

Direct and Indirect Effects of Conditioned Soils and Tissue Extracts of the Invasive Shrub, *Lonicera maackii*, on Target Plant Performance

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ABSTRACT *Lonicera maackii* is an invasive Asian shrub naturalized in North America that has negative effects on forest understory vegetation. Aqueous extracts of leaves and roots of this plant have been shown to inhibit germination of several species. In this study, we examined the extent to which field-collected soils conditioned by *L. maackii* growth, with and without additions of *L. maackii* extracts, had effects on growth, morphology, and reproduction of *Arabidopsis thaliana* in a greenhouse setting. We also examined the effects of nutrient addition to the same soils on the performance of *A. thaliana*, whether plant responses to nutrients varied among *L. maackii*-conditioned and -unconditioned soils, and whether the effects of *L. maackii* extracts varied in soils with different histories and fertility. Plants grown in forest soil collected from a site where *L. maackii* was present grew slower and flowered later than plants grown in an unconditioned soil, but ended up being larger, with more leaves, branches and a higher total seed output. Nutrient addition had a positive effect on performance of *A. thaliana*, but when nutrients were added with extracts of *L. maackii* roots and leaves, the positive effects of nutrients were greatly diminished. Inhibiting plant responses to resource opportunities is a potentially important indirect effect of allelochemicals. Where direct effects of extracts on growth were seen, leaf extracts had a greater effect than root extracts. While the most important impacts of *L. maackii* in the field are undoubtedly due to competition for light and moisture, evidence for allelopathic effects of this plant is growing.

INTRODUCTION Invasive plants may impact native ecosystems in a variety of ways, from exerting resource competition on native plants to altering fire dynamics (Sakai et al. 2001). Identifying mechanisms of invasion and impacts of invasive plants in natural areas is important in order to determine their effect on biodiversity, to predict rates of spread, and to inform control and restoration efforts. Recently, allelopathy as an invasive mechanism has received renewed attention in the literature (Bais et al. 2003, Hierro and Callaway 2003). Plants can be considered allelopathic if they produce secondary metabolites that negatively impact other organisms, including neighboring plants and soil microbes, in an ecologically relevant context. Many examples exist of inhibitory effects of living invasive plants, their intact tissues, or

tissue extracts on germination, growth, and reproduction of other species that indicate the potential for allelopathy (e.g., Lawrence et al. 1991, Roberts and Anderson 2001, Bais et al. 2003, Hierro and Callaway 2003). While allelopathy can be exceedingly difficult to demonstrate in the field because of difficulties in separating its effects from resource competition (Bais et al. 2003), evidence is mounting that direct and indirect effects of chemicals produced by some invasive plants likely contributes to their impacts and spread in novel environments (e.g., Callaway and Aschehoug 2000).

Lonicera maackii (Rupr.) Maxim (Amur honeysuckle: Caprifoliaceae) is an Asian shrub invasive in forests and open areas of eastern and midwestern North America (Luken and Thieret 1995). This shrub has negative effects on understory herbs and tree seedlings that are due in large part to competition for light and soil resources

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(Hutchinson and Vankat 1997, Trisel 1997, Gould and Gorchoy 2000, Collier et al. 2002, Gorchoy and Trisel 2003, Miller and Gorchoy 2004, McClain et al. 2008). However, some of its effects on understory vegetation may be mediated by allelopathic chemicals, either directly or indirectly, but allelopathy has been little studied in this plant (but see Trisel 1997). In a companion paper, we demonstrated the ability of aqueous leachates of fresh leaves and roots of *L. maackii* to inhibit seed germination of the native annual, *Impatiens capensis*, the non-native biennial, *Alliaria petiolata*, and the non-native annual *Arabidopsis thaliana* in laboratory bioassays (Dorning and Cipollini 2006). The inhibitory effect of extracts generally increased with increasing concentration and was more pronounced with application of leaf extract than with root extract. However, when the same extracts were applied to seeds of *L. maackii* itself, germination was unaffected by some concentrations and promoted by others. This implied that *L. maackii* could successfully inhibit the germination of other plants, while not affecting, or even promoting, the germination of its own seeds. A subsequent study revealed the presence of several flavonoids and phenolic acids in methanol extracts of *L. maackii* leaves with potential roles in allelopathy (Cipollini et al. 2008b).

