

## ORIGINAL ARTICLE

# Interpopulation variation in allelopathic traits informs restoration of invaded landscapes

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**Abstract**

Invasive species can show substantial genetic variation in ecologically important traits, across ranges as well within the introduced range. If these traits affect competition with native species, then management may benefit from considering the genetic landscape of the invader. Across their introduced range, *Alliaria petiolata* populations vary in their investment in allelopathic traits according to invasion history, which could lead to gradients of impact on native species. Red oak (*Quercus rubra*) seedlings were transplanted into eight *A. petiolata*-invaded sites that varied in their invasion history and allelochemical concentrations. At each site, an invader removal treatment was crossed with experimental inoculations of native soil biota, to test whether the benefits of these restoration actions differed across invader populations. *Q. rubra* seedlings grew faster in invader populations with a longer invasion history and lower allelochemical concentrations. Invader removal and soil inoculation interacted to determine seedling growth, with the benefits of soil inoculation increasing in younger and more highly allelopathic invader populations. A greenhouse experiment using soils collected from experimentally inoculated field plots found similar patterns. These results suggest that the impact of this invader varies across landscapes and that knowledge of this variation could improve the efficacy and efficiency of restoration activities.

**Introduction**

Although bottlenecks during exotic introductions are often assumed to result in genetically depauperate populations of invasive species, there are many examples of substantial genetic variation among and within invasive populations in their new range (Bossdorf et al. 2005; Durka et al. 2005; Lavergne and Molofsky 2007; Dlugosch and Parker 2008). For instance, multiple introductions from genetically distinct source populations can allow for increased local genetic diversity and novel recombinations (Lavergne and Molofsky 2007; Verhoeven et al. 2011). Heterogeneous selection pressures across the introduced range may also lead to population differentiation in important traits. The development of clines in the invader's new range suggests not only that selection pressures can vary across the range, but that invaders can respond rapidly to this selection (Gilchrist et al. 2004; Keller et al. 2009). The invasion process itself may lead to shifting

selection pressures. On the leading edge of an invasion, traits involved in dispersal, establishment, and competition with native species may be favored, while in long invaded areas fitness may be determined more by intense intraspecific competition as aggressive invaders extirpate native species. For example, invasive cane toads in Australia from the leading edge of the invasion have longer legs and can move farther per night than those in the interior of the range (Phillips et al. 2006; Brown et al. 2007; Phillips 2009), suggesting that selection favors increased dispersal ability at the leading invasion edge. If invaders respond to these shifting selection pressures, this could lead to mosaics of invader impacts across a landscape.

While the presence of genetic variation among and within introduced populations is well documented, less research has addressed how the control of invasive populations, or restoration of invaded landscapes, could take advantage of this genetic structure. If invader impacts

vary across space, then managers might benefit from prioritizing control efforts on the most aggressive populations (Lankau et al. 2011). On the other hand, restoration efforts may be most successful in areas where the per-capita impact of the invader is relatively low. Knowledge of the trait variation across a landscape may also inform the most successful management interventions. For instance, genetic differentiation among invasive California wild radish (*Raphanus sativus*) in response to climatic gradients has resulted in different sensitivities to demographic transitions, such that the optimal life-stage for management differs among the populations (Ridley and Ellstrand 2010).

Allelopathic traits provide an excellent system in which to study variation in invader impact across a landscape. The allelochemicals of exotic species may be especially toxic to natives with no co-evolved resistance traits (Callaway and Ridenour 2004; Callaway et al. 2008). However, the selective value of allelopathic traits may vary across an invasion front, as allelochemicals are predicted to be favored under interspecific, but disfavored under intraspecific, competition (Lankau and Strauss 2007; Lankau 2008). Thus, one could predict an allelopathic invader to have the greatest per capita impact on the invasion edge, where selection maintains allelochemicals at high levels, and weaker impacts in long established populations, where intense intraspecific competition has led to reduced investment in allelochemicals.

*Alliaria petiolata* is one of the most widespread invaders of forest understories in the northeast and Midwestern United States. Its success has been attributed to several factors, including the production of secondary compounds with allelopathic effects on other plants and soil microbes (Rodgers et al. 2008). These novel compounds may be especially effective in the introduced range owing to the naivety of soil microbes (Callaway et al. 2008; Barto et al. 2010a). A number of studies have investigated the allelopathic and/or soil-mediated impacts of this species and found variable results ranging from strong (Roberts and Anderson 2001; Stinson et al. 2006) to relatively weak (Burke 2008) to neutral effects (McCarthy and Hanson 1998). These differences may derive in part from variation among populations in their investment to allelochemicals. Lankau et al. (2009) found lower allelochemical concentrations in *A. petiolata* root tissue in individuals derived from older source populations when grown in a common environment, suggesting that invasive populations tend to reduce investment to allelopathy over time. In a greenhouse study, *A. petiolata* individuals with higher concentrations of a putative allelochemical (glucosinolates) had greater impacts on soil communities and native tree seedlings (Lankau et al. 2009; Lankau 2011b). Analysis of neutral molecular markers suggested

that this pattern was not because of genetically distinct invasions occurring at different times, as there was no relationship between estimated population age and genetic relatedness (Lankau et al. 2009), and there was no relationship between invasion age and 15 soil abiotic variables in a different study (Lankau 2011a). Of course, invasive spread is rarely uniform in space, and populations may be established both along a spreading edge and via propagules from older populations. Additionally, the evolutionary processes resulting in decreased allelochemical concentrations will also not proceed uniformly in all populations. Nevertheless, while invasion history is not a perfect predictor of allelochemical concentrations (explaining about 30% of the variation in chemical traits, Lankau et al. 2009), geographic variation in glucosinolate concentrations among *A. petiolata* populations, due in part to different invasion histories, could lead to variable impacts on native species across a landscape.

Alteration in soil microbial communities has been implicated in the success of numerous invasive plants (Wolfe and Klironomos 2005). However, this research is generally conducted in controlled conditions because of the difficulty of manipulating soil communities in the field. A meta-analysis by Kulmatiski et al. (2008) found only seven published studies of plant–soil feedbacks performed in the field, and none of these studies differentiated between the effects on soil biota versus abiotic factors. Many studies have used fungicide applications to test the role of soil fungi in plant invasions (Callaway et al. 2004a; Nijjer et al. 2007); although these can provide strong experimental evidence for a net effect of fungi on plant growth, they are less suited to distinguishing the more subtle microbial community shifts often associated with invasive plants.

