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Context dependency of the allelopathic effects of *Lonicera maackii* on seed germination

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Abstract Allelopathic effects of invasive plants on native flora may be mitigated by the abiotic and biotic environment into which the allelochemicals are released. *Lonicera maackii* (Amur honeysuckle), an invasive plant of the eastern deciduous forest, suppresses seed germination in laboratory assays. We investigated how *L. maackii* leachate interacts with abiotic conditions and with the soil microbial community. First, we tested the effects of leaf extract from *L. maackii* on germination of the native woodland herb, *Blephilia hirsuta*, under different light and soil conditions. We found that germination of *Blephilia hirsuta* was reduced by *L. maackii* extract, but abiotic conditions did not interact with this effect. We also tested the effects of leaf extract on germination of five native woodland species and *L. maackii* placed in sterile or live soil. There was an overall suppressive effect of *L. maackii* extract on itself and the other five native species tested. However, *L. maackii* extract interacted with live soil in ways that differed with the species being tested and, in some cases, changed over time. Our results indicate that allelopathic potential of *L. maackii* shows context dependency with respect to soil microorganisms and native species identity but not to light conditions or soil type. Our results imply that restoration of invaded areas may require active reintroduction of species sensitive to allelopathy in live soil. Further, laboratory assays of allelopathy should consider the interaction of allelochemicals with biotic and abiotic conditions to more accurately predict the impacts of allelopathy on plant communities.

Keywords Invasion · Allelopathy · Live soil · Leachate · Eastern deciduous forest

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Introduction

The role of allelopathy in plant communities is attracting increasing interest as a mechanism contributing to the success of invasive plants and associated impacts on native plant communities and ecosystem structure and functioning (Callaway and Ridenour 2004; Hierro and Callaway 2003; Stinson et al. 2006). A number of invasive plants have been shown to have direct allelopathic effects on native plants, suppressing seed germination, nutrient uptake and growth (Callaway and Aschehoug 2000; Dorning and Cipollini 2006; Jarchow and Cook 2009; Pisula and Meiners 2010). We are also beginning to appreciate that allelopathic effects of invasive plants may depend on abiotic and/or biotic context.

Abiotic conditions are known to influence the activity of allelochemicals, with the potential to either

diminish or enhance the strength of allelopathic effects. For example, allelochemicals from *Phragmites australis* increase in potency in the presence of UV light and under alkaline conditions (Rudrappa et al. 2009). Interactions with the soil may also alter the strength of allelochemicals. Experiments with catechin, an allelochemical produced by *Centaurea maculosa,* have found that common soil metals influence the concentration of catechin in soils, leading to highly variable effects of this chemical on plant growth (Pollock et al. 2009). Phenolic acids are also likely to be affected by interactions with the soil, due to sorption to soil organic matter (Tharayil et al. 2006).

Allelochemicals may also interact with biotic aspects of the environment, such as soil microorganisms and the identity of co-occurring plant species. For example, glucosinolates released by *Alliaria petiolata* are known to have important effects on plants and plant communities through their effect on mycorrhizal fungi (Anderson et al. 2010; Stinson et al. 2006). However, the direct effects of *Alliaria's* allelochemicals appear to be stronger in sterile conditions than in the presence of an intact soil microbial community (Lankau 2010). Soil microorganisms may degrade allelochemicals, so that in live soil the strength of allelopathic interactions are diminished or eliminated (Kaur et al. 2009; Zhu et al. 2011). For example, *m*-tyrosine and 8-hydroxyquinoline, potential allelochemicals produced by *Festuca rubra* and *Centaurea diffusa,* respectively, inhibit root growth under sterile conditions. However, in live soil, the strength of these effects are diminished (Inderjit et al. 2010; Kaur et al. 2009). Interestingly, 8-hydroxyquinoline may also have a suppressive effect on microbial activity (Inderjit et al. 2010). Alternatively, it is also possible that the byproducts of microbial breakdown may have stronger allelopathic effects than the chemicals produced by plants (Inderjit 2005). Allelochemicals may affect different plant species to varying degrees. Plant species have been shown to differ in the effect of allelochemicals on their germination and growth rates (Gomez-Aparicio and Canham 2008; McEwan et al. 2010). Plants may produce chemicals that protect against allelochemicals released by neighboring plants (Weir et al. 2006), perhaps providing a mechanism for some of the observed variation in plant species sensitivity to allelopathic effects.

