Allelopathic potential of invasive species is determined by plant and soil community context

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Abstract The search for mechanisms to explain the competitive dominance of invasive species has generated much interest in testing for allelopathy as a major mechanism of plant invasion. The Novel Weapons Hypothesis postulates that invasive plants disproportionally impact native plants by producing novel allelochemicals. We studied the allelopathic potential of three invasive and three native species on twelve co-occurring plant species in the Eastern Deciduous Forest of Indiana, USA, using foliar leachate and soil with an intact microbial community. Our bioassay was a full factorial test of two soil treatments and six foliar leachates on the germination and growth of 12 species ($2 \times 6 \times 12$ full factorial). The strength of allelopathic impacts were context dependent, with significant 3-way and 2-way interactions between leachate species, target species, and soil microbial communities (live vs. sterilized). Allelopathic potential was different between life stages of the target species. *Ligustrum vulgare*'s and *Lonicera maackii*'s impacts on some native species supported the Novel Weapons Hypothesis; however, the invasives as a group did not significantly affect growth and had a weaker effect on germination than the effect of certain invasive species individually. For example, native *Cercis canadensis* reduced germination and growth in some conditions. Our results in the live soil treatment indicate that some natives, such as *Elymus hystrix*, should be resistant to *Lonicera maackii* and *Ligustrum vulgare*, but these shrubs' allelopathy could contribute to the decline of susceptible native species.

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Introduction

Allelopathy occurs when a plant releases allelochemicals that inhibit the germination, growth, or fecundity of neighboring competitor species (Rice 1984; Inderjit 2003). In terrestrial systems, these chemicals generally contact soil and soil microorganisms before interacting with a neighboring plant's roots (Inderjit and Rajeswari 2010). The Novel Weapons Hypothesis proposes that non-native plants are particularly allelopathic against native competitors because natives have never been exposed to their novel phytotoxins

(e.g., Callaway et al. 2008). These allelochemicals give the invader a competitive advantage, allowing them to dominate their invaded range (Svensson et al. 2013). However, some native plants produce allelochemicals that could impact the non-native. Also, soil biotic and abiotic properties can change the allelopathic potential of a chemical.

The physical properties of soil (e.g., absorption) can alter allelopathic effects (Tharayil et al. 2006; Pollock et al. 2009). Some studies have found that the allelopathic potential of allelochemicals is uniformly weakened when the target plant is grown in sterile soil (e.g., Krogmeier and Bremner 1989). In other studies, the presence of soil has unpredictable effects on allelopathic potential (Kaur et al. 2009; Zhu et al. 2011). This could be due to the many different factors in soil that can alter allelochemicals (e.g., the concentration of calcium and other metals—Pollock et al. 2009).

The soil microbial community can significantly change the impacts of potential allelochemicals (e.g., Krogmeier and Bremner 1989; Wardle et al. 1998; Cipollini et al. 2012). For example, soil microbes in a plant's new range can reduce allelopathic potential by degrading the allelochemical, reducing its concentration in the soil (Kaur et al. 2009; Zhu et al. 2011). Also, the allelopathic impacts of an invasive plant can depend on the susceptibility of the soil microbes to its allelochemicals: the allelopathic effects of garlic mustard (*Alliaria petiolata*) are significantly higher in its invasive range compared to its native range because the mycorrhizae in garlic mustard's native range have adapted to its allelochemicals, reducing its allelopathic potential on both the mycorrhizae and their plant symbionts (Callaway et al. 2008). Furthermore, the effect of soil microbes on allelopathic potential can depend on the identity of the target species being tested (Bauer et al. 2012). For example, lettuce (*Lactuca* sp.) is particularly sensitive to allelochemicals, so lettuce seed germination will indicate allelopathic potential more often than other species (Kaur et al. 2009). Determining whether soil microbes consistently decrease allelopathic potential will help determine how far allelopathy bioassay results can be extrapolated.

Studies have argued that allelopathy bioassays fail to identify allelopathic effects that would actually occur in nature and would be strong enough to have ecological consequences (e.g., Williamson 1990; Goodall et al. 2010). A few studies have tested whether a species produces allelopathic effects in the field, against its natural competitor species in terrestrial systems (e.g., Stowe 1979). However, scientists often choose to do simpler studies in sterile substrate with limited target species because they are far easier and more efficient. The results from simplified methods are only useful if the researchers follow up a simple preliminary experiment with one in the field (e.g., Jäderlund et al. 1996, 1997). We show that it is possible to conduct a study in the lab that takes into account several major factors that affect allelopathic potential in nature (i.e., the effects of abiotic soil factors, soil microbes, and variation between species). This allows researchers to get ecologically relevant results from a lab-based study.