In this study, I tested whether the growth of native tree seedlings transplanted into a number of *A. petiolata*-invaded sites was influenced by the invasion history or allelochemical traits of the invader population and whether these effects were mediated through soil communities, as has been suggested for this invader (Stinson et al. 2006). To investigate soil biota effects, I planted seedlings of the native tree *Quercus rubra* in eight different sites with either a sterilized or living soil plug from a common, uninvaded site. With this approach, growth in the sterilized treatment should reflect the soil community present at each site, while growth in the live soil treatment will reflect both the local and experimentally added soil communities. Thus, the difference between the treatments can quantify the variation in soil community effects among sites. This is especially appropriate for a species like *A. petiolata*, where the soil community effect is suspected to primarily involve a reduced abundance or altered composition of a specific functional group

(mycorrhizal fungi in this case) (Stinson et al. 2006; Burke 2008; Wolfe et al. 2008; Barto et al. 2011b).

In addition to understanding how invader impacts vary over space and time, this experiment also sought to test whether improved awareness of this variation could inform restoration strategies. At each site, I tested the independent and combined effectiveness of two strategies – local removal of the invader through weeding and restoration of soil communities via the planting of preinoculated native tree seedlings. Soil community inoculations are not commonly used to combat invasive species, but have been used in forestry projects to promote tree seedling establishment and growth (Schwartz et al. 2006; Vellinga et al. 2009; Liang et al. 2010). Specifically, I tested whether these strategies improved the survival and growth of transplanted *Q. rubra* (northern red oak) seedlings overall and whether their relative effectiveness varied among sites according to the invasion history or allelochemical concentration of the *A. petiolata* population. If so, then restoration outcomes could be improved with additional understanding of the variation among invasive populations.

I predicted that (i) transplanted seedlings should grow faster or survive better in older *A. petiolata* populations and/or those with lower allelochemical concentrations, (ii) seedlings should perform better when planted with native soil biota versus sterilized soil plugs and when *A. petiolata* has been experimentally removed, and (iii) the relative effect of these treatments should be greatest in invasive populations with the strongest soil-mediated impacts (i.e., younger and more highly allelopathic populations). Thus, I predict that restoration efforts will be more successful in older invasions or those with lower allelochemical concentrations compared with younger or more highly concentrated sites. Additionally, I predict that the value of the two restoration strategies (invader removal and soil community inoculation) will be relatively greater at sites with more aggressive invader populations (owing to their age or allelochemical concentration).

## Methods

### Study species and sites

*Alliaria petiolata* is a biennial native to Europe and introduced to North America ~150 years ago (Nuzzo 1993). Like other members of the Brassicaceae, it is nonmycorrhizal and produces glucosinolates, a class of secondary compounds derived from amino acids. *Alliaria petiolata* produces two glucosinolates (allyl and benzyl) in its root tissue (Vaughn and Berhow 1999), as well as other unique secondary compounds (alliarinose and iso-6- $\beta$ -vitexin) and high levels of cyanide in its leaves (Haribal and Renwick 1998; Haribal et al. 2001; Cipollini and Gruner 2007).

*Quercus rubra* has wide habitat tolerances and a broad distribution throughout Illinois USDA 2011 and the ability to form connections with both arbuscular and ectomycorrhizal fungi (Dickie et al. 2001). Poor oak regeneration is a serious conservation concern in eastern and central North American forests (Nowacki and Abrams 2008), and *Q. rubra* was previously shown to respond differently to *A. petiolata* genotypes from young versus old populations in a greenhouse setting (Lankau et al. 2009).

The experiment was performed in eight sites spread across north and central Illinois that varied in invasion history and allelochemical concentration. Site locations and attributes of the *A. petiolata* population are presented in Table 1. The invasion history for each site was estimated from herbarium records. Over 600 dated herbarium specimens (Nuzzo 1993) were used to make a spatially interpolated age map by year of first report (see Lankau et al. 2009).

### Quantifying allelochemical concentrations

Allelochemical production was measured both on naturally occurring *A. petiolata* plants at each site and on a set of greenhouse-grown plants to focus on genetic differences among populations. At each site in May of 2010, 10

**Table 1.** Characteristics of eight sites and their resident *Alliaria petiolata* populations used in this study.

Site	Latitude	Longitude	Estimated age (years)	Field (Glucosinolate) ( $\mu\text{mol/g}$ )	Greenhouse (Glucosinolate) ( $\mu\text{mol/g}$ )	% Cover <i>A. petiolata</i>	<i>A. petiolata</i> biomass ( $\text{g/m}^2$ )
Brewer Property	39.45	-88.10	22	3.69	4.36	23.75	27.39
Hidden Springs State Forest	39.32	-88.69	25	3.05	4.37	40.00	106.46
Farmdale Reservoir	40.68	-89.50	27	3.65	3.95	32.50	36.16
Illini Plantations	40.08	-88.21	29	2.60	4.12	47.50	80.90
Lowden State Park	42.03	-89.32	30	2.27		35.00	58.48
Lowden-Miller State Forest	41.96	-89.36	31	1.18	2.86	47.50	70.55
Skokie River Nature Preserve	42.26	-87.85	45	1.17	1.99	65.00	111.26
Healy Road CCFP	42.10	-88.22	48	2.56	3.12	65.00	105.89

first-year rosettes were haphazardly selected from within the experimental area. Plants were carefully dug from the ground, shaken to remove as much soil as possible, and then a subsample of fine roots were collected and placed immediately into 95% methanol. Secondary roots (<1 mm diameter) were pulled from the main taproot randomly from across the root system until approximately 10 mg of tissue had been collected. In many cases, this was an exhaustive sample of all secondary roots.

As part of a larger study (see Lankau et al. 2009), five individuals per population (each from a separate maternal family) were grown in a common greenhouse environment. This sample size is insufficient to determine the levels of genetic variation within populations, but the primary goal was to estimate the relative ranking of genetic investment to these traits among populations. Plants grew in a sterilized background soil with a small inoculum of live soil (50 mL in a 600-mL pot) collected from an uninvaded forest (using the same inocula for all plants). The live soil was collected from forest with no history of *A. petiolata* invasion (the Vermillion River Observatory in eastern Illinois). *Alliaria petiolata* is present in the surrounding area and has been prevented from establishing at this site because of vigilant monitoring (S. Buck, pers. comm.). After 3 months of growth, plants were removed from pots, shaken free of soil, and approximately 10 mg of fine roots collected as described earlier for field samples. Root samples from both field and greenhouse plants were weighed and ground by bead beating prior to extraction and analysis (see Lankau et al. 2009 for details).