Although our initial studies indicated the potential for allelopathy by *L. maackii*, these studies were confined to Petri dish bioassays of leachates on filter paper, and focused only on the germination stage of target plants. Further work is required to add ecological relevance to these findings, including bioassays in field soil across a broader range of plant life stages (Cipollini et al. 2008a). In addition, interactive effects of extracts with relevant environmental factors, including variation in soil history and quality, are needed to predict effects in the field. In the current study, we selected *A. thaliana* as a target plant to further investigate the effects of *L. maackii* allelochemicals under more ecologically realistic conditions. As a self-fertile annual with a rapid life cycle, the use of *A. thaliana* facilitates allelopathic assessments across a range of life history stages in laboratory conditions. It also responds rapidly to environmental stimuli, including changes in soil resource availability. The seed germination response of *A. thaliana* to extracts of *L.*

maackii paralleled that of the native annual *I. capensis* (Dorning and Cipollini 2006). *Arabidopsis thaliana* possesses general allelochemical detoxification mechanisms like most plants (Baerson et al. 2005), but it is considered sensitive to allelochemicals (e.g., Bais et al. 2003; Pennacchio et al. 2005; Cipollini et al. 2008a, b). It is also non-mycorrhizal, thus excluding the possibility of allelopathic effects on mycorrhizae which has been implicated in the effects of some invasive plants (e.g., Roberts and Anderson 2001). While we use *A. thaliana* primarily as a model of a fast-growing annual plant, its range overlaps with that of *L. maackii* throughout its native and introduced ranges in North America, Europe and Asia (United States Department of Agriculture, Natural Resources Conservation Service 2007) and both species can be found growing in close proximity in the same field sites in North America (D. Cipollini, pers. obs.).

In this study, we examined the extent to which field-collected soils conditioned by *L. maackii* growth, with and without additions of *L. maackii* extracts, had effects on growth, morphology, and reproduction of *A. thaliana* in a greenhouse setting. Soils conditioned by *L. maackii* are presumably enriched in leaf- and root-associated molecules from *L. maackii*, but also likely vary from unconditioned soils in nutrient status, microbial composition, and other soil attributes (Blum 1998; Blum et al. 1993, 1999; Kourtev et al. 1998, 2002; Inderjit 2001). Studies using conditioned soils allow the exclusion of the effects of the living invader on light and moisture competition, and focus on changes in soil characteristics exerted previously by the invasive plant. In addition, putatively allelopathic compounds can exert their effects either directly through toxicity or indirectly through interactions with other factors important for plant growth, including soil nutrients (Inderjit 2001). To address the extent to which allelopathic effects of *L. maackii* vary with soil fertility, we also examined the effects of nutrient addition to the same field soils on the performance of *A. thaliana*, whether plant responses to nutrients varied among *L. maackii*-conditioned and -unconditioned soils, and whether the effects of *L. maackii* extracts varied in soils with different histories and fertility. We predicted that performance of *A. thaliana* would be

reduced in *L. maackii*-conditioned soils and in those treated with *L. maackii* extracts. In turn, we predicted that nutrients would generally benefit the performance of *A. thaliana*, and that this effect would be more noticeable in soils conditioned by *L. maackii* in which nutrient availability may have been reduced due to uptake by the invasive plant.

MATERIALS AND METHODS We used *Arabidopsis thaliana* (ecotype Columbia) in this study, as we had in several previous studies to demonstrate allelopathic effects of *Lonicera maackii* extracts and selected secondary metabolites (Dorning and Cipollini 2006; Cipollini et al. 2008a, b). Based on our experience, we consider the Columbia ecotype a "sensitive" ecotype, but variation in allelochemical responsiveness among *A. thaliana* ecotypes is unknown. However, the same level of inhibition of seed germination by extracts of *L. maackii* observed in this ecotype was observed in seeds from two wild populations of *A. thaliana* collected in Ohio (D. Cipollini, unpubl. data). Plants were grown from seed in a temperature-controlled greenhouse under ambient light in soils collected from a site where *Lonicera maackii* was absent (LM absent) and a nearby site where *L. maackii* was present (LM present), both located in the Wright State University Woods, Dayton Ohio. The site where *L. maackii* was absent had no evidence that honeysuckle had ever been growing there. The site where it was present was inhabited by numerous shrubs, the oldest of which had been there for at least 10–15 years, but was depauperate in understory herbs as is common under *L. maackii* thickets (Collier et al. 2002). The composition of the forest overstory was similar otherwise between sites, and the soils were grossly similar in texture and moisture holding capacity. Soil was collected from a ~3 m² area by removing leaf litter and haphazardly collecting the top 10 cm of forest topsoil. Field soils were mixed to break up large clumps and screened to remove rocks, roots, and other debris, placed in 150 ml pots, and moistened with distilled water. Soils were used within one week of collection from the field.

