Plant–soil interactions promote co-occurrence of three nonnative woody shrubs

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Abstract. Ecosystems containing multiple nonnative plant species are common, but mechanisms promoting their co-occurrence are understudied. Plant–soil interactions contribute to the dominance of singleton species in nonnative ranges because many nonnatives experience stronger positive feedbacks relative to co-occurring natives. Plant–soil interactions could impede other nonnatives if an individual nonnative benefits from its soil community to a greater extent than its neighboring nonnatives, as is seen with natives. However, plant–soil interactions could promote nonnative co-occurrence if a nonnative accumulates beneficial soil mutualists that also assist other nonnatives. Here, we use greenhouse and field experiments to ask whether plant–soil interactions (1) promote the codominance of two common nonnative shrubs (*Ligustrum sinense* and *Lonicera maackii*) and (2) facilitate the invasion of a less-common nonnative shrub (*Rhamnus davurica*) in deciduous forests of the southeastern United States. In the greenhouse, we found that two of the nonnatives, *L. maackii* and *R. davurica*, performed better in soils conditioned by nonnative shrubs compared to uninvaded forest soils, which suggests that positive feedbacks among co-occurring nonnative shrubs can promote continued invasion of a site. In both greenhouse and field experiments, we found consistent signals that the codominance of the nonnatives *L. sinense* and *L. maackii* may be at least partially explained by the increased growth of *L. sinense* in *L. maackii* soils. Overall, significant effects of plant–soil interactions on shrub performance indicate that plant–soil interactions can potentially structure the co-occurrence patterns of these nonnatives.

Key words: co-occurring or codominant invaders; invasive species; Ligustrum sinense; Lonicera maackii; *nonnative species; plant–soil interactions;* Rhamnus davurica.

Introduction

Mechanisms promoting invasion by a single nonnative plant species have been well studied (Catford et al. 2009), but few studies have addressed why some communities contain multiple nonnative species (Catford et al. 2012, Kuebbing et al. 2013). Interactions between nonnatives are understudied relative to interactions between natives and nonnatives, and in co-invaded communities it is generally unknown whether nonnative interactions are antagonistic or synergistic (Kuebbing and Nuñez 2015). Understanding the underlying mechanisms that influence patterns of nonnative plant co-occurrence are important for better predictions of what sites are likely to have higher levels of invasion or what suites of nonnatives are most likely to co-occur. Nonnative species are frequently characterized as strong competitors with a greater ability to capture resources than co-occurring natives (van Kleunen et al. 2010),

which indicates that co-occurring nonnatives may be more likely to have negative, rather than positive, effects on one another. However, even if direct interactions among invasive plants are competitive, indirect interactions, such as suppression of co-occurring natives by one invader, could positively influence the occurrence of another nonnative (Flory and Bauer 2014).

Plant–soil interactions can mediate direct and indirect interactions among co-occurring plants (Bever et al. 2010) and might provide a mechanistic explanation for coexistence among nonnatives. The relative strength of plant–soil interactions among plants can predict plant co-occurrence patterns (Bever et al. 1997). Nonnatives frequently experience positive feedbacks in soils conditioned by conspecifics compared to soils conditioned by natives, owing to relatively lower associations with harmful soil pathogens (e.g., enemy release hypothesis; Keane and Crawley 2002, Klironomos 2002) compared to beneficial soil mutualists, such as arbuscular mycorrhizal fungi (AMF; Reinhart and Callaway 2006, Moora et al. 2011, Nuñez and Dickie 2014). Because nonnatives initially experience strong positive feedbacks relative to co-occurring natives, they become dominant in plant communities (Klironomos 2002, Kulmatiski et al. 2008).

This pattern suggests that a nonnative that experiences the greatest release from soil pathogens or the greatest gain in soil mutualists relative to other nonnatives should also dominate a community.

Even if nonnative species are predicted to have strong positive feedbacks in their own home soils, plant–soil interactions could indirectly promote nonnative co-occurrence if one nonnative plant fosters soil communities that contain larger populations of generalist beneficial soil organisms (Reinhart and Callaway 2006) relative to areas uninvaded by that species (Greipsson and DiTommaso 2006). Additionally, plant–soil interactions may explain the co-occurrence of functionally similar nonnative species, such as woody shrubs, that likely compete with one another for resources (Funk et al. 2008). Because woody plants generally require strong microbial associations with mycorrhizal fungi (Nuñez and Dickie 2014), positive plant–soil interactions likely influence nonnative woody shrub co-occurrence patterns.