Glucosinolates and flavonoids were quantified by HPLC (see Lankau et al. 2009). Population mean glucosinolate levels were positively correlated between the greenhouse and field collected plants ( $r = 0.75$ ,  $P = 0.01$ ,  $n = 7$ ). Unfortunately, seeds from one of the eight field sites did not germinate, so data from this site were removed in analyses involving greenhouse determined allelochemical concentrations.

### Field experimental design

One *Q. rubra* seedling was planted into 16 plots at each of eight sites (total of 128). At each site, I factorially crossed an *A. petiolata* removal treatment with a soil inoculation treatment. All seedlings were germinated from seed in March and grown in 165-mL cone-tainers (Stuewe and Sons, Tangent, OR) for 8 weeks prior to planting. Seeds were obtained from a private seed collector, who collected from several naturally occurring trees in central Illinois (Ray Herman, pers. comm.) For plants in the live soil treatment, the seedlings grew in field soil collected from the Vermillion River Observatory. Soil was collected

from several areas underneath a mature oak-hickory canopy and homogenized – *Q. rubra* is a common species at this site, although I did not explicitly attempt to collect soil from directly underneath *Q. rubra* trees. Seedlings in the sterile soil treatment were grown in this same field-collected soil after it had been subjected to repeated rounds of steam sterilization (2 h each round). A subsample of roots taken from eight live and eight sterile soil seedlings just prior to planting found higher rates of ectomycorrhizal root tip colonization on the live soil seedlings ( $9.81 \pm 0.017\%$  SE versus  $5.55 \pm 0.017\%$  SE, LR = 4.77,  $P = 0.03$ , generalized linear model with beta distribution). In this case, four to five sections of fine roots (<2 mm diameter), approximately 8–10 cm long, were collected from the selected seedlings, randomly from across the root system, after removal from the cone-tainer and just prior to planting in the field plot. In previous studies using this same soil, steam sterilization proved effective at reducing or eliminating ecto- and arbuscular mycorrhizal colonization and overall fungal diversity (Lankau 2011b).

In half of the plots, all *A. petiolata* individuals in a 1 m<sup>2</sup> square around the two planted seedlings were weeded in April 2008 and again in May 2009. In both years, the percent cover of *A. petiolata*, other plants, and bare ground was estimated from each plot prior to weeding, and for *A. petiolata* removal plots, the weeded biomass was collected, dried, and weighed.

*Quercus rubra* seedlings were planted in the field in May 2008. Survival, stem height, and diameter were recorded on all seedlings in July and October 2008 and May and September 2009. Seedlings that survived to the last sampling date were left in the field, to allow for the assessment of longer-term effects in the future. Final stem diameter in September 2009 was used in all analyses, as this metric was the best predictor of total above- and below-ground seedling biomass in greenhouse grown seedlings ( $R^2 = 0.40$ ,  $N = 159$ ,  $P < 0.0001$ ;  $R^2 = 0.10$  for stem height). Analysis of stem height and survival is available in the Supporting Information (Tables S1 and S2).

### Greenhouse experimental design

To further explore the role of soil communities in driving patterns in the field experiment and to test whether the soil inoculation treatment lead to persistent changes in soil quality two growing seasons after the original planting, a small greenhouse experiment was performed using soils collected from the field plots in the fall of 2009. Thus, these soil collections had potentially been modified by 2 years of field treatments (removal of *A. petiolata* and planting of inoculated oak seedlings). A soil core (2 cm diameter by 10 cm deep) was taken from each plot, 10 cm away from the experimental oak seedling. A small

volume (50 mL) of live soil from one plot of each treatment combination per site was used to inoculate a 600-mL greenhouse pot, with the remaining volume filled with a steam-sterilized potting soil (for a total of 32 pots). I used soil from a single plot per site per treatment combination so that I could ensure that I only used soils from plots where the experimental *Q. rubra* seedling survived to the end of the experiment (in many sites only one of the original four seedlings survived). Sample size was limited by the availability of germinable acorns. One germinated *Q. rubra* acorn was added to each plot, and the seedlings were allowed to grow for 3 months. After this time, above- and below-ground biomass was collected, dried, and weighed. Additionally, 5–6 sections of secondary roots (approximately 10 cm, with associated higher order roots) were taken for quantification of ectomycorrhizal root tips. For each replicate, the total number of mycorrhizal and nonmycorrhizal root tips was determined under a dissecting microscope. Root sections were placed in a Petri dish with a 1-cm grid, and the number of root intersections with the grid recorded as a measure of root length surveyed for colonization (Newman 1966).

### Analysis

Final stem diameters from the field experiment were analyzed with a series of linear models. Seedlings that did not survive to the final census were entered as zeroes. The four replicates of each treatment combination within a site were averaged prior to analysis, yielding one score for each treatment combination per site to avoid pseudoreplication (eight sites  $\times$  four treatment combinations = 32 averaged points, so that each linear model has 32 degrees of freedom). Three types of linear models were used to address the three main questions of this study:

1 Does *A. petiolata* removal or soil inoculation increase oak seedling growth? I used a factorial ANOVA with *A. petiolata* removal, soil inoculation, and their interaction as factors, with site as a random factor.

2 Does oak seedling growth covary with the estimated age or allelochemical concentration of the *A. petiolata* population? I performed a series of ANCOVAs with *A. petiolata* removal and soil inoculation as factors and either population age or mean allelochemical concentrations as a covariate, with all interactions. Covariates were tested separately as there was not enough power to include multiple covariates and their various interactions in the same model. Stem diameter was also regressed against each covariate separately for each treatment combination ( $n = 7$  or 8 for each separate regression).

3 Does the relative importance of the experimental treatments within sites vary across gradients of population age or allelochemistry? I took the residual values for stem

diameter from an ANOVA with site as the only variable and then ran the same series of ANCOVA's as in 2) with the residual values. As the covariates are population level measures (i.e., one value per population), the variation owing to the main effect of the covariate in the previous models was absorbed into the site effect. Thus, the significant interactions between the covariate and experimental treatments test whether one treatment increases stem diameter relative to the others at that site as the level of the covariate increases or decreases.