These prior studies establish that assessing the importance of allelopathy requires investigation of how the effects of allelochemicals may interact with the biotic and abiotic conditions into which they are released. In this study, we address the context dependency of allelopathic effects of *Lonicera maackii* on the germination of native woodland species.

Lonicera maackii is a highly invasive plant of eastern deciduous forest (Hartman and McCarthy 2008; Hutchinson and Vankat 1997). Competitive suppression of native plants, likely due to reduced light availability under the dense canopies formed by this species, is frequently cited as the cause of *L. maackii*'s effects on native plant species (Gorchov and Trisel 2003; McEwan et al. 2009; Miller and Gorchov 2004). However, allelopathy may also contribute to suppression of native plant species following *L. maackii*'s invasion. Previous work employing tests of seed germination on filter paper has found that *L. maackii* leachate reduces germination of several native and non-native species, but increases germination of *L. maackii* seeds (Dorning and Cipollini 2006; McEwan et al. 2010). Leaf extracts from *L. maackii* have also been found to reduce growth and reproduction of *Arabidopsis thaliana* plants grown in field soil, and constrained this species ability to respond to increases in soil fertility (Cipollini et al. 2008). Further, *Arabidopsis* has been reported to grow and flower more slowly, but eventually reach greater size and reproductive output, in soils from a site invaded by *L. maackii* as compared to non-invaded control soils (Cipollini and Dorning 2008). Overall, these studies have reported stronger effects of leaf extracts than root extracts (Cipollini and Dorning 2008; Dorning and Cipollini 2006).

Here, we present the results of two experiments on the effects of leachates from *L. maackii* leaves on the germination of native woodland species. Our study builds on previous allelopathy work with *L. maackii* by exploring the abiotic and biotic context dependency of its allelopathic potential. Furthermore, given previous research indicating that germination responses to *L. maackii* leachate may vary with species identity (McEwan et al. 2010), we expanded our test to additional native woodland species. Our objective was to determine whether the allelopathic potential observed with *L. maackii* in previous studies was likely to affect native germination in natural conditions. In the first experiment, we tested the factorial effects of *L. maackii* extract, light level (uninvaded forest canopy vs. shade of *L. maackii* canopy), and soil type (silt loam vs. sand) on percent germination of a native woodland

herb, *Blephilia hirsuta*. In the second experiment, we tested the effect of *L. maackii* extract on the time course of germination for five native species and *L. maackii* in sterile soil versus soil inoculated with a live microbial community. We expected that allelopathic effects of *L. maackii* would vary with the abiotic and biotic conditions into which they are released. In particular, we predicted that allelopathic effects would be stronger on sand—a substrate less likely to react with allelochemicals, and under shaded conditions associated with *L. maackii* dominance in the field (Experiment 1). We also predicted that live soil would mitigate allelopathic effects of *L. maackii* and that native species would vary in their germination responses to *L. maackii's* allelochemicals (Experiment 2).

Methods

Experiment 1

Our first experiment tested the effects of *L. maackii* extract on germination of a woodland mint (*Blephilia hirsuta*) as a function of substrate and light conditions. We used a full $3 \times 2 \times 2$ factorial design ($n = 6$, per treatment) with three liquid treatments (*Lonicera maackii* extract, distilled water, and pH-adjusted distilled water), two substrates (sterile soil and sterile sand), and two light levels (2.8 and 1.7% full sun).

We used topsoil (Crider silt loam, Monroe County, IN, USA; 2% organic carbon, 9 μg/g nitrate, 6 μg/g P, pH = 6.3) and commercially available play sand. Local topsoil, collected from uninvaded areas of deciduous woodland, was chosen to best mimic relevant ecological conditions, and sand was chosen to provide a less reactive substrate, to contrast with the local silt loam soil, and explore the generality of our results. The soil was screened through a 2-mm sieve, and both substrates were sterilized at 110°C for 60 min prior to use. We filled 18,100 × 15 mm glass Petri plates with 40 mL of each prepared substrate.

We procured seeds of the native woodland herb *Blephilia hirsuta* from Prairie Moon Nursery (Winona, MN, USA). Seeds were surface-sterilized (1 min 95% v/v ethanol followed by 1 min 50% v/v bleach), rinsed in distilled water, and allowed to dry overnight before adding to plates in batches of 20 seeds per plate.