Leaf and root material for extracts were collected from a single mature *L. maackii* shrub in the Wright State University Woods in September 2003. Aqueous extracts were prepared from leaves and roots as in Dorning

and Cipollini (2006). Briefly, leaves were incubated without homogenization in distilled water (5 mL per g plant material) for 48 hours at room temperature. The leachate was then decanted, vacuum filtered through Whatman® 42 filter paper, and stored at 4°C (Roberts and Anderson 2001). Leaves were rapidly frozen and stored at –20°C until extraction, and extracts were stored at 4°C until their use in bioassays. Roots were first rinsed briefly in distilled water to remove soil, and then extracted following a similar protocol.

In each pot, several *A. thaliana* seeds were sown and thinned to one seedling per pot one week after germination. For each soil type (LM present or absent), eighteen pots were assigned to receive one of six treatments: 1) nutrient application [0.4 g/L Peter's 20-20-20 N-P-K, plus micronutrients (Grace-Sierra, Milpitas, California)], 2) *L. maackii* root extract application, 3) *L. maackii* leaf extract application, 4) nutrient and *L. maackii* leaf extract applications, 5) nutrient and *L. maackii* root extract applications, and 6) controls given only water. Each plant received 2 ml of each of its assigned treatment(s) pipetted into the soil near the base of the plant once per week starting when plants were thinned. Otherwise, pots were given distilled water as needed to keep the growing medium evenly moist.

The rosette diameter and total numbers of leaves present on plants in each pot were recorded six, eight, and ten weeks after the seeds were planted. The first appearance of flowers for each plant was recorded. All seeds from each plant were collected as they matured, and were dried and weighed as in Cipollini (2007). At the cessation of flowering, the height of the main stem was measured to the nearest 1.0 mm. The numbers of primary branches emerging from the main stem were counted along with the number of secondary branches present on primary branches, and the number of additional bolts arising from the base of the plant. Survival to maturity was also recorded throughout the experiment. Germination was not considered in this experiment, as our extract and nutrient treatments began after seedlings had emerged and were thinned.

Effects of the two soil types and six treatments on the size, morphology, phenology, and fitness of *A. thaliana* were first analyzed using multiple analysis of variance

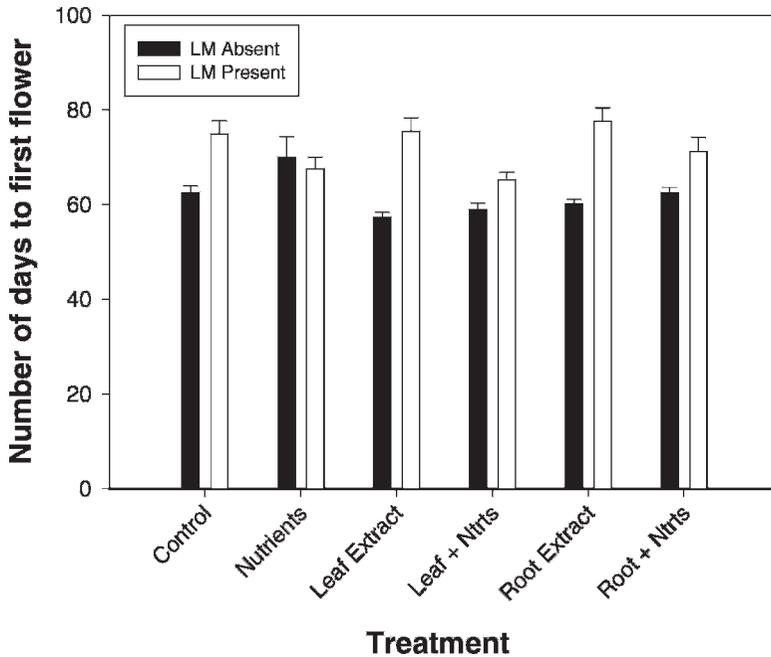


Figure 1. Number of days to first flower for *Arabidopsis thaliana* plants grown in forest soil conditioned by *Lonicera maackii* growth and treated with *L. maackii* leaf and root extracts and nutrients. Bars in this and all other figures represent the mean (\pm SE) of 16–18 replicates. LM Absent = forest soil not conditioned by *L. maackii* growth, LM Present = forest soil conditioned by *L. maackii* growth.

using the Wilk's Lambda test statistic. This was followed by a two-way analysis of variance for each variable separately, with the fixed factors soil type and treatment. The absence of treatments including both root and leaf extracts with and without additional nutrients precluded using a four-way full factorial design. Means were compared using Tukey's tests. All statistical analyses were conducted on SAS (Version 8.0, SAS Institute, Cary, North Carolina).