Here, we explore plant–soil interactions among three nonnative, invasive woody shrubs to test whether plant–soil interactions promote their co-occurrence. Two of the shrubs, *Ligustrum sinense* Lour. (Chinese privet) and *Lonicera maackii* (Rupr.) Herder (bush honeysuckle), are ubiquitous forest invaders in this region and frequently form codominant stands where both species attain high relative abundance (Kuebbing et al. 2014). The third shrub, *Rhamnus davurica* Pall. (Dahurian buckthorn) is regionally uncommon but can be locally abundant at sites where it is present (distribution information *available online*).[6] *Rhamnus davurica* is generally associated with the nonnative shrubs, *L. sinense* and *L. maackii*, but the reason for this association is unknown (see Plate 1). The native range of all three species is in eastern Asia, and the native range of *L. maackii* overlaps with those of *R. davurica* and *L. sinense*. In their nonnative ranges, both *L. sinense* and *L. maackii* associate with AMF (Greipsson and DiTommaso 2006, Shannon et al. 2014), but it is unknown whether *R. davurica* establishes soil mutualisms. However, its nonnative congener, *R. cathartica*, forms AMF mutualisms in its native and nonnative ranges (Knight et al. 2007). Using these species, we ask the following questions in a paired greenhouse and field plant–soil feedback experiment: (1) Do plant–soil interactions promote the codominance of the two common nonnative woody shrubs *L. sinense* and *L. maackii*? (2) Do plant–soil interactions facilitate invasion by a less-common nonnative woody shrub (*R. davurica*)?

METHODS

Greenhouse plant–soil feedback

To test plant–soil interactions between the codominant shrubs *Ligustrum sinense* and *Lonicera maackii*, we

[6] http://www.eddmaps.org/Species/subject.cfm?sub=6304

established a two-phased plant–soil feedback experiment (Bever et al. 2010) in greenhouses at the University of Tennessee, Knoxville, Tennessee, USA. We established four soil treatments for this experiment: *L. sinense*-conditioned soils, *L. maackii*-conditioned soils, uninvaded control soils, and sterilized soils (Appendix: Fig. A1). To create the *L. sinense*- and *L. maackii*-conditioned soils, we introduced *L. sinense* or *L. maackii* seedlings to uninvaded forest soil microbial communities to allow communities to differentiate in response to each invader during an initial soil-conditioning phase. We collected uninvaded soils from the upper 10 cm of mineral soil from three uninvaded forested areas that were more than 150 m apart in the I. C. King Natural Area (35°53′58.88″ N, 83°56′41.65″ W) in Knoxville. The surrounding flora in the uninvaded area included *Acer* L. (maple), *Fagus grandifolia* Ehrh. (American beech), *Fraxinus* L. (ash), and *Quercus* L. (oak). We homogenized and sieved field-collected soils (10-mm mesh) to remove any branches, large roots, or debris. We filled 2.5-L pots (13.34 × 13.34 × 13.97 cm; Gage Durapot 550S; Gage Industries, Lake Oswego, Oregon, USA) with a 9:1 ratio of twice-autoclaved potting soil (Fafard 2 Mix; Fafard, Anderson, South Carolina, USA) and field soil, filling pots in the following order: 1.92 L potting soil, 0.24 L uninvaded forest soil inoculum, and capped with 0.24 L potting soil. We planted one six-week-old seedling of *L. sinense* or *L. maackii* in each pot. We collected fruit from a minimum of 10 maternal plants in I. C. King in the fall of 2009. We removed the pulpy fruit exterior and air-dried seeds for two weeks. Processed seeds were stored in brown paper bags at 4°C until sown for germination. We germinated surface-sterilized seeds (3.0% hydrogen peroxide, H_2O_2) in trays of twice-autoclaved sand (Quikrete Hardscapes Play Sand, item #212779; Quikrete, Marcellus, New York, USA) in growth chambers (12:12 h day : night photo regime, 18°:22°C). To create the uninvaded control soil treatment, we filled pots with the same sterile soil-to-inoculum soil ratio but did not add a seedling to the pot. Control soils had the same greenhouse exposure as seedling-conditioned pots. We randomized all pots on greenhouse benches and watered pots as necessary. After 12 months, we clipped seedlings at the root collar and harvested coarse roots from each pot. We rinsed roots to remove excess soil and weighed roots and shoots after drying in a forced-air oven at 60°C for at least 72 h.

We prepared the pre-conditioned soils (i.e., *L. sinense*-conditioned soils, *L. maackii*-conditioned soils, uninvaded control soils) for the second phase of the experiment by filling each pot with a 9:1 ratio of twice-autoclaved potting soil and a portion of the conditioned soil from that pot. We refreshed pots with sterilized soils to reduce the influence of differences in nutrient uptake rates among individual seedlings in the conditioning phase (Kulmatiski and Kardol 2008, Brinkman et al. 2010). We did not replace sterilized soils in control pots,

PLATE 1. Seedlings of three nonnative woody shrubs, *Ligustrum sinense*, *Lonicera maackii*, and *Rhamnus davurica* form a dense ground cover in a deciduous forest understory in Knoxville, Tennessee, USA. Photo credit: S. E. Kuebbing.

which did not receive any seedlings during the initial phase, but we did mix soils in each pot by hand to replicate the soil disturbance in the planted pots. At this point, we created the fourth soil treatment, sterilized soils, which were pots filled with 2.4 L of twice-autoclaved sterilized potting soils.