To produce the *L. maackii* leaf extract, we used a modified version of the protocol described by Dorning and Cipollini (2006), harvesting whole *L. maackii* leaves in September and incubating the whole leaves in 5-mL distilled water for 72 h. We filtered the extract through a Whatman 2 filter using a vacuum pump and stored the extract at 5°C while preparing the plates. We chose to use extracts from leaves, rather than roots, due to previous reports that leaf extracts appear to more strongly inhibit seed germination (Cipollini and Dorning 2008; Dorning and Cipollini 2006).

Since our previous research indicated that *L. maackii* leaf extract has a lower pH than distilled water (methods below), we added hydrogen chloride to distilled water until it matched the average pH of the extract. This third liquid treatment (in addition to the extract and distilled water) thus allowed determination of whether pH alone could affect germination. We added 15 mL of liquid treatment to each seeded plate. To ensure that seeds were evenly moist across substrates, we added an additional 5-mL distilled water to each plate with soil substrate because the soil absorbed more liquid than the sand.

The assembled plates were cold stratified in the dark at 4°C for 7 weeks before initiating germination under fluorescent lights on a 12 h per day light cycle at room temperature. Half the plates received 42.6 μmol/m^2/s of photosynthetically active radiation, PAR), comparable to nearby forested areas following tree leaf expansion (Bauer and Shannon, unpublished data). Four layers of cheesecloth were taped to the lids of the other half of the plates to reduce the light reaching the seeds by 50%, mimicking the shading of seeds under an *L. maackii* canopy (McKinney and Goodell 2010). After 21 days under these conditions, we recorded the total number of seeds per plate that had germinated.

Initial pH measurements

Initial tests indicated that *L. maackii* extract has a lower pH than the distilled water we intended to use as a control. To confirm this effect and to determine the pH buffering capacity of different substrates and whether a live soil microbial community could alter the pH of *L. maackii* extract, we filled 120-mL specimen cups with one of the following substrates (obtained and prepared as described above): sterile sand, sterile soil, and live soil (six replicates for each substrate). An additional six specimen cups were left empty. Three cups of each substrate were filled with 30 mL of distilled water, while the remaining three

cups of each substrate were filled with 30 mL of fresh *L. maackii* leaf extract. All specimen cups were sealed and placed on a shaker for 24 h at room temperature. After this 24-h incubation time, we measured the pH of each sample using a Thermo Orion model 420 A + pH meter. Our pH measurements confirmed that *L. maackii* extract had a substantially lower pH than distilled water (Table 1). This pH difference was reduced in the presence of sterile or live soil compared to sand (liquid × substrate interaction; ANOVA $F_{3, 16} = 8.718$, $P = 0.0011$), but in all cases remained significant.

Experiment 2

Our second experiment compared germination responses to *L. maackii* extract in sterile versus live soil, where microbial activity could potentially mitigate or exacerbate extract effects, and expanded our tests of *L. maackii* extract effects to five woodland species plus *L. maackii* itself. We employed a full 2 × 2 × 6 factorial experiment ($n = 6$, per treatment) with two substrates (live soil and sterile soil), two liquid treatments (*L. maackii* leaf extract and distilled water), and five native woodland species plus *L. maackii*. Based on results from Experiment 1 (described below), we dropped the pH, shade, and substrate treatments.

We filled 100 × 15 mm glass Petri plates with 70 mL of sterile topsoil that had been filtered through a 4.75-mm sieve and sterilized as above. We then created a microbial wash by mixing approximately 10 L of fresh forest soil from non-invaded sites within local deciduous forest (Indiana University Research and Teaching Preserve, Bloomington, Indiana, USA) with 10 L of distilled water, and successively filtering it through 1.18-mm and 355-μm sieves. The goal of the filtering process was to prevent germinating seeds and moss spores from interfering with our treatment manipulations. Ten milliliters of the microbial wash was added to half the plates to create a live soil substrate, while the other half of the plates received 10-mL distilled water.