RESULTS In the MANOVA, size, morphology, phenology, and fitness of *Arabidopsis thaliana* plants varied among soil types ($F_{65,595} = 3.87$, $P < 0.0001$), treatments ($F_{85,556} = 4.17$, $P < 0.0001$), and the interaction of soil type and treatment ($F_{85,556} = 4.17$, $P < 0.0001$).

Overall soil conditioning effects. *Arabidopsis thaliana* plants grown in LM-absent soil flowered 11 days earlier on average than those grown in LM-present soil (Figure 1, Table 1). At six weeks, plants grown in the LM-absent soil had three more leaves on average than those grown in the LM-present soil (Figure 2A, Table 1). By week ten of the experiment (Figure 2C, Table 1), plants in the

LM-present soil had many more leaves than those in the LM-absent soil, a pattern that began to emerge by week eight (data not shown). Plants grown in the LM-absent soil had a 25% larger rosette diameter at week six than those grown in the LM-present soil (Figure 2B, Table 1). This relationship was reversed by week ten, when plants in LM-present soil had an 18% larger rosette diameter than those in LM-absent soil (Figure 2D, Table 1), also a pattern that began to emerge by week eight (data not shown). Plants grown in LM-present soil grew to be 3 cm taller on average than those grown in the LM-absent soil (Figure 3A, Table 2) and had a greater total seed mass than those in the LM-absent soil (Figure 3B, Table 2). While there was no significant effect of soil type or treatment on survival to reproduction, plants grown in the LM-absent soil tended to have higher survival (79%) than those in the LM-present soil (69%) (Figure 3C, Table 2). While the number of primary branches was similar overall in each soil type (Figure 4A, Table 2), plants grown in the LM-present soil had more secondary branches than those in the LM-absent soil (Figure 4B, Table 2). Plants in the LM-present soil averaged nearly six secondary

Table 1. F-values from ANOVA of the effects of *Lonicera maackii*-conditioned and unconditioned soils, nutrient and extract treatments, and their interaction on days to first flower and vegetative growth traits of *Arabidopsis thaliana*. Asterisks indicate significant effects: * $p = 0.05 < > 0.01$, ** $p = 0.01 < > 0.001$, *** $p = < 0.001$

Factor	Days To First Flower	Rosette Diameter Week 6	Number of Leaves Week 6	Rosette Diameter Week 10	Number of Leaves Week 10
Soil Type	65.81***	30.12***	24.39***	12.94***	63.02***
Treatment	3.33**	6.05***	6.67***	16.25***	13.52***
Soil × Trt	5.75***	3.70**	3.04*	2.99*	9.20***

branches while those in the LM-absent soil averaged just under two. Plants grown in the LM-present soil also had more additional bolts than those in the LM-absent soil (Figure 4C, Table 2).

Interactions between soils, extracts, and nutrients. In LM-absent soil, plants given nutrient application alone flowered later than those in all other treatments (Figure 1, Table 1). In contrast,

in LM-present soil, nutrient addition tended to speed flowering overall and plants treated with leaf extract plus nutrients flowered significantly faster than those treated with root extract.

At week six in the LM-absent soil, plants given root extract alone had more leaves than those given leaf extracts with or without nutrients (Figure 2A, Table 1). At the same time, plants in the LM-present soil given root