The second phase of the experiment consisted of planting six-week-old seedlings into each of the four soil treatments. We planted 15 replicates of each of the three nonnative shrubs in three of the four soil treatments (*L. sinense*-conditioned soils, *L. maackii*-conditioned soils, sterilized soils), but only six replicates for each shrub in the uninvaded control soil treatment. We also planted the locally common native shrub, *Lindera benzoin* (L.) Blume (northern spicebush) into all four soil treatments (15 replicates per treatment except for the uninvaded control soil, which had only six replicates) to compare the response of a native shrub to invaded and uninvaded soils. This gave us a total of 204 pots in the second phase. Fruits from all species were collected from a minimum of 10 maternal plants in I. C. King in the fall of 2010. We prepared *L. sinense* and *L. maackii* as in the initial conditioning phase of the experiment. *Rhamnus davurica* and *L. benzoin* seeds were cleaned, sterilized,

placed in sterilized containers of autoclaved sand and distilled water, and cold stratified at 4°C for nine months prior to germination (Bonner and Karrfalt 2008). After 12 months, we measured number of leaves, final plant height, aboveground biomass, and belowground biomass. Seedling biomass was assessed as in the first phase of the experiment.

We assessed the AMF and soil pathogen colonization of *L. sinense* and *L. maackii* roots. We haphazardly subsampled five root samples for each species and soil treatment. We clipped roots into 2 cm lengths and randomly selected root fragments from across the root system. We placed root fragments into tissue cassettes (Fisher Brand Catalog #22-272; Thermo Fisher Scientific, Pittsburgh, Pennsylvania, USA) and cleared roots with a 10% KOH solution followed by a 1:110% KOH and 1.5% H_2O_2 solution to aid clearing, and we stained roots with trypan blue (Koske and Gemma 1989). We mounted stained root segments horizontally on glass microscope slides using polyvinyl-lacto-glycerol (PVLG) and measured AMF and pathogen colonization using the magnified intersection method (McGonigle et al. 1990). We assessed a minimum of 50 random root intersections for each slide and determined the percent-

age of AMF colonization as the proportion of intersections containing AMF hyphae, arbuscules, and vesicles, and the percentage of pathogen colonization as the proportion of intersections containing pathogenic structures. We were unable to bleach the natural pigment coloration from *R. davurica* roots adequately and could not assess root colonization for this species.

Field plant–soil feedback

To test feedbacks in the field, we planted seedlings of each nonnative shrub underneath the canopies of mature (fruiting) shrubs of each of the three invasive shrub species. In May 2012, we selected 10 individuals of each species growing within a 150×150 m area at I. C. King. This location was heavily invaded by these species, and there were no other shrubs or open spaces in the area. We established a 1.5×1.5 m plot underneath each shrub canopy and used the main stem(s) as our plot center. All understory plants in the plot were clipped at the root collar, and the aboveground biomass was collected, identified, and dried in a forced-air oven at 60°C for at least 48 h before weighing. Recruitment of new plants to plots was low, but we cleared plots of aboveground biomass in September 2012 and March 2013.

We measured shrub and plot characteristics that can affect woody seedling performance: (1) Understory light availability (AccuPAR PAR-80; Decagon Devices, Pullman, Washington, USA). To account for differences in sun location, we took measurements on a cloudless day in the morning (09:00–11:00) and afternoon (14:00–15:00) in August 2012. (2) Soil moisture (Hydrosense CS620 volumetric water content probe; Campbell Scientific, Logan, Utah, USA) in May, June, August, and October 2012. (3) Soil pH; we collected, sieved (2-mm mesh size), and homogenized four 10-cm mineral soil cores (5.08 cm diameter) in each plot. We measured the soil pH of slurries of 10 g field moist soil and 20 mL deionized water with a pH conductivity meter (Denver Instruments Model 220, Bohemia, New York, USA). For each mature shrub, we measured the following characteristics: (1) number of stems, (2) diameter at root collar (DRC), (3) plant height with an optical clinometer, and (4) plant canopy area as an ellipse.