Table 1 Mean ± standard error of the pH of distilled water (DW) and *L. maackii* extract control solutions and solutions buffered with sterile soil, soil with a live microbial community, and sand

Substrate	DW	*L. maackii* extract
Control	6.58 ± 0.47 a	4.96 ± 0.01 c
Sterile soil	7.33 ± 0.02 b	6.08 ± 0.02 a
Live soil	7.39 ± 0.11 b	6.23 ± 0.12 a
Sand	8.39 ± 0.18 d	6.24 ± 0.01 a

Letters indicate significant differences ($P < 0.05$)

To better approximate leaf litter leachate that spring germinating seeds would be exposed to, we created a leaf extract similar to experiment 1 using *L. maackii* leaves collected in November and frozen at −20°C. The leaves were thawed and incubated in 5 mL of distilled water per gram of leaf for 72 h, after which we filtered the extract through a Whatman 2 filter using a vacuum pump. We added 15 mL of liquid (either extract or distilled water) to each seeded plate.

We used seeds from the following woodland species, placing 20 seeds in each plate after all liquid treatments and soil had been added: *L. maackii, Blephilia hirsuta, Bromus pubescens, Elymus hystrix, Eupatorium rugosum,* and *Penstemon calycosus*. All species other than *L. maackii* are native perennial woodland herbs and grasses known to occur in the same habitats as *L. maackii*. While vegetative spread is an important means of recruitment for perennial herbs and grasses (Grime 2002), these species also flower and set abundant seed. Recruitment from seed is important for the maintenance of genetic variation in populations and for longer distance dispersal and colonization of open sites, such as woodland gaps (Hughes and Fahey 1991). Furthermore, recruitment from seed has important application in woodland restoration, where seed sowing has been shown to restore a diversity of herbs and grasses (Brudvig et al. 2011). The native seeds were obtained from Prairie Moon Nursery and Spence Restoration Nursery (Muncie, IN, USA). The *L. maackii* seeds were collected from naturally growing plants in a municipal forest preserve (Lower Cascades Park, Bloomington, Indiana, USA).

All plates were cold stratified at 4°C for 4 weeks before initiating germination under fluorescent lights (42.6 μmol/m^2/s of photosynthetically active radiation, PAR) at room temperature.

Data analysis

All analyses were performed in SAS (SAS Institute Inc. 2010). We analyzed effects of *L. maackii* extract, soil type, and light and all possible interactions on germination in experiment 1 using three-way ANOVA. pH measurements among the different

substrate treatments were compared using one-way ANOVA. Separate repeated measures ANOVAs were used for each species in experiment 2 to analyze the effects of *L. maackii* extract, soil inoculum, time, and all possible interactions on germination. Pairwise comparisons of means were made using LS means.

Results

Experiment 1

Extract from *L. maackii* leaves significantly depressed germination of *B. hirsuta* (ANOVA, $F_{2,\ 24} = 7.06$, $P = 0.004$; Fig. 1a). Germination was lower with extract added compared to distilled water controls ($P = 0.005$) and pH-adjusted controls ($P = 0.023$). Distilled water controls were not significantly different from pH-adjusted water ($P = 1.00$).

We did not detect interactions between the effects of *L. maackii* extract on *B. hirsuta* germination and either substrate type or light (ANOVA, $F_{2,\ 24} = 0.83$, $P = 0.447$). Light and substrate did, however, interact with one another to affect the germination of *B. hirsuta* (ANOVA, $F_{1,\ 24} = 3.95$, $P = 0.058$; Fig. 1b). There was higher germination in high light than in low light in topsoil ($P = 0.048$), but not sand. Allelopathic potential and shading effects of *L. maackii* were comparable in magnitude; both reduced germination of *Blephilia* seed to about 20% (Fig. 1).

Experiment 2

Overall, we found highly variable effects of *L. maackii* leaf extract, soil inoculum, and time on the species we tested, although all species tended to germinate best under the combination of sterile soil and distilled water (Fig. 2; Table 2). *L. maackii* leaf extract typically depressed germination, but this effect interacted with soil inoculum and time in different ways across species.

Lonicera maackii

Germination of *L. maackii* seeds was low in this experiment, with <20% of seeds germinating overall. Compared to distilled water *L. maackii* extract did, however, have a significant negative effect on germination of its own seeds over time (Fig. 2a; Table 2: time × extract, $P < 0.001$). There was also a tendency for soil inoculum and *L. maackii* extract to interact: in the absence of *L. maackii* extract, germination in live soil was reduced relative to sterile soil (Fig. 2a; Table 2: soil inoculum × extract, $P = 0.050$).