Residuals of all models met assumptions of normality and homoscedasticity without transformation, with the exception of survival, which was analyzed with a generalized linear model with a binomial distribution and logit link. Residual values were normally distributed and homoscedastic, despite high mortality, primarily because I used averaged values of four subsamples as my replicates, such that there were no structural zeroes in the final averaged data set.

Several potential allelochemicals were measured, but only glucosinolate concentration in root tissue was a significant predictor of seedling growth, so analyses using the other potential allelochemicals (flavonoids in leaf or root tissue and glucosinolates in leaf tissue) are not presented. This finding is consistent with previous studies in this system (Lankau et al. 2009). After 8 weeks of growth in the greenhouse (but prior to planting), seedlings grown in live soil had significantly thinner stems ( $3.11 \pm 2.01$  mm vs  $3.26 \pm 3.75$  mm,  $P = 0.001$ ). Therefore, initial stem diameter and height at planting were included in all linear models.

The greenhouse experiment was analyzed with models similar to 2) previously. As there was only one seedling per treatment combination per site, it was not necessary to average replicates prior to analysis. Additionally, as the seedlings grew in a common greenhouse environment, adding a site effect did not increase explanatory power. Seedling biomass was analyzed with linear models as the data were normally distributed. Percent ectomycorrhizal colonization (# ectomycorrhizal root tips/total # of tips) was analyzed with a generalized linear model with a beta distribution and log link, as the observed variance was substantially greater than predicted by a binomial distribution. Significance of each effect in the generalized linear model was assessed with likelihood ratio tests, comparing the improvement of fit of a model including the effect and one without the effect (as well as any interactions that include the given effect). As with the field experiment, models included the experimental treatments and all interactions with one of the three covariates (field glucosinolate concentration, greenhouse glucosinolate concentration, or estimated population age). The position of the pot in the greenhouse racks was included as a

covariate for both seedling biomass and ectomycorrhizal (ECM) colonization. Additionally, the number of root-grid intersections was included as a covariate in the ECM analysis to control for variation in the root length measured. All analyses were performed in the R statistical language, using either the `lm` function in the base package Team 2005 or the `betareg` function in the `betareg` package (Cribari-Neto and Zeileis 2010).

## Results

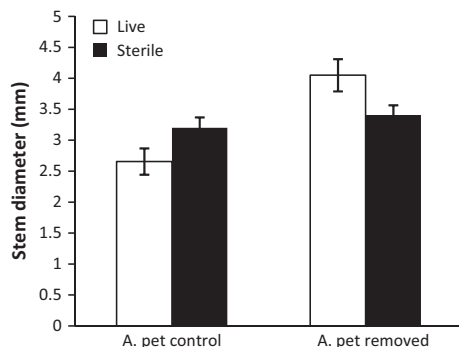
### Field experiment

1 Does *A. petiolata* removal and/or soil inoculation improve oak seedling growth?

In the field experiment, red oak seedlings grown in live soil grew larger than those grown in sterile soil, but only when *A. petiolata* was removed from the immediate area (Fig. 1). When the *A. petiolata* individuals were left undisturbed, oak seedlings in live soil grew less than those in sterile soil (Fig. 1), resulting in a significant interaction between the two treatments (*A. petiolata* removal,  $F_{1,32} = 11.46$ ,  $P = 0.003$ ; Soil restoration,  $F_{1,32} = 0.046$ ,  $P = 0.833$ ; *A. pet* \* Soil,  $F_{1,32} = 6.33$ ,  $P = 0.02$ ).

2 Does oak seedling growth vary with the age and/or allelochemical concentration of the *A. petiolata* population?

In 'natural' field conditions (ambient *A. petiolata* densities and no outside soil biota inoculation), oak seedlings grew larger in sites with a longer history of *A. petiolata* invasion (Table 2) and lower mean root glucosinolate concentrations, whether measured from field or greenhouse-grown plants (Table 2). These patterns were in the same direction for all four treatment combinations and explained a large amount of variation in seedling growth among sites (Fig. 2, Table 2).



**Figure 1** Final stem diameter of *Quercus rubra* seedlings by experimental treatment (*Alliaria petiolata* control or removed, live or sterilized soil inoculation) in the field experiment. For visual clarity, absolute means are graphed with standard errors from a model that controls for average differences among sites. For statistical comparisons, see text.

However, the greenhouse glucosinolate trend was significantly stronger for seedlings planted in sterilized versus live soil (Table 3). These patterns were broadly similar for *Q. rubra* stem height and survival (see Supporting Information) and were primarily driven by differences in survival, as the patterns were weaker when dead replicates were removed from the analysis (see Supporting Information).

In a partially observational study such as this, interpretations of covariates can be confounded by correlated gradients. For each site, I had data on field and greenhouse glucosinolate concentrations, estimated population age, latitude, longitude, % cover of *A. petiolata*, and % cover of other plants, resulting in 21 possible correlations. After correcting for multiple comparisons using the false discovery rate (Benjamini and Hochberg 1995), significant positive correlations were found between % cover of *A. petiolata* and population age, and significant negative correlations were found between greenhouse glucosinolate concentrations and latitude, and % cover of other plant species with both % cover of *A. petiolata* and population age ( $r = 0.92$ ,  $-0.93$ ,  $-0.86$ , and  $-0.85$ , respectively,  $n = 8$ ,  $P < 0.01$  for all). When these six covariates were entered into the same model, greenhouse glucosinolate concentration was the only one to remain a significant predictor of oak stem diameter. However, there were not sufficient degrees of freedom to test the interactions of all the covariates with the experimental treatments in the same model.

3 Does the relative importance of the experimental treatments within sites vary across gradients of population age and/or allelochemistry?

When controlling for overall variation among the field sites, seedlings planted with live soil outperformed those in sterilized soil in young and high glucosinolate populations, when *A. petiolata* was removed. However, in older and low glucosinolate populations, growth in the various treatments was more similar (Fig. 2). Overall, the relative effect of soil inoculation tended to decrease with population age and increase with glucosinolate concentrations, resulting in significant interactions between the two covariates and the soil treatment (Table 3). Again, these results were broadly similar for stem height and were primarily driven by mortality (Supporting Information).