We used a soil substrate with and without a live forest soil microbial community to test the impact of soil microbes and microfauna on allelopathic potential. We compared the effects of foliar leachates from three invasive species to foliar leachates from three dominant native species. This allowed us to test whether allelopathy could reduce native seedling establishment, contributing to the reduced native plant diversity found in invaded sites (Gould and Gorchov 2000; Merriam and Feil 2002). Our study also incorporated multiple life stages: seed germination and seedling growth. Our objectives were to determine

(1) Are the invasive species more allelopathic than native species with a similar abundance, growth form, habitat, and phenology?
(2) Do soil microbes and microfauna significantly change allelopathic effects of the foliar leachate?
(3) Are species-specific allelopathic effects consistent across a variety of target species?

Methods

We tested the effects of leachates from the leaves of the six woody species on the germination and growth of twelve co-occurring forest species. Our tests were conducted in soil in the presence or absence of a soil microbial community, resulting in a two-way, full factorial design for each of the 12 target species (6 leachate species × 2 microbial treatments × 10 replicates, $n = 1440$). We first tested how the treatments affected seed germination in Petri plates. We then transplanted the seedlings to sterile 49-mL pots

and continued the treatments to measure their effect on seedling growth. All tools, work surfaces, and pots were sterilized with 0.6 % sodium hypochlorite.

Target species

We used species and soil from an Eastern Deciduous Forest habitat to test our hypothesis that allelopathy contributes to the reduced native seedling recruitment in invaded areas (reduced native seedling recruitment was observed in Hutchinson and Vankat 1997; Gould and Gorchov 2000). Three dominant exotic invasive species in this habitat are the shrubs *Lonicera maackii* (Rupr.) Maxim, *Ligustrum vulgare* (L.), and *Elaeagnus umbellata* (Thunb.) (Since two of these species are easily confused when abbreviated normally as L. species, we will use genus names hereafter.). *Lonicera*, from Southeast Asia (Hutchinson and Vankat 1997), is considered allelopathic (Dorning and Cipollini 2006; Cipollini and Dorning 2008). Less is known about *Ligustrum* and *Elaeagnus* (from Europe and Southeast Asia, respectively; Miller 2003). *Elaeagnus* leaf extracts have been shown to inhibit tree germination and tree seedling growth (Orr et al. 2005), and some studies of *Ligustrum* show results consistent with the presence of allelochemicals (e.g., Morris et al. 2002; McEwan et al. 2009). We compared foliar leachates from these three species to those from three co-occurring common native woody understory species: *Cercis canadensis* (L.), *Acer saccharum* (Marshall), and *Lindera benzoin* (L.) Blume. None of these species have been tested for allelopathic potential. These natives were chosen because all six species (three natives and three exotic invasives) have a similar growth habit (*Acer* is common as a short understory tree as well as a canopy tree at this site), habitat, and phenology.

Our target species included the six woody species described above and six native herbaceous species that co-occur in the Eastern Deciduous Forest in the Indiana University Research and Teaching Preserve. However, *Ligustrum* had insufficient germination, despite high germination in pilot studies, so it was dropped as a target species from the study, resulting in eleven target species ($n = 1320$). The six herbaceous species included two grasses (*Elymus hystrix* L. and *Bromus purgans* L.), one sedge (Carex frankii Kunth), and three forbs (*Penstemon calycosus* Small, *Eupatorium rugosum* Houtt., and *Blephilia hirsuta* (Pursh)

Benth.). Some of the herbaceous species (particularly *Penstemon*) appear to occur only in uninvaded areas, but *Elymus* and *Eupatorium* are also common in invaded areas at the IURTP (personal observation). We collected the seeds of *Lindera*, *Cercis*, *Ligustrum*, *Elaeagnus*, *Lonicera*, and *Elymus* from the IURTP and purchased seeds of *Bromus*, *Carex*, *Penstemon*, *Eupatorium*, *Blephilia*, and *Acer* from commercial midwestern collections (Spence Restoration Nursery in Muncie, IN, USA; Sheffield's Seed Company in Locke, NY, USA).

Leachates

An emerging seedling is likely to experience two types of leachate as it grows. During germination in the spring, chemicals are released from leaf litter frozen over the winter. To mimic this, we collected senescing leaves during October and November of 2010 and stored them at −20 °C. In May of 2011, we soaked the leaves in 15 mL of water per gram of leaf for 72 h at room temperature to mimic the saturation of leaf litter with early spring rain. We then vacuum-filtered each foliar leachate through Whatman® 1 filter paper (11 μm pore size) to remove the majority of fungal spores and microbes bound to leaf particles. Foliar leachates were stored in sealed containers at 4 °C until use. After the spring thaw finishes, the major source of allelochemicals would be leachate from live leaves and roots. This is because chemical compounds from leaf litter do not remain in the seedlings' rhizosphere for long. Some allelochemicals break down within 24 h (Blair et al. 2005; Zhu et al. 2011), and water-soluble compounds can be washed from the surface soil. To mimic this situation, we created a second type of leachate for the growth portion of the experiment. We collected fresh leaves in June of 2011 and soaked them in 5 mL of water per gram of leaf for 72 h at room temperature, without freezing. This protocol produces a foliar leachate with similar allelopathic effects to root leachate (Dorning and Cipollini 2006). These foliar leachates were also vacuum-filtered and stored at 4 °C until use. All leaves were collected from the IURTP, which contains both invaded and uninvaded areas in a maple-beech habitat.