Blephilia hirsuta

We found significant interactive effects of time and *L. maackii* extract (time × extract, $P < 0.001$) and soil inoculum and *L. maackii* extract (Table 2: soil inoculum × extract, $P = 0.002$) on germination of *Blephilia*. *Lonicera maackii* extract had a negative impact on germination of *Blephilia*, and by the 6th day all treatments were significantly different. Within extract treatments, germination was increased by live soil in the presence of *L. maackii* extract, but live soil reduced germination in plates without *L. maackii* extract (Fig. 2b).

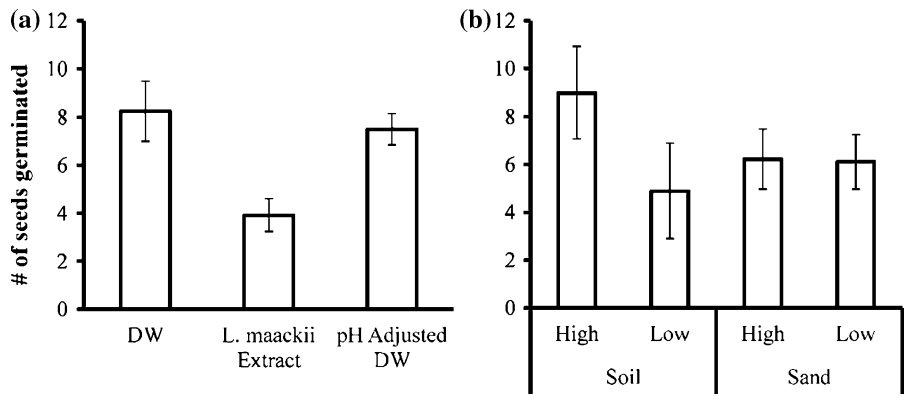

Fig. 1 Mean (±S.E.) number of *Blephilia hirsuta* seeds out of 20 that had germinated at the end of the experiment. **a** Effects of *L. maackii* extract and pH-adjusted distilled water on germination averaged across light and soil treatments, and **b** effects of different high and low light levels on germination in soil and sand

Table 2 Repeated measures ANOVA results for the effect of time, soil inoculum, *L. maackii* extract and interactions on germination of five native species and *L. maackii*

		Lonicera		*Blephilia*		*Bromus*	
	df	*F*	*P*	*F*	*P*	*F*	*P*
Soil inoculum	1, 20	4.37	0.050	0.42	0.526	3.75	0.067
Extract	1, 20	28.77	<0.001	72.28	<0.001	18.89	<0.001
Soil inoculum × extract	1, 20	4.37	0.050	13.51	0.002	0.34	0.567
Time	6, 120	11.91	<0.001	45.37	<0.001	149.14	<0.001
Time × soil inoculum	6, 120	1.12	0.354	2.35	0.035	1.07	0.382
Time × extract	6, 120	11.91	<0.001	4.73	<0.001	5.94	<0.001
Time × soil inoculum × extract	6, 120	1.12	0.354	0.56	0.765	6.50	<0.001
		Elymus		*Eupatorium*		*Penstemon*	
	df	*F*	*P*	*F*	*P*	*F*	*P*
Soil inoculum	1, 20	3.02	0.098	27.61	<0.001	3.85	0.064
Extract	1, 20	0.27	0.611	15.14	0.001	27.05	<0.001
Soil inoculum × extract	1, 20	40.41	<0.001	6.02	0.023	16.72	<0.001
Time	6, 120	88.34	<0.001	43.1	<0.001	74.47	<0.001
Time × soil inoculum	6, 120	2.98	0.010	0.75	0.608	2.49	0.026
Time × extract	6, 120	1.04	0.403	3.98	0.001	7.21	<0.001
Time × soil inoculum × extract	6, 120	20.03	<0.001	2.7	0.017	10.82	<0.001

Bromus pubescens

The effects of *L. maackii* extract and soil inoculum on *Bromus* varied over time (Fig. 2c; Table 2: time × soil inoculum × extract, $P < 0.001$). Early in the experiment, seeds in sterile soil with distilled water showed greater germination than seeds in the other three treatments. By the middle of the experiment, this interaction was no longer significant, but a negative effect of *L. maackii* extract on seed germination persisted through the rest of the experiment.

Elymus villosus

The effect of *L. maackii* extract was dependent on soil inoculum with some change in this effect over time (Table 2: time × soil inoculum × extract, $P < 0.001$). There was little germination initially, and initially no significant differences between treatments. However, by day 6, treatments had diverged into two groups that persisted through the end of the experiment (Fig. 2d), with germination highest in live soil plates with extract and sterile plates without extract.