### Greenhouse experiment

Oak seedling growth in greenhouse pots inoculated with soils from field plots mirrored the pattern observed in field grown seedlings. In soils from low glucosinolate *A. petiolata* populations, seedlings tended to grow larger in soils from plots with sterile versus live inoculations (Fig. 3A,

**Table 2.** Coefficients of determination, correlation coefficients, and *P*-values for correlations of *Quercus rubra* stem diameter in the field experiment versus *Alliaria petiolata* population age or glucosinolate concentrations measured from naturally occurring field plants or plants grown in a common greenhouse (each site as a unique replicate, *n* = 8 for estimated age and field glucosinolate concentrations, and *n* = 7 for greenhouse glucosinolate concentrations). (A) refers to correlations to raw diameter data, while (B) refers to correlations between covariates and residual values for stem diameter after removing overall site differences.

Treatment		Predictor								
A. pet	Soil	Estimated age			Field (Glucosinolate)			Greenhouse (Glucosinolate)		
		<i>R</i> <sup>2</sup>	<i>r</i>	<i>P</i>	<i>R</i> <sup>2</sup>	<i>r</i>	<i>P</i>	<i>R</i> <sup>2</sup>	<i>r</i>	<i>P</i>
(A) <i>Q. rubra</i> stem diameter (raw)										
Con	Live	0.42	0.65	0.080	0.53	-0.72	0.042	<b>0.84</b>	<b>-0.92</b>	<b>0.004</b>
Con	Ste	0.54	0.74	0.038	0.45	-0.67	0.070	<b>0.86</b>	<b>-0.93</b>	<b>0.003</b>
Rem	Live	0.36	0.60	0.113	0.47	-0.69	0.061	0.70	-0.84	0.018
Rem	Ste	0.59	0.77	0.026	<b>0.65</b>	<b>-0.80</b>	<b>0.016</b>	<b>0.94</b>	<b>-0.97</b>	<0.0001
(B) <i>Q. rubra</i> stem diameter (residual)										
Con	Live	0.11	-0.33	0.422	0.03	0.18	0.661	0.17	0.41	0.366
Con	Ste	0.23	0.48	0.225	0.01	0.09	0.841	0.13	-0.35	0.435
Rem	Live	0.24	-0.49	0.223	0.13	0.37	0.371	0.28	0.53	0.222
Rem	Ste	<b>0.67</b>	<b>0.82</b>	<b>0.013</b>	<b>0.75</b>	<b>-0.87</b>	<b>0.005</b>	<b>0.94</b>	<b>-0.97</b>	<b>&lt;0.0001</b>

Italicized values are significant at *P* < 0.05 (uncorrected), while bold values are those that retain significance after correcting for the false discovery rate Benjamini and Hochberg 1995; A. pet, *A. petiolata* removal treatment (Con, control, Rem, removed), Soil, soil inoculation treatment (Live, live inoculum, Ste, sterile inoculum).

Table 4). However, this situation reversed in soils from high glucosinolate *A. petiolata* populations, resulting in a significant soil treatment by glucosinolate concentration interaction, regardless of whether glucosinolate concentrations were estimated from field- or greenhouse-grown *A. petiolata* plants (Table 4). Population age was not a significant predictor of seedling growth (Table 4).

The patterns of oak seedling growth may be partially explained by the abundance or infection potential of ectomycorrhizal fungi in the soil inocula. The proportion of ectomycorrhizal root tips showed a significant interaction between soil inoculation treatment and greenhouse measured glucosinolate concentrations, as seen for seedling biomass (Table 4, Fig. 3B). In soils from sterile plots, ectomycorrhizal abundance was high in low glucosinolate plots but decreased with increasing glucosinolate concentrations (Fig. 3B). In soils from plots with a living inoculation, ectomycorrhizal abundance was relatively constant across the glucosinolate gradient (Fig. 3B). However, these patterns were not significant when using the glucosinolate concentrations of field-grown plants. Additionally, there was a surprising pattern of lower ECM colonization in the *A. petiolata* removal plots, when controlling for other factors, although this difference did not appear to translate into higher seedling growth in those soils.

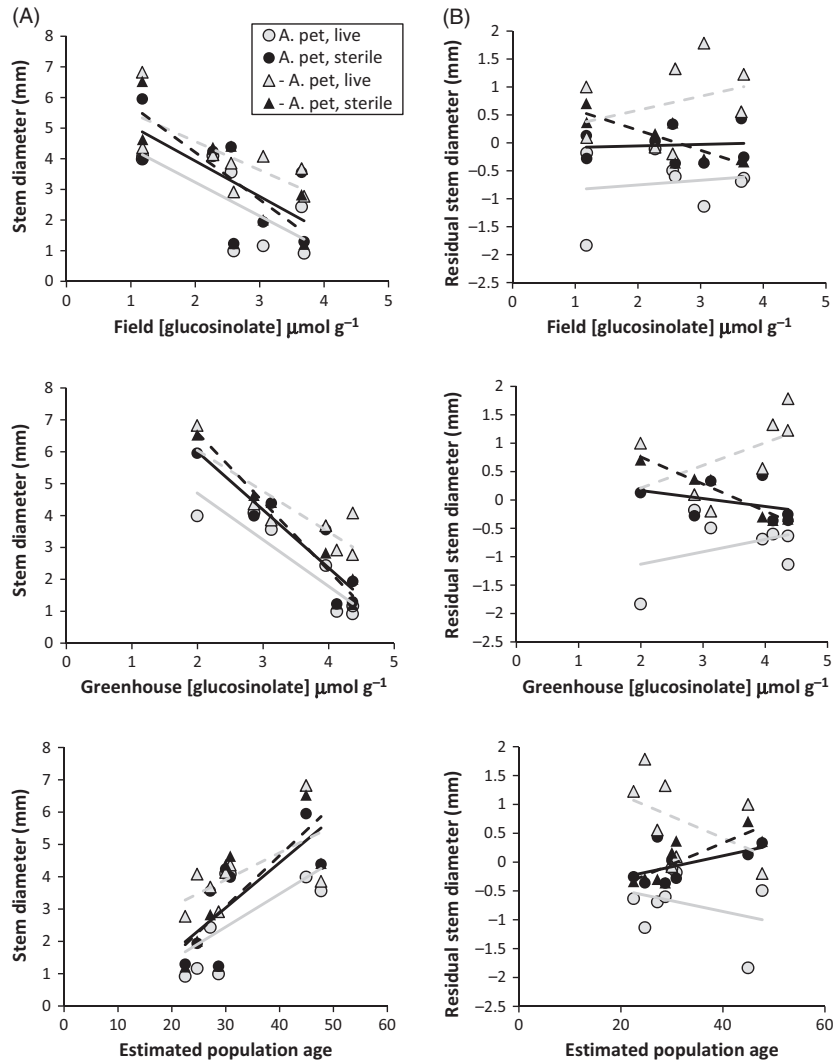
## Discussion

Genetic variation among invasive populations may lead to gradients or mosaics in their impact on native species. In