In May 2012, we planted eight-week-old seedlings in each plot. We collected fruit from mature shrubs at I. C. King in fall 2011 and germinated seeds using the same greenhouse methods. Seed start dates were staggered to accommodate differences in germination time and to insure all individuals were the same age at time of planting. We planted 360 seedlings of each species, for a total of 1080 seedlings. We subdivided each plot into 36 0.25×0.25 m squares and planted a single seedling in the center of each square. We planted 12 seedlings of each shrub in each plot and determined seedling arrangement with a random number generator. We replanted dead individuals after two weeks, assuming

seedlings had died from transplant shock. We recorded the initial height of each seedling at planting (or replanting) and monitored seedlings weekly for the first month of the experiment and monthly thereafter. At each recording period, we measured the height and number of leaves for each surviving seedling and noted dead or missing seedlings. After one year of growth, we measured seedling height, stem diameter, and number of leaves of surviving seedlings. We clipped seedlings at the root collar and dried shoots in a forced-air oven at 60°C for 48 h before weighing final aboveground biomass.

Statistical analysis

For the greenhouse experiment, we tested for the effects of soil treatment on seedling growth (height, number of leaves, above- and belowground biomass) with linear models followed by a priori orthogonal paired contrasts (Brinkman et al. 2010). Response variables were transformed as necessary to meet model assumptions (Appendix: Table A1). Because the final size of the seedling in the initial conditioning phase could affect the size of the seedling in the second feedback test phase (Brinkman et al. 2010), we tested for a relationship between seedling biomass in the initial conditioning phase and the second experimental phase with Pearson's correlation test. We found no significant correlation ($P > 0.1$) between seedling biomass in the experimental phases, suggesting that feedback responses were due to differences in the microbial community and not to nutrient availability (Kulmatiski and Kardol 2008, Brinkman et al. 2010).

When we found a significant ($P < 0.1$) effect of soil treatment, we calculated plant–soil feedbacks between soil treatment groups as $(S1_i - S2_i)/\max(S1_i, S2_i)$ where $S1_i$ is the plant performance in pots with soil type 1 and $S2_i$ is plant performance in pots with soil type 2 (Brinkman et al. 2010). Because we did not have natural pot pairings, we adapted this method to accommodate the random pairings of pots in our tests by calculating the feedbacks of all possible combinations of pot pairs. This plant–soil feedback calculation method allows us to compare feedbacks between greenhouse and field experiments and centers feedback values around 0 (i.e., maximum feedback values are −1 and 1), which allows for easier comparison of negative and positive feedback values (Brinkman et al. 2010). In the figures, we present the mean feedback value and 95% confidence intervals from feedback calculations, but we derive the statistical significance of soil treatments from the a priori contrasts (Appendix: Table A1).

We also tested for community plant–soil feedback, which accounts for the net pairwise dynamics of individual feedbacks between two plant species grown in soils conditioned by each species (Bever et al. 1997). We calculated the interaction coefficient I_s, which indicates the direction of the net pairwise feedback, as $I_s = G(A)_\alpha - G(A)_\beta - G(B)_\alpha + G(B)_\beta$ where $G(A)_\alpha$ and

$G(B)_\beta$ are the growth of plants A and B, respectively, in soils conditioned by conspecifics and $G(A)_\beta$ and $G(B)_\alpha$ are the growth of plants A and B, respectively, in soils conditioned by the other species. The magnitude of the feedback was tested with a "home vs. away" comparison (Bever et al. 1997). Community plant–soil feedbacks incorporate the relative strength of individual species feedbacks, and thus the sign of an individual species feedback does not necessarily predict the overall community feedback. For community feedback models, negative I_s values indicate that both species will coexist under current feedback dynamics while positive I_s values indicate one species will eventually be lost from the system (Bever et al. 1997). Finally, to assess differences in percentage of root colonization of AMF and root pathogens of *L. sinense* and *L. maackii* shrubs, we used generalized linear models with a Poisson error distribution and log-link function.

For the field experiment, we tested how the identity of the adult shrub (i.e., indication of feedback), characteristics of the adult shrub, and environmental characteristics of the plot affected final seedling performance (height, stem diameter, shoot mass, and number of leaves) and mortality with mixed-effect regression models and spatial linear models. Because we found high collinearity among plot and shrub characteristics, we used principal components analysis (PCA) to derive orthogonal summary combinations of the data. We split the variables into two separate data sets for PCA analysis: shrub variables that characterized the size and structure of the central shrub (DRC, number of stems, canopy area, and plant height) and plot variables that characterized the plot abiotic environment (soil moisture, light levels, and soil pH). We used plot and shrub PCA axes as independent plot characteristic variables, which allowed us to distinguish between characteristics of the adult shrub and environmental characteristics of the plot. Additionally, because we found that initial seedling height was significantly related to final seedling performance (i.e., significant Pearson's correlation coefficient, $P < 0.05$), we included the initial seedling height as a random effect in all models. We accounted for the variation in initial seedling height as a random term in the model's error structure by defining initial seedling height as a correlation structure in the models. To select the best model to describe seedling performance and mortality, we first ran full models with shrub species, shrub and plot PCA axes, and initial seedling height. Because mortality data (i.e., number of surviving seedlings) had a Poisson error distribution, we used generalized linear mixed-regression models ("glmer" function) with a Poisson error distribution and a log-link function. We selected the best model using backward stepwise selection and the "anova" function with maximum likelihood (Crawley 2012). We used the nlme (Pinheiro et al. 2014) and lme4 (Bates et al. 2014) packages for mixed-effect models and the vegan package

(Oksanen et al. 2013) with R software (R Development Core Team 2013).