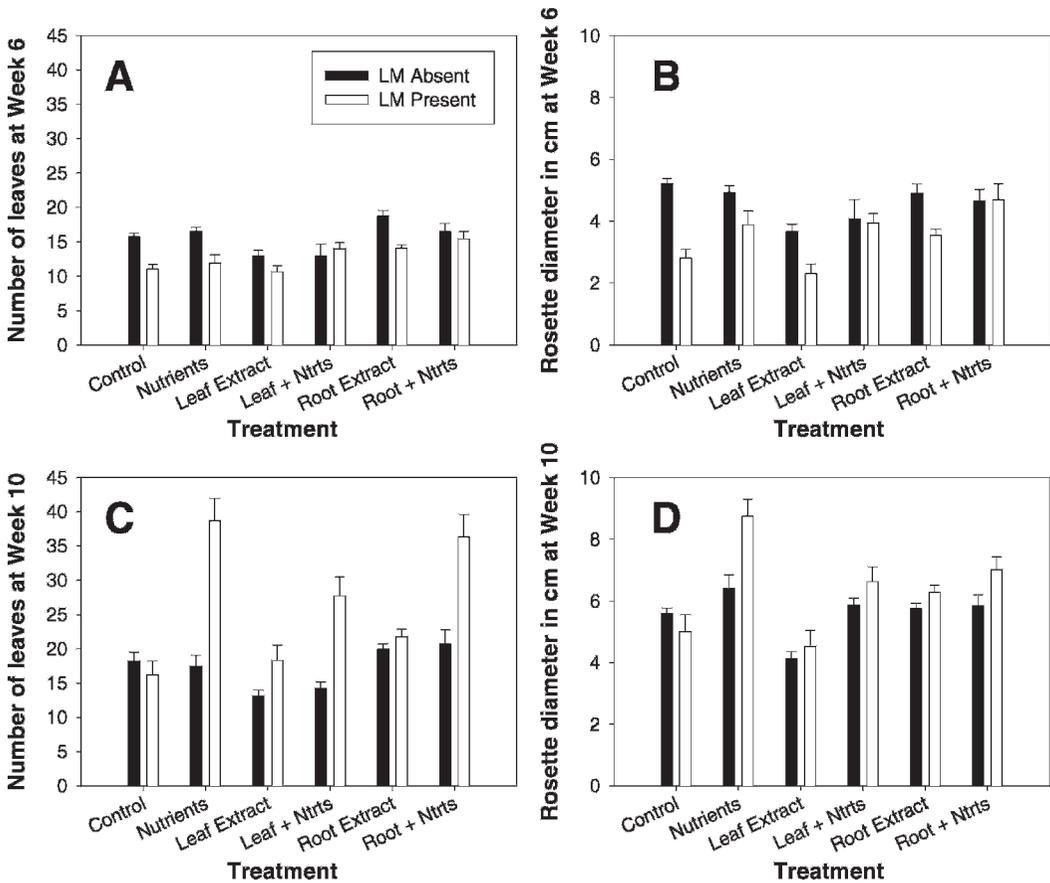


Figure 2. Vegetative growth characteristics of *Arabidopsis thaliana* plants grown in forest soil conditioned by *Lonicera maackii* growth and treated with *L. maackii* leaf and root extracts and nutrients. A. Leaf number at 6 weeks, B. Rosette diameter at 6 weeks, C. Leaf number at 10 weeks, and D. Rosette diameter at 10 weeks.

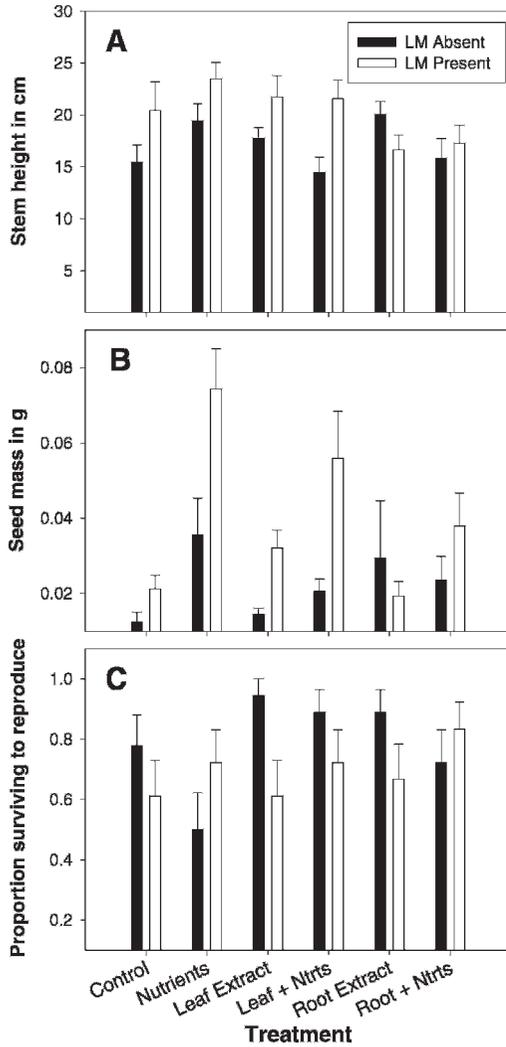


Figure 3. End of season growth and reproductive characteristics of *Arabidopsis thaliana* plants grown in forest soil conditioned by *Lonicera maackii* growth and treated with *L. maackii* leaf and root extracts and nutrients. A. Main stem height, B. Total seed mass, C. Proportion surviving to reproduce.