Some studies compare the effects of plant leachate to water (e.g., Nilsson and Zackrisson 1992; Cipollini et al. 2008b; Pisula and Meiners 2010). However, ecologically, it is more appropriated to compare the

effects of invasive plant leachate to the effects of leachate from dominant native species. Using water as a control in allelopathy bioassays increases the odds of finding allelopathic potential because water has a different osmotic potential than leachate, making it easier for seeds to imbibe the water control (Anderson and Loucks 1966; Haugland and Brandsaeter 1996). Also, the ecological implications of being more allelopathic than water are unclear. Plants are not generally exposed to pure water in nature; they are exposed to water-carrying leachates and exudates from neighboring species. Using native species' leachates for comparison, we tested whether the allelopathic potential of the invasive leachates could be responsible for the reduced native diversity seen in invaded sites compared to uninvaded sites. Therefore, we used native plant leachates, not water, as the control treatments to determine the relative impact of invasive plant leachate.

Soil treatments

Our experiment included a live soil treatment and a sterilized soil treatment. We used Crider silt-loam topsoil from an old-field site one mile south of the IURTP as background soil for this experiment. The background soil was sifted through a 2-cm sieve, mixed 1:1 with sand, and sterilized twice at 121 °C (2 h each time, following the protocol used by Bever 1994; Bever 2002; Shannon et al. 2012). Mixing the soil with sand prevented it from solidifying during the sterilization process.

The live soil treatment was identical to the sterilized treatment except for the addition, after sterilization, of a microbial wash modeled after a wash used in previous studies to add bacteria to the soil (Koide and Li 1989; Larimer et al. 2012). We collected the top 10 cm of soil from an uninvaded, relatively bare site in the IURTP (only mature *Acer* was present). To include a larger variety of soil microbes, we used five arbitrary collection points separated from each other and any invasive plant by at least 5 m. We mixed the soil with 2 L of water per liter of soil, then filtered it through a 250-um sieve to create our microbial wash. The 250-um aperture of the sieve allowed soil bacteria, fungal spores, and microfauna into the solution but no plant matter. This microbial wash was immediately applied to the appropriate Petri plates (germination portion of the study) or pots (seedling growth portion

of the study). To use fresh microbial wash each time, we collected soil from this site eight times between April and October of 2011. Pots and Petri plates in the sterilized soil treatment received distilled water instead of microbial wash.

To preserve their chemical compounds, foliar leachates were not sterilized, so the sterilized soil treatment was not sterile. Thus, all treatments in the project contained a foliage-derived microbial community; however, the sterilized soil treatment contained only foliar microbes, not microbes adapted to the soil.

Germination experiment

This experiment consisted of six leachates and two soil treatments in a 6 × 2 full factorial design for each of 11 target seed species (*Ligustrum* had insufficient germination). Ten replicates per treatment combination resulted in 1,320 Petri plates. Each plate contained 50 mL sterile background soil and 20 seeds (except for *Elymus*, which had ten seeds per plate due to insufficient seed availability). We then added 10 mL leachate and either 10 mL microbial wash (live soil treatment) or 10 mL water (sterilized soil treatment). This produced 120 Petri plates per target species, from six leachates crossed with two soil treatments. All plates were cold/moist stratified in the dark at 4 °C for 30 days (herbaceous seeds) or 90 days (woody seeds) based upon previous germination trials. Due to space constraints, the herbaceous and woody species were tested separately. After stratification, each replicate was placed on a fluorescently lit shelf with plates independently randomized on each shelf. We counted germination 4, 8, 16, and 32 days after initial exposure to light and removed germinated seedlings.

We analyzed the germination results using a negative-binomial generalized linear model, due to the distribution of the data ('glm.nb' in package 'MASS' ver. 7.3-22 in R ver. 2.15.2, as a type 3 test in package 'car' R Core Team 2012). The response variable was a count of germinated seeds in a Petri plate. The predictor variables were two levels for soil (live vs. sterilized), six different leachate species and the 11 target species. The model included all interactions of these three factors. To address the specific biological comparisons of most interest, we used four orthogonal contrasts within this model. The contrasts were all invasive leachates compared to all native

leachates, then each invasive leachate compared to the group of all native leachates.