We also found evidence of spatial autocorrelation in some of our response and explanatory variables (Appendix: Table A2), which violates the assumption of independently distributed errors and inflates Type I error (Legendre 1993). To account for spatial autocorrelation, we used a simultaneous autoregressive spatial error model (SAR_{err}) that accounts for spatial dependence in both the response and explanatory variables (Kissling and Carl 2008). We used the "spdep" package (Bivand et al. 2005) and defined our spatial neighborhood as any plot with a neighbor distance of less than 10 m (i.e., less than 10 m between plot centers). The spatial weights matrix was calculated with a row standardizing code (style = "W"), which scales the neighbor covariances by the number of neighbors for each region (Kissling and Carl 2008). We report reduced model variables, standardized regression coefficients, and Moran's I values from the mixed-effect and SAR_{err} models for comparison (Appendix: Table A3). When we found evidence for feedback between seedling performance and adult shrub identity (i.e., adult shrub identity was significant [$\alpha > 0.05$] in final model), we calculated feedbacks from the average seedling performance (shoot mass, number of leaves, final height, stem diameter) of surviving seedlings in each plot using the same method as in the greenhouse experiment. All statistical analyses were performed with R software (R Development Core Team 2013).

RESULTS

Greenhouse plant–soil feedback

Two of the nonnative shrubs, *Rhamnus davurica* and *Lonicera maackii*, performed better in soils conditioned by the nonnatives *L. maackii* or *Ligustrum sinense* compared to uninvaded soils (Fig. 1; Appendix: Table A1). The shoot biomass and height of *R. davurica* were on average 1.8 and 1.5 times higher, respectively, and the mean shoot biomass and number of leaves of *L. maackii* were 1.5 and 1.3 times higher, respectively, in invaded soils compared to uninvaded control soils (Fig. 1; Appendix: Table A4). The other codominant nonnative shrub, *L. sinense*, and the native woody shrub, *L. benzoin*, exhibited no differences in growth between invaded and uninvaded soils ($P > 0.1$; Appendix: Table A1).

We found insignificant feedbacks between the codominant nonnative shrubs in home soils vs. soils conditioned by the other invader (Fig. 2; Appendix: Table A1). The mean root mass of *L. maackii* in soils conditioned by a conspecific was moderately higher ($P < 0.1$) compared to soils conditioned by *L. sinense*, and its growth trended higher in home soils compared to *L. sinense*-conditioned soils (Fig. 2). The nonnative *L. sinense* showed no significant response ($P > 0.1$), though its root mass and height trended higher in soils
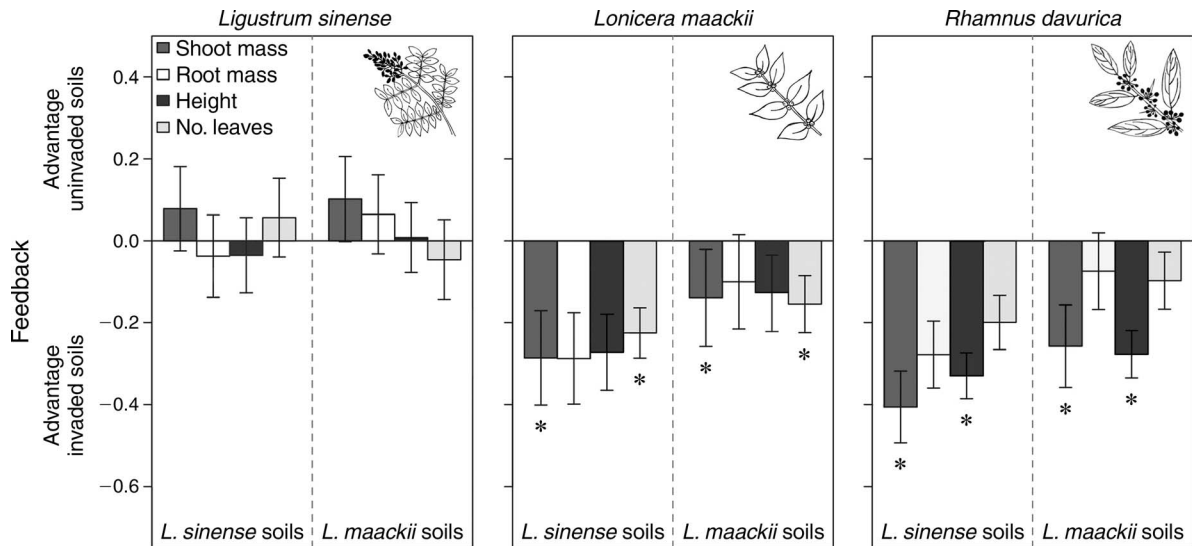
Fig. 1. The nonnative shrubs *Lonicera maackii* and *Rhamnus davurica* performed better in soils conditioned by codominant nonnative shrubs, *Ligustrum sinense* and *L. maackii*, compared to uninvaded control soils in a greenhouse experiment. Error bars are 95% confidence intervals from feedback calculations and asterisks represent significance ($P < 0.05$) from a priori orthogonal contrast tests.

conditioned by *L. maackii* compared to its home soils (Fig. 2).