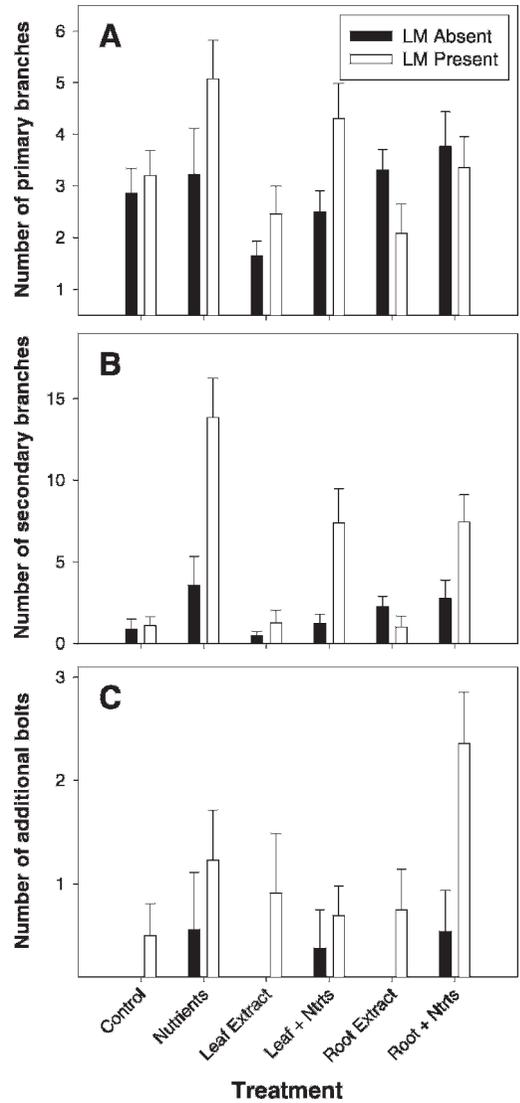


Figure 4. End of season architectural traits of *Arabidopsis thaliana* plants grown in forest soil conditioned by *Lonicera maackii* growth and treated with *L. maackii* leaf and root extracts and nutrients. A. Number of primary branches on the main stem, B. Number of secondary branches on primary branches, C. Number of additional bolting stalks arising from the base of the plant.

Table 2. F-values from ANOVA of the effects of *Lonicera maackii*-conditioned and unconditioned soils, nutrient and extract treatments, and their interaction on survival to reproduction and final height, seed production, and architectural traits of *Arabidopsis thaliana*. Asterisks indicate significant effects: * p = 0.05 < > 0.01, **p = 0.01 < > 0.001, *** p = < 0.001

Factor	Proportion Surviving	Stem Height	Total Seed Mass	Number of 1° Branches	Number of 2° Branches	Number of Additional Bolts
Soil Type	2.49	9.45**	20.87***	2.64	22.02***	13.85***
Treatment	1.05	1.83	7.51***	3.25**	8.10***	3.44**
Soil × Trt	2.19	2.45*	3.72**	2.42*	5.02***	1.35

extract plus nutrients had more leaves than those in the control and those given only leaf extract. At week ten in the LM-absent soil, plants treated with root extract alone or with root extract plus nutrients had more leaves than those treated with leaf extract alone or leaf extract plus nutrients (Figure 2C, Table 1). Plants treated with leaf extract also had fewer leaves than the control. At the same time in the LM-present soil, plants treated with nutrients alone or with root extract plus nutrients had many more leaves than those treated with both extracts alone and the control. Plants treated with leaf extract plus nutrients also had more leaves than those in the control. The tendency for leaf extracts to dampen plant responses to nutrients can be seen.

Patterns in the response of rosette diameter to the treatments largely paralleled those in leaf number. At six weeks in the LM-present soil, plants given leaf extract had a smaller rosette diameter than those in all nutrient treatments, regardless of extract addition (Figure 2B, Table 1). Control plants were also smaller than those given root extract plus nutrients. At the same time in the LM-absent soil, plants given leaf extract were smaller than those in all other treatments except the leaf extract plus nutrient treatment. At week ten in the LM-present soil, plants given nutrients alone were larger than those in all other treatments except the root extract plus nutrient treatment (Figure 2D, Table 1). Plants in the root extract plus nutrient treatment were larger than those in the control and leaf extract treatments, and those in the leaf extract plus nutrient treatment were larger than those in the leaf extract treatment. The tendency for root or leaf extracts to dampen responses to nutrients can be clearly seen in this soil type. At the same time in the LM-absent soil, plants treated with nutrients alone had the largest rosette diameter and those in the leaf extract treatment had the smallest diameter, by far. All other treatments were intermediate.