Growth experiment

Allelopathic impacts to germination do not necessarily predict the impacts to seedling establishment and growth (Stowe 1979; Orr et al. 2005; Goodall et al. 2010); therefore, it was important for us to test the impact of the leachates on seedling growth as well.

We washed, weighed, and transplanted one seedling from each Petri plate into sterile background soil in a 49-mL pot (Ray Leach RLC3 Cone-tainer pot sterilized with 0.6 % sodium hypochlorite). Abnormal seedlings, i.e., without roots, were not used in the growth experiment. Seedlings were grown in a greenhouse under natural light and watered twice daily with tap water. Seedlings from the live soil treatment received 10 mL fresh microbial wash 48 h after transplant. Seedlings from the sterilized soil treatment received only tap water. In the greenhouse, pot order was randomized and pots were spaced far enough apart to avoid cross-contamination of soil microbes and foliar leachate microbes. All seedlings were watered with 10 mL foliar leachate (20 % of pot volume) 24 h after transplant and an additional 5 mL leachate seven times evenly dispersed over the next 28 days. This pulsed exposure to leachate mimics the pattern of allelochemical concentrations found in the field (Inderjit et al. 2008). After 28 days, we harvested and weighed seedlings (fresh weight). Some seedlings did not survive to harvest. Percent survival results mentioned in the text are the number of seedlings that survived to harvest divided by the total number of seedlings that were transplanted.

To analyze final seedling biomass (a proxy for seedling growth), we used a general linear model of total (above and below ground) fresh biomass (via the 'glm' command in R ver. 2.15.2). Analyzing seedling growth (increase in size after transplant) was statistically problematic because of some individuals that lost mass after transplant. This 'negative growth' produced errors in any transformation to meet the normality assumption. Therefore, we analyzed seedling biomass in place of growth, since weight at transplant correlated strongly with target seed species (species with larger seeds had larger seedlings at the time of transplant). Using final biomass as our response variable therefore measured seedling growth, with

the variation due to initial seedling size captured by the "Target Species" term.

For this second life stage, the independent variables we investigated were again live versus sterile soil and the six leachates used on seven target species. Although germination was studied with 11 target species, four species had too few individuals that germinate or survive the experiment (*Carex* and *Penstemon* overall (13 and 19 seedlings out of 120, respectively), while *Eupatorium* and *Blephilia* had low success only in sterile soil (15 and 0 seedlings out of 60, respectively). They were therefore excluded from this seedling biomass analysis. Also, due to germination and survival, we had unbalanced replication. We addressed this with the type 3 sums of squares (using 'Anova' in R package 'car' ver. 2.0-15). The same contrasts were used in this analysis as in the germination analysis. To meet the assumptions of the general linear model, seedling size was transformed to the 0.25th power, as suggested by the Box-Cox procedure.

Results

Are the invasive leachates more allelopathic than native leachates?

Foliar leachate species significantly inhibited or promoted germination and growth of some target species (a significant Leachate × Target sp. effect on germination ($P < 0.001$; Table 1) and seedling size ($p < 0.001$; Table 2). Specifically contrasting invasive and native leachates, invasive leachates inhibited germination of some target species ($P = 0.001$; Table 3), but there was no difference between native

Table 1 The effects of treatments on seed germination were tested using a negative-binomial generalized linear model, in this case including all leachate species separately (glm.nb; $n = 1320$)

	Chi sq	df	P
Soil microbes	10.55	1	0.001
Leachate	2.77	5	0.735
Target sp.	349.49	10	<0.001
Soil microbes × leachate	21.06	5	0.001
Soil microbes × target sp.	25.72	10	0.004
Leachate × target sp.	133.80	50	<0.001
Soil mic. × leachate × target sp.	177.13	50	<0.001

and invasive leachate for growth ($P = 0.095$; Table 4). Examining Figs. 1 and 2, it becomes clear that there is no consistent effect of invasive versus native origin of the leachate. For both germination and growth, the invasive species *Elaeagnus* frequently had an impact comparable to the three native species' leachates. The effects of invasive status that do reach significance seem, therefore, to be driven by *Lonicera* or *Ligustrum* having a larger effect, rather than a consistent trend of all invasive species being allelopathic.

Do soil microbes and microfauna significantly change allelopathic effects of the foliar leachate?