Eupatorium rugosum

Eupatorium germination was similarly low in the presence of *L. maackii* extract, live soil or both, but was significantly higher in sterile soil with distilled water throughout the experiment (Fig. 2e). However, the difference between the sterile soil and distilled water treatment and the other three treatments lessened over time (time × soil inoculum × extract, $P = 0.017$, Table 2).

Penstemon calycosus

There was a significant interaction between *L. maackii* extract, soil inoculum, and time on germination of *Penstemon* seeds (Table 2: time × soil inoculum × extract, $P < 0.001$). Initially, there were no differences between treatments, but treatments began to diverge early in the experiment. By day 8, we found negative effects of *L. maackii* germination of *Penstemon,* but only in sterile soils. Germination in live soils, with or without *L. maackii* extract, and sterile soil without extract were not significantly different (Fig. 2f).

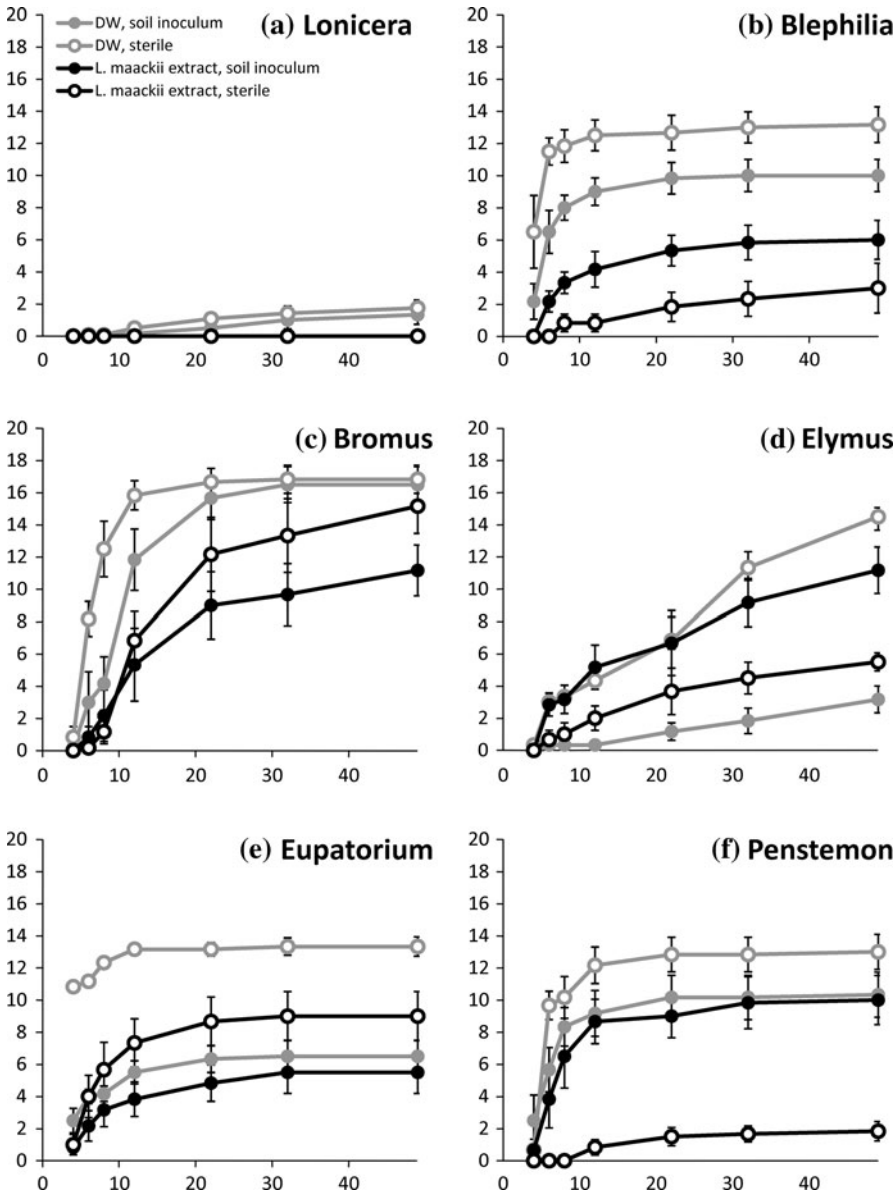

Fig. 2 Mean (±S.E.) number of seeds of **a** *Lonicera maackii*, **b** *Blephilia hirsuta*, **c** *Bromus pubescens*, **d** *Elymus hystrix*, **e** *Eupatorium rugosum*, and **f** *Penstemon calycosus* that germinated over time in topsoil with and without live soil inoculum and *L. maackii* extract. No *Lonicera* seeds germinated in the presence of *L. maackii* extract (the *L. maackii* extract, soil inoculum treatment is obscured by the *L. maackii* extract, sterile treatment in the *Lonicera* figure)