All four shrub species had substantial positive feedbacks in live soil treatments compared to sterilized soils (Appendix: Fig. A2, Table A1). The average shoot and root growth of *L. sinense* and *L maackii* were five and eight times higher, respectively, across all live soil treatments compared to sterilized soils (Appendix: Table A4). The nonnative *R. davurica* and native shrub *Lindera benzoin* also had strong positive feedbacks in live soils, but these feedbacks were less substantial than those of the codominants (Appendix: Fig. A2). The average shoot and root biomass of *R. davurica* and *L. benzoin* were 2.5 and 1.6 times higher, respectively, in live compared to sterilized soils (Appendix: Table A4). Similarly, mean root colonization by AMF for *L. sinense* and *L. maackii* were 30 and 26 times higher, respectively, in live soils compared to sterilized soils (Fig. 3). The root colonization (<0.01% of root fragments; Fig. 3) in sterilized soils was likely due to contamination during the 12-month period in the greenhouse; however, the overall reduction in plant growth in sterilized soils indicates that this treatment was still effective in testing how a dramatic reduction in AMF affects plant growth. Root pathogen colonization among soils did not differ significantly ($P > 0.1$).

Field plant–soil feedback

Growth and survival of *L. sinense* and *L. maackii* seedlings in the field were affected by the identity of the mature nonnative shrub the seedling grew underneath, indicating the influence of plant–soil feedbacks while accounting for the variation in plot environment, shrub size, and spatial autocorrelation across plots (Table 1, Fig. 4). Growth of *L. sinense* was strongly and significantly greater in soils conditioned by the other codominant shrub *L. maackii* (Table 1, Fig. 4). Seedling shoot mass was 1.4 times higher, and seedling height, stem diameter, and number of leaves were 1.2 times higher in *L. maackii* field plots compared to *L. sinense* field plots. The only influence of mature *L. sinense* shrubs on *L. maackii* seedlings was on seedling stem diameter (Table 1, Fig. 4). Using growth parameters from our field feedback experiment, we calculated the net pairwise feedback interaction coefficient (I_s) between the codominant shrubs (Bever et al. 1997). Net pairwise feedback values were always negative (shoot biomass, I_s = −0.01, $P = 0.03$; height, $I_s = −1.54$, $P = 0.05$; number of leaves, $I_s = −1.92$, $P = 0.09$; stem diameter, $I_s = −0.14$, $P = 0.11$), which indicates that the codominant invaders will coexist under current feedback dynamics (Bever et al. 1997).

Growth of all three nonnative woody shrubs was influenced by variation in plot environment and shrub size (Fig. 4; Appendix: Table A3). We accounted for variation among plot abiotic features (i.e., soil moisture, soil pH, and light availability) and among mature shrub features (i.e., canopy size, shrub height, number of stems, diameter at root collar) using principal components analysis (PCA), which accounted for collinearity among plot and shrub variables. We used the first two principal components from the each PCA as independent variables in the models. The axes from the abiotic feature PCA captured 68% of the measured environ-
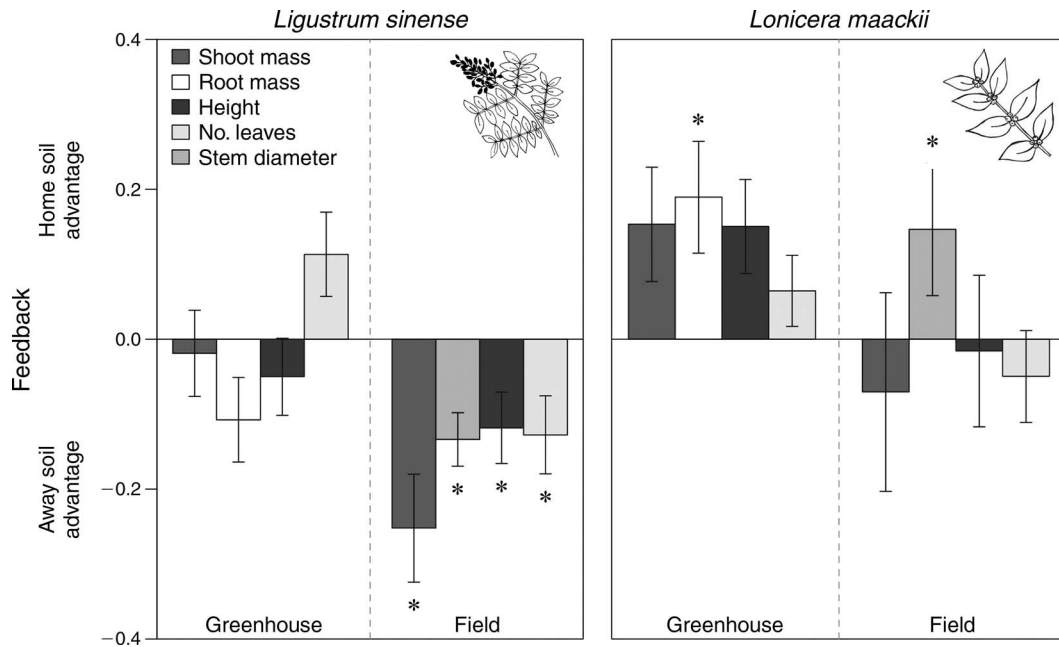
Fig. 2. Two codominant nonnative shrubs had similar directional but different magnitude feedbacks in greenhouse and field experiments. Root mass and stem diameter data were collected only in the greenhouse or field, respectively. Error bars and asterisks are as in Fig. 1.