Soil microbes significantly altered germination rates in a complex manner, enhancing allelopathic effects in some treatments while reducing them in others (Fig. 1). There was considerable variability in how target species were affected by combinations of treatments (i.e., a significant 3-way interaction; Table 1; Fig. 1). Conversely, microbes did not significantly alter the allelopathic effect of leachates on the size of seedlings ($P = 0.297$ and 0.409 for 3- and 2-way interactions with microbes and leachate, respectively; Table 2). Soil microbes did affect seedling survival in some herbaceous species, with *Blephilia* and *Penstemon* seedlings surviving better in live soil than in sterile soil (*Blephilia*: 46 % vs. 11 %; Penstemon 88 % vs. 44 % survival). On the other hand, Carex seedlings survived better in sterile soil (40 % survival in live soil vs. 92 % in sterile soil).

Are species-specific allelopathic effects consistent across a variety of target species?

There was a significant 3-way interaction between soil treatment, leachate species, and target species on

Fig. 1 Average number of germinated seedlings for each target ▶ species in live (*gray*) and sterilized (*white*) soil treatments. *Error bars* are ±1 SE. Leachate species listed in the *horizontal axis* are abbreviated as follows: Invasives: Ela *Elaeagnus umbellata*, and Lon *Lonicera maackii*, Lig *Ligustrum vulgare*; and Natives: Ace *Acer saccharum*, Cer *Cercis canadensis*, and Lin *Lindera benzoin*. *Dotted lines* separate the invasive species' leachates (Ela, Lon, and Lig) from the native species' leachates (Ace, Cer, and Lin)

germination ($P < 0.001$; Table 1) and a significant 2-way interaction between leachate species and target species on seedling size ($P < 0.001$; Table 2), indicating that the effects on germination and growth depended strongly on which target species was exposed to which leachate species (Figs. 1, 2). Some leachates inhibited germination/growth in one target species while promoting germination/growth in other species (e.g., *Acer* leachate inhibited *Carex* germination in live soil, but promoted *Bromus* germination in live soil relative to leachate from other species in the community; Fig. 1). Based orthogonal contrasts and Fig. 1, *Ligustrum* leachate inhibited germination in the most target species relative to the native leachate species (Leachate x Target sp. $P < 0.001$, Table 3). In sterile soil, *Ligustrum* inhibited germination for *Blephilia*, *Bromus*, *Eupatorium*, and *Penstemon*. In live soil, *Ligustrum* inhibited germination for *Acer*, *Bromus*, *Eupatorium*, and *Penstemon*. *Lonicera* overall had the strongest effect on final seedling biomass relative to the native leachate species (Table 4: Leachate $P = 0.098$, Leachate × Target sp. $P = 0.002$). *Lonicera* inhibited growth for *Blephilia*, *Bromus*, *Cercis*, *Elaeagnus*, *Elymus*, and *Lindera* (Fig. 2). In some treatments with *Lonicera* leachate, seedlings did not survive to be harvested. This was generally due to root inhibition and occurred with *Blephilia* (20 % survival), *Eupatorium* (20 % in live

Table 2 The effects of the treatments on the growth of seedlings as shown in their size (average final biomass), tested using a general linear model ($n = 723$)