this study, I found that *A. petiolata*'s impact on transplanted *Q. rubra* seedlings declined with increasing population age and decreasing allelochemical concentrations. Experimental manipulations of *A. petiolata* density and soil microbial communities suggest that the well-documented impacts of *A. petiolata* on soil communities can translate into field conditions. The experiment also suggests that restoration activities can improve the success of native plants in invaded areas, but their relative efficacy and efficiency depended on the specific qualities of the invasive population.

Across sites, transplanted red oak seedlings grew substantially better in sites with a longer history of *A. petiolata* invasion. This is consistent with the pattern seen in vegetation surveys around Illinois, where the change in abundance of woody seedlings over a 5-year period was positively correlated with the age of the *A. petiolata* population (increasing in the oldest, but decreasing in the youngest, populations) (Lankau et al. 2009). *Alliaria petiolata* individuals descended from older populations have been shown to exert weaker impacts on soil fungal communities (Lankau 2011b) and several tree species (Lankau et al. 2009). These age-related effects are likely a result of a strong negative correlation between population age and the concentration of several putative allelochemicals (including two glucosinolates and a flavonoid glycoside, alliarin, (Lankau et al. 2009).

The population mean glucosinolate concentration was a strong predictor of oak seedling growth among sites, regardless of whether the chemicals were measured in naturally occurring plants in the field or in plants grown



**Figure 2** Final stem diameter of *Quercus rubra* seedlings versus the estimated age or mean glucosinolate concentration of the *Alliaria petiolata* population (measured on either field- or greenhouse-grown plants) for each treatment combination in the field experiment. Each point is the mean of four replicates. (A) raw stem diameters across sites, (B) residual values for stem diameter after removing overall site differences. *A. pet.*, *A. petiolata* control treatment, *-A. pet.*, *A. petiolata* removal treatment, live, live soil inoculation treatment, ste, sterilized soil inoculation treatment. Gray symbols and lines, live soil inoculation treatments, Black symbols and lines, sterilized soil inoculation treatments. Circles and solid lines, *A. petiolata* control treatments, Triangles and dashed lines, *A. petiolata* removal treatments.

in a common environment. This suggests that these patterns are not the result of a shared environmental correlation between glucosinolate expression in *A. petiolata* and oak seedling growth. However, it is still possible that environmental differences among sites could both select for genetic differences in glucosinolate concentrations among *A. petiolata* populations and determine oak seedling growth. However, a separate study measured a suite of 16 abiotic soil variables (including measures of plant macro- and micronutrients, pH and conductivity, and soil texture) and found no significant correlations between any variables and mean glucosinolate concentrations

(Lankau 2011a). Additionally, while glucosinolate concentrations were correlated with several other variables (including population age, latitude, longitude and *A. petiolata* cover), when all covariates were entered into the same model, only glucosinolate concentration measured in a common environment remained a significant predictor of oak seedling growth.

Oak seedlings grown and planted in live soil plugs grew better than those in sterilized plugs when the surrounding *A. petiolata* individuals were removed, but this pattern reversed in the presence of the invader. As the live soil inoculums likely included pathogens as well as mutualists,

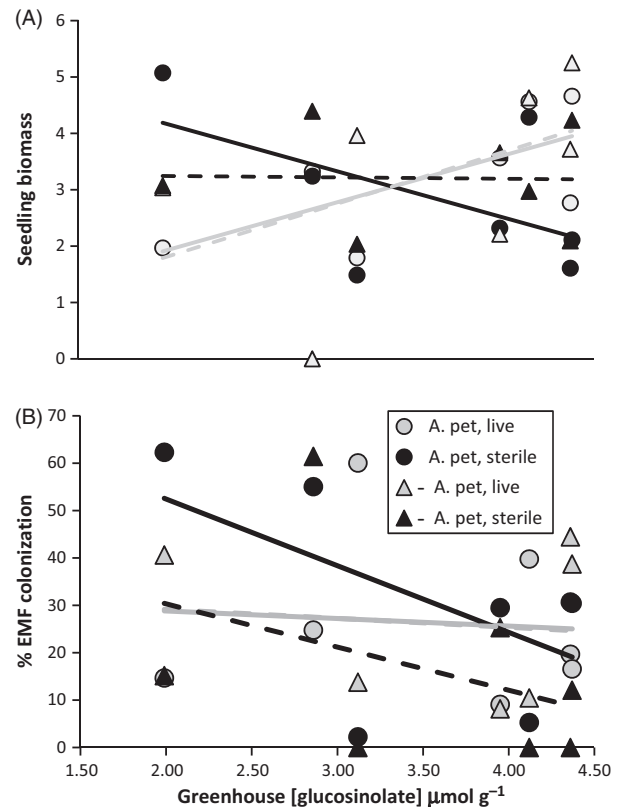


**Table 3.** Statistical results of linear models relating raw *Quercus rubra* stem diameters (first column) and residual *Q. rubra* stem diameters after controlling for overall site effects (second column) from the field experiment, in anova models with either estimate population age (A), or population average glucosinolate concentration, measured from either field- (B) or greenhouse-grown plants (C) as the covariate.