mental variation across plots; the first axis described variation in soil moisture and pH (PC1 plot, 53%; Appendix: Fig. A3a), and the second axis described variation in understory light availability (PC2 plot, 15%; Appendix: Fig. A3a). The first two axes of the shrub PCA captured 71% of the measured variation, and the first axis described differences in shrub size (PC1 shrub, 41%; Appendix: Fig. A3b), while the second shrub PCA axis described shrub structure (PC2 shrub, 30%; Appendix: Fig. A3b). While all four axes were important variables (i.e., included in best reduced models) for most growth metrics for all three nonnative shrubs (Fig. 4), we failed to find significant models to explain the differences in shoot mass, height, or number of leaves of *L. maackii* seedlings or the shoot mass of *R. davurica* seedlings (Table 1).

Seedling survival was highly variable among the three nonnative woody shrubs. At the end of one year of growth in the field, 18% of *L. maackii* seedlings ($N = 67$), 29% of *L. sinense* seedlings ($N = 104$), and 64% of *R. davurica* seedlings ($N = 230$) were still alive. Survival of *L. maackii* seedlings was 1.5 and 1.9 times higher in *L. sinense* plots compared to *L. maackii* and *R. davurica* plots, respectively, and number of surviving seedlings decreased as plot moisture and pH decreased (Table 1; Appendix: Table A3b). Light availability (PC2 plot) was the only variable that affected survival of *L. sinense* seedlings in the field, and survival was higher in plots with high afternoon light and low morning light (Table 1; Appendix: Table A3a). We did not find a significant

model to describe survival of *R. davurica* seedlings in the field (Table 1).

DISCUSSION

Plant–soil feedbacks provide a mechanistic explanation for the co-occurrence of three nonnative woody shrubs in forests in eastern Tennessee, USA. Greenhouse and field experiments showed consistent signals that the codominance of the ubiquitous nonnative shrubs *Ligustrum sinense* and *Lonicera maackii* may be at least partially explained by the increased growth of *L. sinense* in *L. maackii* soils. Furthermore, the regionally rare nonnative shrub *Rhamnus davurica* performed better in soils conditioned by either codominant shrub compared to uninvaded forest soils, which suggests that these codominant invaders could facilitate the spread of a rarer nonnative. We found the strongest feedbacks in our field experiments, which indicates that plant–soil feedbacks can be important mechanisms regulating plant performance in their nonnative range even under varying environments.