	Chi sq	df	P
Soil microbes	0.50	1	0.478
Leachate	6.22	5	0.286
Target sp.	183.26	6	<0.001
Soil microbes × leachate	5.05	5	0.409
Soil microbes × target sp.	5.27	6	0.510
Leachate × target sp.	63.61	30	<0.001
Soil mic. × leachate × target sp.	33.60	30	0.297
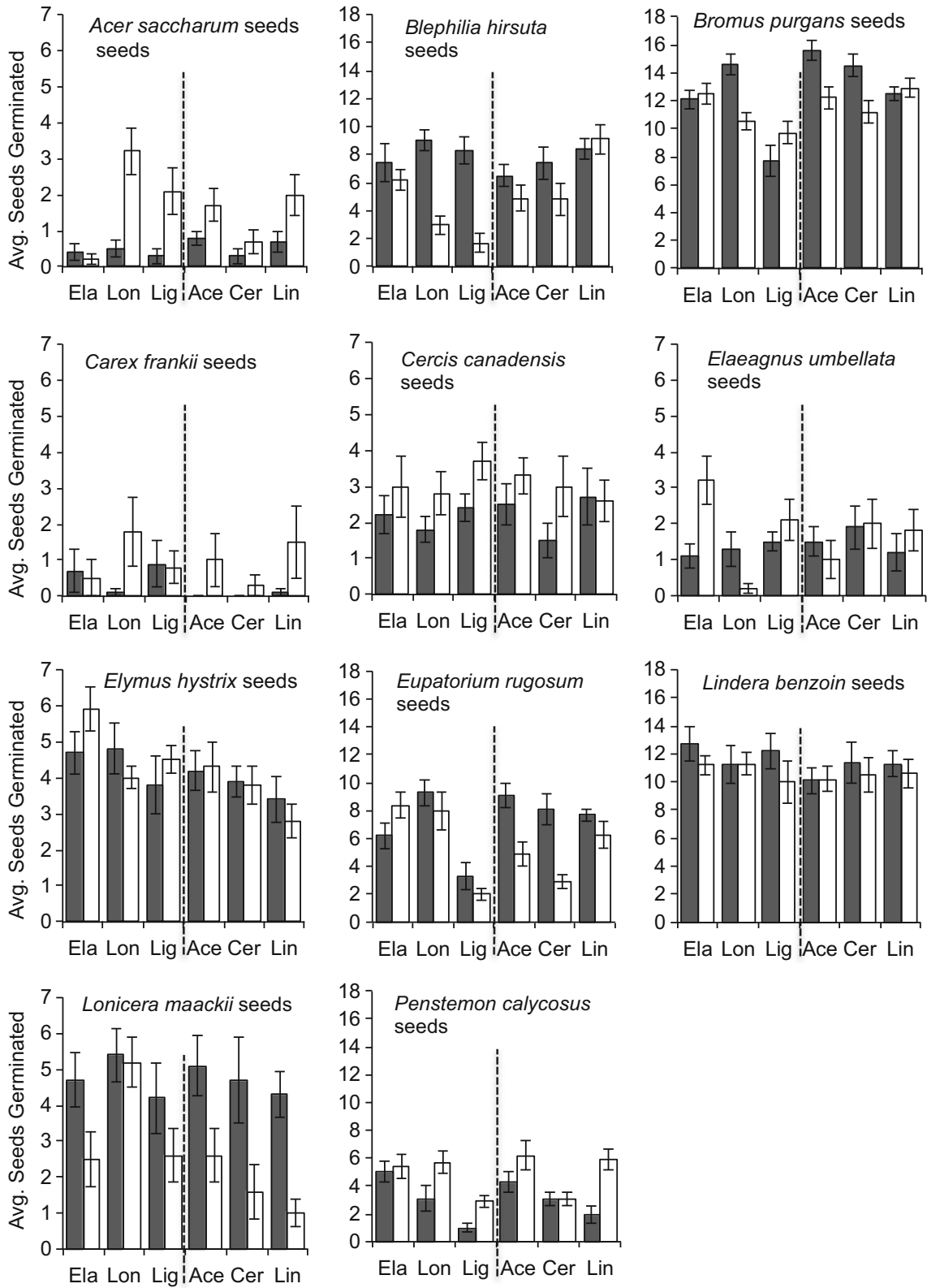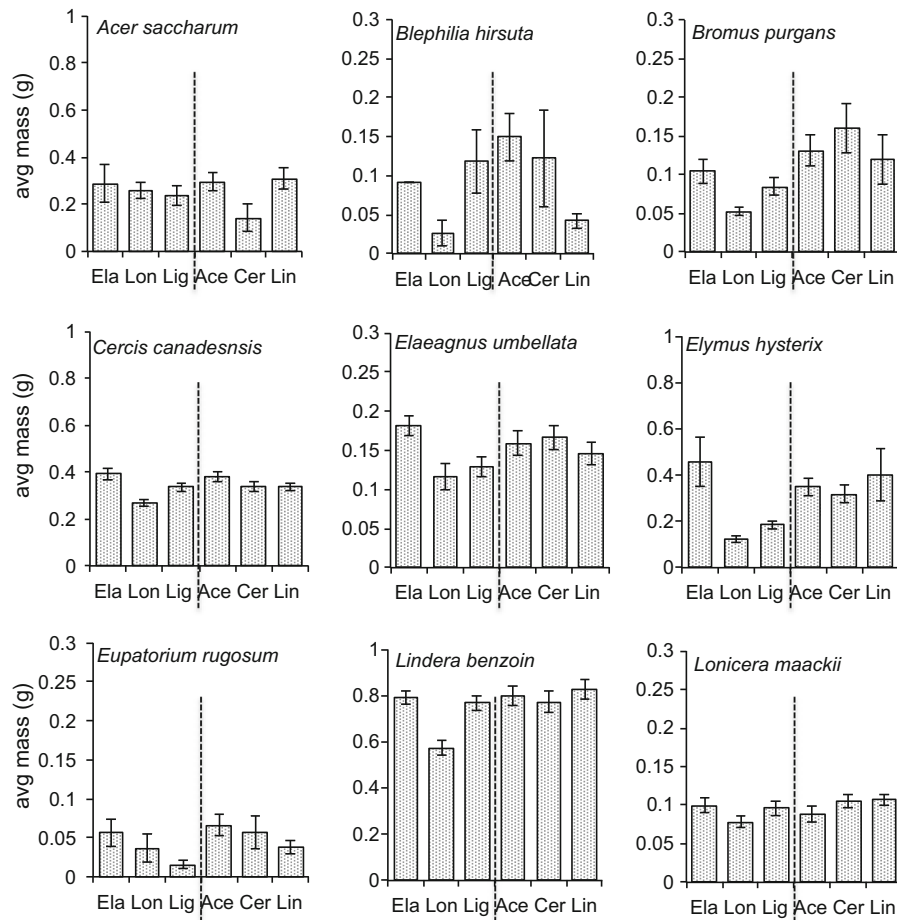
Fig. 2 Average mass of seedlings grown in both soil types. *Error bars* are ±1 SE. Leachate species listed in the *horizontal axis* are abbreviated as follows: Invasives: Ela *Elaeagnus umbellata*, Lon *Lonicera maackii*, and Lig *Ligustrum vulgare*; and Natives: Ace *Acer saccharum*, Cer *Cercis canadensis*, and Lin *Lindera benzoin*. *Blephilia* and *Eupatorium* are shown here, but could not be included in the growth analysis because fewer than three seedlings germinated and survived to harvest in sterilized soil (*Blephilia*) or in *Ligustrum* and *Cercis* leachates (*Eupatorium*). *Dotted lines* separate the invasive species' leachates (Ela, Lon, and Lig) from the native species' leachates (Ace, Cer, and Lin)

