in lettuce and radish treated with *Alliaria* and *Ailanthus* extracts and in lettuce, radish, fowl bluegrass, and big bluestem treated with *Melilotus* extracts corresponding to data shown in Figures 1 – 3.

Seed Species	Trait	Char	Figure	ANOVA terms		
<i>Alliaria</i> extracts				E	B	ExB
Lettuce	germination	BC1	1a	<0.001	<0.001	<0.001
Lettuce	germination	BC2	1b	<0.001	0.011	<0.018
Radish	germination	BC1	1c	<0.001	<0.001	<0.001
Radish	germination	BC2	1d	<0.001	0.002	0.002
<i>Ailanthus</i> extracts						
Lettuce	germination	BC1	2a	<0.001	0.393	0.597
Lettuce	germination	BC2	2b	<0.001	0.662	0.631

Radish	germination	BC1	2c	<0.001	0.689	0.457
Radish	germination	BC2	2d	<0.001	0.995	0.450
<i>Melilotus</i> extracts						
Lettuce	germination	BC3	3a	0.201	0.537	0.537
Lettuce	germination	BC4	3b	0.323	0.382	0.382
Radish	germination	BC3	3c	<0.001	0.009	0.002
Radish	germination	BC4	3d	<0.001	0.086	0.085
Big Bluestem	germination	BC3	3e	<0.001	0.013	<0.001
Big Bluestem	germination	BC4	3f	<0.001	0.027	0.020
Fowl Bluegrass	germination	BC3	3g	NA	NA	NA
Fowl Bluegrass	germination	BC4	3h	<0.001	<0.001	0.994

Figure legends:

Fig. 1. Percent germination after 7 days in seeds of two agricultural species exposed to *Alliaria* leaf extracts and biochar treatments. Distilled water (dashed line) and biochar leachate controls are compared with a factorial combination of two concentrations of *Alliaria* extract (0.1 and 0.3 g/ml), and three biochar treatments. Means are plotted ± 1 SE; differences significant at $p < .05$ (Tukey HSD post hoc tests) are indicated by different letters. (a) *Alliaria* extract, BC1: lettuce, (b) *Alliaria* extract, BC2: lettuce, (c) *Alliaria* extract, BC1: radish, (d) *Alliaria* extract, BC2: radish

Fig. 2. Percent germination after 7 days in seeds of two agricultural species exposed to *Ailanthus* leaf extracts and biochar treatments. Distilled water (dashed line) and biochar leachate controls are compared with a factorial combination of two concentrations of *Ailanthus* extract (0.1 and 0.3 g/ml), and three biochar treatments. Means are plotted ± 1 SE; differences significant at $p < .05$ (Tukey HSD post hoc tests) are indicated by different letters. (a) *Ailanthus* extract, BC1:

lettuce, (b) *Ailanthus* extract, BC2: lettuce, (c) *Ailanthus* extract, BC1: radish, (d)

Ailanthus extract, BC2: radish

Fig. 3. Percent germination after 7 days in seeds of two agricultural species and two grass

species exposed to *Melilotus* leaf extracts and biochar treatments. Distilled water

(dashed line) and biochar leachate controls are compared with a factorial

combination of two concentrations of *Melilotus* extract (0.1 and 0.3 g/ml), and

three biochar treatments. Means are plotted ± 1 SE; differences significant

at $p < .05$ (Tukey HSD post hoc tests) are indicated by different letters.

(a) *Melilotus* extract, BC1: lettuce, (b) *Melilotus* extract, BC2: lettuce,

(c) *Melilotus* extract, BC1: radish, (d) *Melilotus* extract, BC2: radish,

(e) *Melilotus* extract, BC1: big bluestem, (f) *Melilotus* extract, BC2: big bluestem,

(g) *Melilotus* extract, BC1: fowl bluegrass, (h) *Melilotus* extract, BC2: fowl

bluegrass

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