Source	Stem diameter		Residual stem diameter	
	F	P	F	P
<b>(A)</b>				
<i>Alliaria petiolata</i> removal	3.24	0.086	<b>16.17</b>	<b>0.001</b>
Soil inoculation	0.01	0.915	0.53	0.473
<i>A. pet</i> × Soil	2.488	0.130	<b>13.42</b>	<b>0.001</b>
Estimated Pop Age	<b>21.54</b>	<b>&lt;0.001</b>	0.05	0.834
Age × <i>A. pet</i>	0.01	0.937	0.13	0.718
Age × Soil	1.31	0.265	<b>8.18</b>	<b>0.009</b>
Age × <i>A. pet</i> × Soil	0.09	0.762	1.18	0.290
Initial diameter	0.59	0.449	1.05	0.316
Initial height	0.03	0.856	0.33	0.571
<b>(B)</b>				
<i>A. petiolata</i> removal	2.57	0.123	<b>12.77</b>	<b>0.002</b>
Soil inoculation	0.58	0.453	2.02	0.170
<i>A. pet</i> × Soil	2.16	0.156	<b>11.35</b>	<b>0.003</b>
Field (Glucosinolate)	<b>23.30</b>	<b>&lt;0.001</b>	0.13	0.725
Gluc × <i>A. pet</i>	0.12	0.736	0.65	0.428
Gluc × Soil	1.02	0.324	<b>5.05</b>	<b>0.035</b>
Gluc × <i>A. pet</i> × Soil	0.47	0.499	2.93	0.101
Initial diameter	1.13	0.299	1.29	0.268
Initial height	0.37	0.552	2.11	0.161
<b>(C)</b>				
<i>A. petiolata</i> removal	<b>8.80</b>	<b>0.008</b>	<b>17.71</b>	<b>0.001</b>
Soil inoculation	0.55	0.470	2.07	0.167
<i>A. pet</i> × Soil	<b>9.33</b>	<b>0.007</b>	<b>17.74</b>	<b>0.001</b>
Greenhouse (Glucosinolate)	<b>113.94</b>	<b>&lt;0.001</b>	0.14	0.710
Gluc × <i>A. pet</i>	0.34	0.567	0.93	0.349
Gluc × Soil	<b>4.71</b>	<b>0.044</b>	<b>11.62</b>	<b>0.003</b>
Gluc × <i>A. pet</i> × Soil	1.29	0.270	3.15	0.093
Initial diameter	1.93	0.182	2.48	0.133
Initial height	0.18	0.679	1.89	0.186

Bold values are significant at  $P < 0.05$ . *A. pet.*, *A. petiolata* removal treatment, Soil, soil inoculation treatment.

the presence of *A. petiolata* may have shifted the balance of the inocula to net pathogenic. Additionally, seedlings grown with mycorrhizal fungi may have had altered root development (Hetrick 1991). If the fungal symbionts were reduced by the presence of *A. petiolata* in the field, the altered root morphology could have left these seedlings at a disadvantage. Finally, the cost/benefit balance of mycorrhizae may have been altered if the presence of *A. petiolata* reduced the abundance of mycorrhizal fungal hyphae in soil but left behind the carbon sinks on plant roots. This pattern is opposite to that seen for arbuscular mycorrhizal infection, in which precolonization by arbuscular mycor-



**Figure 3** Seedling biomass (A) and ectomycorrhizal colonization (B) of *Quercus rubra* grown in greenhouse pots inoculated with soil from experimental field plots, versus the glucosinolate concentration of the *Alliaria petiolata* population from the site where the soil was collected. *A. pet.*, *A. petiolata* control treatment,  $-A. pet.$ , *A. petiolata* removal treatment, live, live soil inoculation treatment, ste, sterilized soil inoculation treatment. Gray symbols and lines, live soil inoculation treatments, Black symbols and lines, sterilized soil inoculation treatments. Circles and solid lines, *A. petiolata* control treatments, Triangles and dashed lines, *A. petiolata* removal treatments.

rhizal fungi (AMF) protected a native annual from the allelopathic effects of *A. petiolata* (Barto et al. 2010b). Barto et al. (2010a) concluded that the allelopathic effects of *A. petiolata* are likely due to independent effects on plant and mycorrhizal fungal growth prior to the development of the symbiosis. It may be that ectomycorrhizal fungi (EMF) do not provide the same protective effect or that the pattern in my study was driven more by interference with continuing colonization postplanting rather than the alteration in symbioses developed prior to planting.

Surprisingly, soils collected from *A. petiolata* removal plots led to lower ectomycorrhizal colonization when used to inoculate greenhouse grown seedlings, although this difference did not translate into lower growth of seedlings in those soils. It is also possible that *A. petiolata* shifts the composition, as well as the abundance, or ECM communities, as has been seen for arbuscular mycorrhizal

**Table 4.** Statistical results of models relating *Quercus rubra* seedling biomass (first column) and ectomycorrhizal colonization (second column) in the greenhouse experiment to experimental treatments and glucosinolate concentrations of the field plots from which the soil inoculations were collected.

Source	Seedling biomass		Percentage of ectomycorrhizal colonization	
	F	P	$\chi^2$	P
<b>(A)</b>				
<i>Alliaria petiolata</i> removal	0.01	0.935	<b>5.18</b>	<b>0.023</b>
Soil inoculation	0.15	0.703	1.11	0.292
<i>A. pet</i> × Soil	0.09	0.764	1.79	0.183
Estimated Pop Age	0.44	0.513	1.71	0.191
Age × <i>A. pet</i>	0.01	0.923	0.08	0.776
Age × Soil	0.64	0.386	0.03	0.863
Age × <i>A. pet</i> × Soil	0.78	0.386	1.16	0.314
Greenhouse position	0.00	0.997	<b>5.03</b>	<b>0.025</b>
Root length measured	NA	NA	0.19	0.661
<b>(B)</b>				
<i>A. petiolata</i> removal	0.00	0.993	<b>5.40</b>	<b>0.020</b>
Soil inoculation	0.43	0.519	1.00	0.317
<i>A. pet</i> × Soil	0.34	0.565	2.40	0.122
Field (Glucosinolate)	0.03	0.869	1.75	0.186
Gluc × <i>A. pet</i>	1.30	0.265	0.46	0.500
Gluc × Soil	<b>5.32</b>	<b>0.030</b>	0.23	0.630
Gluc × <i>A. pet</i> × Soil	0.02	0.889	0.22	0.642
Greenhouse position	0.65	0.427	1.94	0.161
Root length measured	NA	NA	0.00	0.969
<b>(C)</b>				
<i>A. petiolata</i> removal	0.00	0.958	<b>6.10</b>	<b>0.014</b>
Soil inoculation	0.66	0.426	0.09	0.771
<i>A. pet</i> × Soil	0.03	0.859	1.09	0.296
Greenhouse (Glucosinolate)	0.65	0.432	<b>3.91</b>	<b>0.048</b>
Gluc × <i>A. pet</i>	1.19	0.288	0.46	0.500
Gluc × Soil	<b>7.89</b>	<b>0.011</b>	<b>3.88</b>	<b>0.049</b>
Gluc × <i>A. pet</i> × Soil	0.08	0.783	1.22	0.269
Greenhouse position	2.31	0.145	<b>7.36</b>	<b>0.007</b>
Root length measured	NA	NA	0.61	0.436