soil, 29 % in sterile soil), and *Bromus* in sterile soil (50 %). The overall average survival rate for this study was 67 % in live soil and 76 % in sterile soil.

Discussion

The Novel Weapons Hypothesis (Callaway and Ridenour 2004) was supported by *Ligustrum's* and *Lonicera's* impacts on native germination or growth, though these impacts were not consistent for all target species. However, not all of our invasive species showed evidence of novel weapons. Analyzed as a group, invasive species did not significantly affect germination or final biomass. *Lonicera* and *Elaeagnus* had positive effects on germination compared to native leachates. Native *Acer* growth was most strongly inhibited by the commonly co-occurring native *Cercis*, and *Acer* germination was inhibited equally by both *Cercis* and the invasive *Ligustrum*. This shows that it is insufficient for an invasive plant to simply have allelopathic potential. It must be more allelopathic than the co-occurring natives for allelopathy to give it an advantage.

Ligustrum inhibited germination the most frequently, while *Lonicera* inhibited growth the most

Table 3 Orthogonal contrasts within the negative-binomial generalized linear model are shown below investigating specific biological hypotheses. Tables showing the complete results from the orthogonal contrasts are available in the Supplement

Orthogonal contrasts	Chi sq	df	P
Native leachates versus invasive leachates			
Leachate origin	0.52	1	0.469
Leachate origin × target sp.	31.08	10	0.001
Soil mic. × leachate Origin × target sp.	47.05	10	<0.001
Elaeagnus versus all natives			
Leachate	0.96	1	0.328
Leachate × target sp.	22.85	10	0.011
Soil mic. × leachate × target sp.	33.55	10	<0.001
Ligustrum versus all natives			
Leachate	0.01	1	0.941
Leachate × target sp.	55.78	10	<0.001
Soil mic. × leachate × target sp.	47.61	10	<0.001
Lonicera versus all natives			
Leachate	0.27	1	0.604
Leachate × target sp.	3.64	10	0.962
Soil mic. × leachate × target sp.	36.11	10	<0.001

Table 4 Orthogonal contrasts within the general linear model are shown below investigating specific biological hypotheses. Tables showing the complete results from the orthogonal contrasts are available in the Supplement

Orthogonal contrasts	Chi sq	df	P
Native leachates versus invasive leachates			
Leachate origin	0.50	1	0.479
Leachate origin × target sp.	10.80	6	0.095
Soil mic. × leachate Origin × target sp.	1.85	6	0.933
Elaeagnus versus all natives			
Leachate	0.57	1	0.449
Leachate × target sp.	3.78	6	0.707
Soil mic. × leachate × target sp.	2.35	6	0.885
Ligustrum versus all natives			
Leachate	0.46	1	0.499
Leachate × target sp.	10.21	6	0.116
Soil mic. × leachate × target sp.	4.42	6	0.621
Lonicera versus all natives			
Leachate	2.74	1	0.098
Leachate × target sp.	21.31	6	0.002
Soil mic. × leachate × target sp.	3.06	6	0.801

frequently compared to the native leachate species. Since both germination rates and growth showed a significant interaction between leachate species and test species, the results must be interpreted cautiously. Therefore, our results indicate that *Ligustrum*

(1) inhibits germination more than the other leachate species against this community of test species;

(2) inhibits germination for several species (e.g., *Bromus*, *Penstemon*, and *Eupatorium*) but not others (e.g., *Cercis* and *Lindera*); and

(3) has a higher probability of inhibiting germination of an untested seed species than the other leachate species tested.