In the LM-absent soil, plants given nutrients or root extracts alone were taller at the end of the growing season than those given leaf extract plus nutrients, and plants given nutrients alone tended to be taller than the controls (Figure 3A, Table 2). In the LM-present soil, plants given root extract with and without nutrients tended to be shorter than those in the other treatments.

In the LM-absent soil, plants given nutrients alone had a greater total seed mass than those in the leaf extract and control treatments (Figure 3B, Table 2). In the LM-present soil, plants given nutrients alone had a greater total seed mass than those in all other treatments except the leaf extract plus nutrient treatment. Plants in the leaf extract plus nutrient treatment had a greater total seed mass than those in the root extract treatment. In both soil types, the tendency for extract addition to dampen increases in total seed mass in response to nutrients can be seen.

In the LM-absent soil, plants given nutrients alone tended to have poorer survival than those in the root extract, leaf extract, and leaf extract plus nutrient treatments (Figure 3C, Table 2). The proportion surviving to reproduce was similar among treatments in the LM-present soil.

In both soil types, plants in the leaf extract treatment had fewer primary branches than those in the nutrient and root extract plus nutrient treatments (Figure 4A, Table 2). In the LM-present soil, plants in the root extract treatment also had fewer primary branches than those in the nutrient treatment. Plants in the LM-present soil also tended to have the most primary branches when treated with nutrients, while those in the LM-absent soil had the most if they were given root extract (the treatment with the fewest branches in the LM-present soil).

In the LM-present soil, all plants receiving additional nutrients had many more secondary branches than plants not receiving additional nutrients (Figure 4B, Table 2). In the LM-absent soil, secondary branching was similar across treatments.

In both soil types, plants in the root extract plus nutrient treatment had more additional bolts than those in the leaf extract, root extract, and control treatments (Figure 4C, Table 2). In addition, plants grown in the LM-absent soil only had additional bolts in the three treatments where additional nutrients were applied.

DISCUSSION Invasive plants are known to influence the condition of the surrounding soil with effects on neighboring vegetation (Olson and Wallander 2002, Kourtev et al. 2002, Kourtev et al. 1998, Wolf et al. 2004). We show here that conditioning of forest soils by *Lonicera maackii* growth had marked effects

on size, morphology, phenology, and reproduction of *Arabidopsis thaliana*. In addition, the impact of added nutrients and *L. maackii* extracts also varied in soils with different histories.

In our study, *A. thaliana* plants grew faster and flowered earlier in soil collected from the site where *L. maackii* was absent, indicating that conditioning by *L. maackii* somehow influenced the ability of the soil to support rapid plant growth. In rosette species such as *A. thaliana*, vegetative growth (i.e., production of new rosette leaves) largely stops when flowering commences, and reproductive output is strongly correlated with rosette size (e.g., leaf area) attained at flowering (Cipollini et al. 2008a). With adequate access to light and moisture, plants grown in the soil collected from the site where *L. maackii* was present grew slower and flowered later than plants grown in unconditioned soil, but ended up being larger, with more leaves, branches and a higher total seed output. A similar pattern was seen in a study of the effects of lateral shading on *A. thaliana*, where laterally shaded plants (with no overhead shading and adequate access to moisture) grew more slowly than control plants, but attained a larger size before flowering and produced more seeds (Cipollini 2005). Plants grown in the LM-present soil also tended to have lower survival rates than those in the LM-absent soil. Coupled with the negative effects of *L. maackii* extracts and selected metabolites on germination (Dorning and Cipollini 2006, Cipollini et al. 2008b), our findings indicate that soil conditioned by *L. maackii* will inhibit or delay germination, possibly reduce survival of seedlings, and delay reproduction. However, surviving plants of annual species such as *A. thaliana* could produce more seeds given an extended vegetative growing period prior to reproduction. While this would appear to be beneficial, resource limitation caused by the invader itself in the field (i.e., reduced light to the understory) would likely prevent developmentally-delayed plants from achieving the increased size and seed production seen in the greenhouse. In addition, delayed phenology may alter a plant's ability to react to stimuli such as weather conditions and alter the plant's exposure to herbivores, pollinators, and competing plants. These indirect effects could have a dramatic effect on growth, survival, and reproduction in the

field (Singh and Pal 2003, Fathi et al. 2003). Effects of *L. maackii* on target plant phenology would have been missed if only selected endpoints, such as seed production, were examined.