F statistics are presented for seedling biomass (which was analyzed with a linear model) and  $\chi^2$  statistics for mycorrhizal colonization (which was analyzed with a generalized linear model with a beta error distribution). Bold values are significant at  $P < 0.05$ . *A. pet*, *A. petiolata* removal treatment, Soil, soil inoculation treatment, Age, estimated population age, Gluc, population average glucosinolate concentration, measured either from field- (B) or (C) from greenhouse-grown plants.

communities (Burke 2008) and fungal communities in general (Callaway et al. 2008), and this effect may be as or more important than total ECM colonization on seedling growth.

Soil inoculation had the strongest effects in younger populations with higher allelochemical concentrations. As glucosinolates and their breakdown products have short

half-lives in soil (Gimsing et al. 2009), in the *A. petiolata*-removed plots, seedling growth likely reflected the legacy of that particular *A. petiolata* population on the soil community. This suggests that in the older/less toxic populations, where seedling growth was similar between live and sterilized soil treatments in the absence of *A. petiolata*, the *in situ* soil community may be recovering in its ability to support plant growth. This is consistent with results from a study in which soil microbial communities (including bacterial, fungal, and arbuscular mycorrhizal fungal communities) were characterized with molecular methods from 15 sites along a chronosequence of *A. petiolata* invasion (Lankau 2011a). This interpretation is also supported by the small, follow-up greenhouse experiment performed here, in which seedling growth and ectomycorrhizal colonization were high in soils collected from low glucosinolate *A. petiolata* populations, but declined with increasing glucosinolate concentrations. However, this pattern was only observed in soils collected from plots that lacked the live soil inoculation; in inoculated plots, seedling growth and mycorrhizal colonization were relatively constant across the glucosinolate gradient.

Allelopathy and the alteration in soil communities have been implicated in a number of invasions (Klironomos 2002; Bais et al. 2003; Hierro and Callaway 2003; Callaway et al. 2004b; Wolfe and Klironomos 2005; Callaway and Vivanco 2007; van der Putten et al. 2007; Jordan et al. 2008; Pringle et al. 2009). Nevertheless, it has been difficult to determine the importance of these effects under natural field conditions or to apply this knowledge to management or restoration. However, some studies have found evidence for these effects using exogenous applications of the putative allelochemical (Thorpe et al. 2009) or experimental additions of activated carbon (Gomez-Aparicio and Canham 2008; Kulmatiski 2011). I used a combination of observational and experimental approaches to investigate the role of allelopathy and altered soil communities in *A. petiolata* – native species interactions. The correlational approach used here offers less causal information than an experimental approach. However, experimental approaches to allelopathy in the field introduce potentially severe artifacts that can make interpretation difficult. Exogenous applications of putative allelochemicals may not realistically simulate the relevant concentrations, exudation processes, or breakdown kinetics of chemicals. Activated carbon has indirect effects on plant growth even in simplified greenhouse conditions (Lau et al. 2008). Combining a correlational approach with experimental manipulations may allow for stronger interpretations. Here, the fact that the negative relationship between glucosinolate concentrations and oak seedling growth was partially eliminated by the soil restoration treatment provides additional evidence that

*A. petiolata*'s allelochemicals act to reduce the quality of the soil community for oak seedlings.

The results of this study have several implications for restoration of forests invaded by *A. petiolata*. First, inoculation with soil microbial communities can improve the growth of tree seedlings, but only when carried out in combination with removal of the invader. Secondly, all else being equal, when land managers must choose between sites for restoration projects, restoration is predicted to be most successful in sites with an older (and thus likely less toxic) *A. petiolata* populations. Finally, when restoring a particular site, knowledge about the *A. petiolata* population present could help make restoration decisions. In young, highly toxic sites, both *A. petiolata* removal and soil restoration are likely to improve outcomes. However, in older, less toxic populations, native transplants may perform well even without expending resources on invasive removal or soil inoculation.

Knowledge of the spatial and temporal variation in invader traits may be useful to the management of many invaders. Comparisons of quantitative traits are often made between introduced and native populations, to study postintroduction evolution in growth, herbivore resistance, etc (Blair and Wolfe 2004; Joshi and Vrieling 2005; Lavergne and Molofsky 2007; Keller and Taylor 2008; van Kleunen and Fischer 2008; Barney et al. 2009). These experiments typically also provide data on the among population genetic variation within introduced ranges, which could be harnessed to tailor regionally or locally specific management strategies. Additionally, there may be predictable changes in invasive populations through time, both because of evolution within the invasive species and because of adaptation and acclimation in native species (Carroll et al. 2005; Siemann et al. 2006; Strauss et al. 2006; Strayer et al. 2006). While more research is necessary before general recommendations can be made, management priorities (for instance, invasive removal versus native planting) may vary consistently in older versus newly invaded sites.

Invasive species can harbor substantial genetic variation among populations, owing to random and selective forces (Bossdorf et al. 2005; Dlugosch and Parker 2008). Here, I show that gradients in genetic investment to allelopathic traits, related to the history of invasion, resulted in gradients in impact on native tree seedlings in natural field conditions. This suggests that the impact of this invader could lessen over time. Such a phenomenon could have important implications for the long-term outcome of an invasion (i.e., whether it leads to the extinction of native species or the integration of the new species into the native community), as well as provide opportunities to improve control and restoration efforts by capitalizing on

these evolutionary trends. Data for this study are available as online Supporting Information.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Data S1.** Raw data from the field and greenhouse experiments.

**Table S1.** Statistical analysis of alternative measures of *Q. rubra* performance in the field experiment (raw values).

**Table S2.** Statistical analysis of alternative measures of *Q. rubra* performance in the field experiment, using residual values after removing mean differences among sites.

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