Discussion

We found partial support for our hypothesis that allelopathic potential would interact with abiotic and biotic conditions. The abiotic conditions we manipulated, shading and soil type, had significant effects on seed germination, but did not interact with the effects of *L. maackii* extract. In contrast, soil inoculum did interact with the allelopathic potential of *L. maackii*, although live soil did not have the consistently mitigating effects on allelopathy that we predicted. The effects of *L. maackii* extracts, soil inoculum, and the interactions between the two treatments were also dependent upon the species of seeds that were being tested and often varied over the course of the experiment. Overall, compared to the sterile soil, distilled water control, *L. maackii* extract reduced seed germination.

Studies of other species have reported that allelopathy interacts with abiotic conditions, either by neutralizing allelochemicals (Inderjit et al. 2010) or by increasing the strength of allelochemicals (Rudrappa et al. 2009). Since previous work has found that *L. maackii* reduces light availability in forest

understories, and this may suppress growth and reproduction of native herbaceous species (Luken et al. 1997; Miller and Gorchov 2004), we tested whether *L. maackii*'s allelopathic effects on native germination interacted with its abiotic effects on light levels. We found that *L. maackii* leachate and shade had similar negative effects on *Blephilia* seed germination. However, we found no evidence that allelopathic potential interacted with light levels in this study. Rather, *L. maackki* extracts resulted in consistently reduced germination of *Blephilia* seed across light and soil conditions. We also observed that the reduced light associated with dense *L. maackii* canopy reduced germination of *Blephilia* only in field soil. Our study therefore suggests that both allelopathy and shading contribute to *L. maackii*'s effects on native plant species in the field.

Some invasive plants have already been shown to alter soil pH (Cumming and Kelly 2007; McGrath and Binkley 2009), and low pH is known to act as a filter on plant species composition (Gough et al. 2000). After finding that extract from *L. maackii* leaves was significantly more acidic than distilled water, we tested whether the pH of *L. maackii* extracts affect germination. Germination in *L. maackii* extract was significantly lower than pH-adjusted water or distilled water controls, indicating that the effects of *L. maacki* extract on *Blephilia* germination cannot be attributed to changes in pH, strengthening support for a role of allelochemicals in suppression of seed germination. Despite the lack of effect of pH on germination, the potential for *L. maackii* or other invasive species to decrease the pH of rainwater or the soil may lead to effects on plant communities and warrants further investigation (Cumming and Kelly 2007 and McGrath and Binkley 2009). We also found that the acidity of *L. maackii* extract was reduced in soil or sand compared to distilled water, indicating that the effect of soil chemistry on plant exudates is worth considering in future experiments (Pollock et al. 2009).

We found that the presence of live soil altered the observed allelopathic effects of *L. maackii* extract. Although the role of micro-organisms and allelopathy in plant invasions are both receiving increased attention (Inderjit and van der Putten 2010), the interaction between the two has only recently been investigated. Interactions may occur when soil micro-organisms degrade allelochemicals into compounds without allelopathic properties (Kaur et al. 2009; Lankau 2010), or when the byproducts of microbial breakdown have stronger allelopathic effects (Inderjit 2005). However, previous research has not investigated the effects of interactions between allelopathy and soil microorganisms on seed germination.