While our results provide evidence for residual allelopathic effects in soils where *L. maackii* has grown, the mechanism of these effects is unclear. Delayed development could be a sub-acute effect of allelochemicals in the soil, or the result of alterations in nutrient availability or beneficial microbial activity in conditioned soils. It is possible that allelochemicals in conditioned soils are metabolized by soil microbes over time (barring fresh inputs from living *L. maackii* plants) and become a source of nutrients to plants or to microbes that indirectly benefit plants. This would explain why conditioned soils seemed to be "toxic" to plant growth during the early parts of the experiment, but ended up fostering the growth of large plants later in the experiment. It is well known that some common compounds associated with allelopathy, such as phenolic acids of the type found in *L. maackii*, can be readily metabolized by soil microbes to less toxic forms (Ohno 2001). Plants also display some general mechanisms to detoxify xenobiotic compounds (Baerson et al. 2005), and perhaps plants become more resistant to the effects of xenobiotics as they age. This also suggests that residual allelopathy in areas where *L. maackii* is removed in the field are unlikely to persist beyond one growing season. It is important to mention that our experiment was conducted on soils collected from only one site where *L. maackii* was absent and one where it was present. The two sites were close together, with broadly similar attributes, but differences in soil quality unrelated to the presence of *L. maackii* were possible.

The effects of adding *L. maackii* extracts or nutrients on the performance of *A. thaliana* were largely dependent on soil history. However, some general effects were seen across both soil types. For example, nutrient addition generally had a positive effect on growth and reproduction of *A. thaliana*, as expected. However, when nutrients were added with extracts of *L. maackii* roots and leaves, the positive effects of nutrients were greatly diminished. Although we have seen direct effects of *L. maackii* extracts on the performance of *A. thaliana* when added at higher

rates (Cipollini et al. 2008a), the most important effect of extract addition observed here was to constrain plant responses to nutrients, rather than direct effects. Extracts may have directly bound nutrients and inhibited their uptake, promoted soil microbial activity that competed for nutrients, or altered nutrient availability through changes in soil pH (e.g., Blum et al. 1993, Blum 1998, Blum et al. 1999, Inderjit 2001).

When direct effects of extracts on plant traits were seen, such as on rosette diameters and leaf numbers at ten weeks, leaf extracts had a greater effect than root extracts. This parallels previous findings on seed germination (Triesel 1997, Dorning and Cipollini 2006). *Lonicera* species are known to produce a variety of flavonoids, phenolic acids, and iridoids in their leaves (e.g., Flamini et al. 1997, Skulman et al. 2004). We identified two major flavones and their glucoside derivatives and several phenolic acids in methanol extracts of *L. maackii* leaves (Cipollini et al. 2008b), which are thought to be among the compounds responsible for allelopathic effects seen here. These compounds (or other unidentified compounds) may either reach higher concentrations in leaves than in roots or they may leach more readily from leaves than roots when incubated in water.

Despite having some general effects across soil types, certain effects of nutrient and extract addition varied with soil history. For example, nutrient addition had a much greater positive effect on growth and fitness in the *L. maackii*-conditioned soil than in the unconditioned soil, as predicted if *L. maackii* effectively extracted nutrients from the soil. Nutrient addition also sped flowering and enhanced survival in the conditioned soil, while slowing flowering and reducing survival in the unconditioned soil. This further suggests that conditioning by *L. maackii* growth not only contributed allelochemicals to the soil, but affected nutrient availability. In turn, because of the stronger response to nutrients alone observed in the conditioned soil, constraints on the response to nutrients by *L. maackii* extracts were more apparent in this soil type. This pattern is similar to the "soil sickness" phenomenon seen in agricultural situations, where previous growth by a given crop and practices associated with its culture lead to depressed crop yields in subsequent plantings. Alterations in nutrient availabili-

ties and allelopathy are among the factors believed to contribute to this phenomenon (Politycka 2005).

In conclusion, we have demonstrated that a soil conditioned by *L. maackii* growth altered size, phenology, survival, architecture, and reproduction of a target species. In turn, leaf and root extracts of *L. maackii* were capable of inhibiting the positive response of the plants to nutrient availability, a potentially important indirect effect of allelochemicals. Allelopathic effects mediated by mycorrhizae were excluded through the use of a non-mycorrhizal target plant in this study, but additional effects mediated by mutualistic fungi or bacteria may exist. While the most important impacts of *L. maackii* in the field are undoubtedly due to competition for light and moisture, evidence for direct and indirect allelochemical effects of this plant on neighboring plants and soils is growing.

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