The same interpretation applies for the effect of *Lonicera* on seedling growth. We observed that seedlings exposed to *Lonicera* leachate tended to have stunted roots, which suggests that *Lonicera's* allelochemical affects root growth but not germination. Root stunting in target species after exposure to leachate has been observed with other invasive

species, such as *Centaurea maculosa* (Kaur et al. 2009) and *Campuloclinium* (Goodall et al. 2010).

Lonicera and *Ligustrum* could reduce native diversity by reducing the germination or growth of heterospecific seedlings. These effects on germination and growth may give these two species a competitive advantage, especially when combined with *Lonicera's* ability to reduce native plant fecundity (Gould and Gorchov 2000; Cipollini et al. 2008a). However, the leachate species × target species interaction poses substantial challenges to predicting the consequences of allelopathy in a specific invaded site, as allelopathy would inhibit only a portion of the community. For example, *Ligustrum* would have very little effect on *Blephilia*, *Carex*, *Cercis*, and *Lindera* in live soil. If these species are not inhibited in other ways, such as shading, they may become more dominant at the site compared to an uninvaded site. Our results may explain why some of the target species at the study site only occur in uninvaded areas, while species such as *Elymus* also occur in invaded areas (personal observation).

The effects of the six leachates on the target species were not consistent over the two life stages. Within

live soil, seedlings grown in *Lonicera* leachate reduced growth more often than native leachates; however, *Lonicera* leachate did not inhibit germination of these species. Conversely, *Ligustrum* leachate had a stronger negative impact on germination than on growth. This is similar to other researchers' observations for *Lonicera maackii* (McEwan et al. 2010), *Lolium arundinaceam* (Orr et al. 2005), *Campuloclinium macrocephalum* (Goodall et al. 2010), and *Elaeagnus umbellata* (Harnden et al. 2011). In these studies, the results of germination tests did not predict the effects of leachate on shoot or radicle growth. While it is possible that the differences between the life stages in our study were due to different leachate preparation methods (senesced leaves vs. fresh leaves), our findings are consistent with the results of these other studies, which used the same leachate for all life stages (Goodall et al. 2010; Harnden et al. 2011). Our results provide further support for the need to study the impacts of allelopathy on as many life stages as possible, from germination to seed set, in order to accurately predict the effects of an allelopathic species on the neighboring plant community.

Our study found that it is not possible to predict allelopathic effects on germination in soil with a live microbial community based on a bioassay using sterile substrate. Our results using, for example, *Lonicera* leachate led to markedly different outcomes for live and sterilized soil on the germination of *Blephilia*, *Penstemon*, and *Acer* (Fig. 1). Similarly, the sensitivity of target species to the different leachates clearly varied between live and sterilized soil. These idiosyncratic results could be due to multiple soil microbes interacting with the chemicals in the leachate. The soil microbial community consists of mutualists, parasites, and pathogens, all of which could impact seedling's resistance to the chemicals in the leachate. Additionally, these soil microbes break down leachate chemicals, either increasing or decreasing the impact of any allelochemicals (Kaur et al. 2009; Cipollini et al. 2012). This indicates that, to understand the ecological implication of a putative allelochemical, allelopathy assays in terrestrial systems need to account for the effects of soil microbes. Future research should test whether soil microbial communities from different sites or habitats have similar effects on allelopathic potential.

The significant interaction between target species and leachate species (for seedling size) or target species and both leachate and soil treatments (for germination) shows that the strength of allelopathic effects can be context dependent (as in Orr et al. 2005; Bauer et al. 2012), limiting researchers' ability to extrapolate results. We found that the strength of allelopathic impacts also varied among different target plant species and soil treatments. Therefore, allelopathy studies should expand beyond overly simplified methods. For example, the effects of soil microbes are large and of unpredictable direction. Also, using only one species may lead to spurious conclusions because of idiosyncratic species-specific responses to leachates. Further, allelopathic potential against germination does not reliably predict allelopathic potential against growth or other fitness components. Therefore, allelopathic potential cannot be lightly asserted based upon simplified tests. While the more complex bioassays involving soil microbial communities, multiple test species, and native leachate controls may reduce the number of discoveries of allelopathic interference, it will help ensure that those discoveries are more likely to represent ecological phenomena.

Our results support the Novel Weapons Hypothesis as a mechanism supporting the dominance of *Ligustrum* and *Lonicera* over some, but not all, native species. It is possible that the reduction in plant diversity observed in invaded sites (Gould and Gorchov 2000; Merriam and Feil 2002) is driven by a subset of particularly susceptible plant species and that the abundance of more resistant species remains unchanged post invasion. If this is the case, non-susceptible species could be used early in a restoration to re-establish an abundant native community as the invasive shrub is being removed.

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