We found that live soil had important effects on allelopathic inhibition of seed germination, but these effects varied by species. For example, *L. maackii* extracts had dramatic negative effects on germination of *Penstemon.* However, live soil inoculum mitigated these effects, so that only sterile soil with extract experienced reduced germination. In the case of *Blephilia,* live soil reduced germination, but mitigated the effect of *L. maackii* allelochemicals. In contrast, live soil and extract both inhibited germination of *Bromus*, but live soil did not mitigate the effects of allelochemicals. Live soil and extract also suppressed germination of *Eupatorium.* For this species, the greatest germination occurred in sterile distilled water. The effects of treatments on *Elymus* were unusual. The effects of *L. maackii* extract and live soil appeared to neutralize each other, but extract or live soil on their own suppressed germination of *Elymus.*

Together, these results indicate an important, but variable, role of the soil microbial community in allelopathy. Recently, a new conceptual framework for exotic plant–soil microorganism interactions has been proposed, wherein exotic plant–soil feedbacks are partitioned into direct effects of soil biota on the invasive plant and indirect plant–soil effects that operate through allelochemicals, litter decomposition or changes to the soil microbial community (Inderjit and van der Putten 2010). Alteration (enhancement or dampening) of allelochemical effects by soil microbes represents a distinct new pathway of interaction in this conceptual framework that will be helpful in interpreting and directing future research. Tests of allelochemicals conducted in Petri plates with filter paper and distilled water may assess potential for allelopathic activity, but our results indicate that this activity will vary based on biotic conditions including soil microorganisms.

Variation in effects of allelochemicals may have important consequences at the community level. Although some previous work suggests that *L. maackii* has a general suppressive effect on native vegetation (Hutchinson and Vankat 1997; Miller and Gorchov 2004), other work suggests that in addition to reduced diversity and abundance of native plant species there is

also a shift in community composition associated with *L. maackii* invasion (Hartman and McCarthy 2008). Although there is not sufficient overlap in the species included in our studies to allow direct comparison with Hartman and McCarthy's (2008) findings, the variation in germination of native species in response to *L. maackii* extracts may help to explain how *L. maackii* alters the composition of native plant communities. Species that are more tolerant of *L. maackii*'s allelochemicals may be more likely to persist after invasion. Even if a plant species is unable to tolerate direct competition with *L. maackii*, species that are most tolerant of *L. maackii*'s allelochemicals may be the first to re-colonize areas where *L. maackii* is being managed, but where litter, roots and re-sprouts continue to input allelochemicals into the soil.

Although previous authors have reported no autotoxic effect of *L. maackii* (Dorning and Cipollini 2006), our experiment did find reduced germination of *L. maackii* seeds watered with *L. maackii* extract. *Lonicera* germination rates were, however, very low overall in our study (<20%), so the lower rate in the presence of extract may not be biologically significant and this result could easily change under more favorable conditions for germination than achieved with our seeds/experimental conditions. Dorning and Cipollini argued that the lack of autotoxic effects of *L. maackii*, but strong effects on other species, could explain its invasion success. However, an invasive species would not be forced to deal with autotoxic effects immediately after colonizing a site. In addition, *L. maackii* fruits are widely distributed by birds so that many seeds are not forced to contend with their parent's allelochemicals. Lack of passage through a bird gut might explain the very low germination we observed for *L. maackii* in this study (Bartuszevige and Gorchov 2006). In any case, we argue that autotoxic effects do not rule out allelopathy as a strong explanation for negative effects of *L. maackii* on native plants and for the dominance of *L. maackii* in many invaded natural areas. Nevertheless, autotoxicity has the potential to limit the ability of *L. maackii* to maintain dominance of a site by reducing or preventing germination of *L. maackii* seeds near established plants.

We also found that examining germination over time may be quite important in determining effects of allelopathy. For example, *Bromus* showed relatively similar total germination at the conclusion of the experiment, but more dramatic differences early. Overall, allelopathic effects on germination may not absolutely reduce the number of seeds that germinate but may delay their germination. These delays in germination have the potential to be ecologically significant, with species missing optimum times for establishment and growth (Verdu and Traveset 2005). In the case of *L. maackii*, these delays may be further complicated by altered patterns of growth and reproduction of herbaceous species in soils previously occupied by *L. maackii* (Cipollini and Dorning 2008).

For restoration ecologists managing invasions of *L. maackii*, this study could have important implications. Previous work has shown delays in the response of native plants to management of *L. maackii*, if native plants recover at all (Luken et al. 1997; Vidra et al. 2007). Delays may be explained by persistence of allelochemicals, or continued release from plants that persist in management areas. Our results also indicate that release from light competition in managed areas would not alleviate the strength of these allelopathic effects. Consequently, restoration of invaded areas may require re-introduction of native species, but only after the invasion is controlled and allelochemicals have broken down.

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