The presence of *L. maackii* in these forests could be prolonging the dominance of *L. sinense* on the landscape. *Ligustrum sinense* had strong negative feedbacks in its home soils relative to *L. maackii* soils, suggesting a possibility that the dominance of *L. sinense* in these forests could have declined if not for the later introduction and spread of *L. maackii*. In East Tennessee, the arrival of *L. sinense* predates that of *L. maackii* by four decades (University of Tennessee
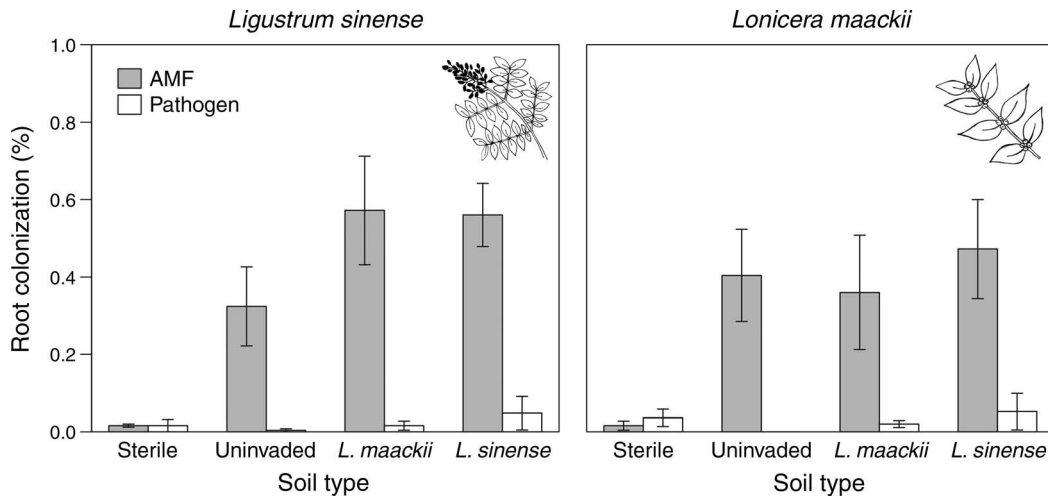
FIG. 3. Two codominant nonnative woody shrubs had higher percentage of root colonization by arbuscular mycorrhizal fungi (AMF) relative to bacterial pathogens in a greenhouse experiment. Soil treatments were sterilized potting soil (sterile) or mixtures of sterilized potting soils plus the following soil inocula: soils collected from uninvaded forested areas (uninvaded) or soils conditioned previously by an individual *L. sinense* or *L. maackii* plant. Pathogen and AMF colonization rates did not differ significantly between live soil treatments ($P > 0.1$).

Herbarium records; E. Wofford, *personal communication*). We found that both the older invader, *L. sinense*, and the newer invader, *L. maackii*, performed better in *L. maackii* soils than in *L. sinense* soils, which is consistent with the hypothesis that invaders with a longer residency time in the nonnative range should have more negative feedbacks in their home soils (Diez et al. 2010). While many nonnative plants experience strong positive feedbacks at the outset of the invasion process (Klironomos 2002, Reinhart and Callaway 2006, but see Andonian et al. 2011), there is an expectation that feedback strength should decrease through time as native soil and plant communities adapt to the invader's presence (Diez et al. 2010, Lankau 2012).

TABLE 1. Growth and survival of three nonnative, invasive, woody shrub seedlings in field plots were affected by a combination of abiotic and biotic variables.

Traits by species	Variables retained in best reduced model	Model χ^2
Ligustrum sinense		
Survival (no. alive)	PC2 plot***, *L. maackii*, *R. davurica*	11.56**
Mass (g)	*L. maackii**, PC1 plot*, PC2 plot†, *R. davurica*, PC2 shrub	17.44**
Height (cm)	PC1 plot*, *L. maackii*, *R. davurica*, PC2 plot	10.49*
Stem diameter (mm)	*L. maackii***, *R. davurica**, PC2 shrub**	11.68*
Number of leaves	*L. maackii*†, PC1 plot**, *R. davurica*	13.20**
Lonicera maackii		
Survival (no. alive)	PC1 plot**, *L. sinense*, *R. davurica*	12.30**
Mass (g)	*L. sinense*, *R. davurica*, PC1 shrub, PC2 shrub	3.96
Height (cm)	*L. sinense*, *R. davurica*, PC1 shrub, PC2 shrub	5.16
Stem diameter (mm)	*L. sinense*†, PC1 shrub†, PC1 plot	8.30†
Number of leaves	*L. sinense*, *R. davurica*, PC2 plot	2.14
Rhamnus davurica		
Survival (no. alive)	*L. sinense*, *L. maackii*, PC1 plot, PC2 plot	5.12
Mass (g)	*L. sinense*, *L. maackii*, PC1 shrub	2.28
Height (cm)	PC1 shrub*, *L. sinense*, *L. maackii*	7.11†
Stem diameter (mm)	PC2 plot**, PC1 shrub†, *L. sinense*, *L. maackii*	16.38**
Number of leaves	PC1 plot**, *L. sinense*, *L. maackii*, PC2 plot	11.64**

Notes: Variables included invasive canopy shrub identity (*Lonicera maackii*, *Ligustrum sinense*, or *Rhamnus davurica*), plot environmental variation, as analyzed by principal component (PC) analysis (PC1 plot [soil moisture and pH], PC2 plot [light availability]) and shrub characteristics (PC1 shrub [shrub size], PC2 shrub [shrub shape, number of stems]), that explain seedling performance (mass, height, stem diameter, number of leaves). We tested for model significance by comparing the best reduced linear mixed-effect model to a null model with the random effect of initial seedling height using chi-squared tests. Model χ^2 not marked with a footnote were not significant.

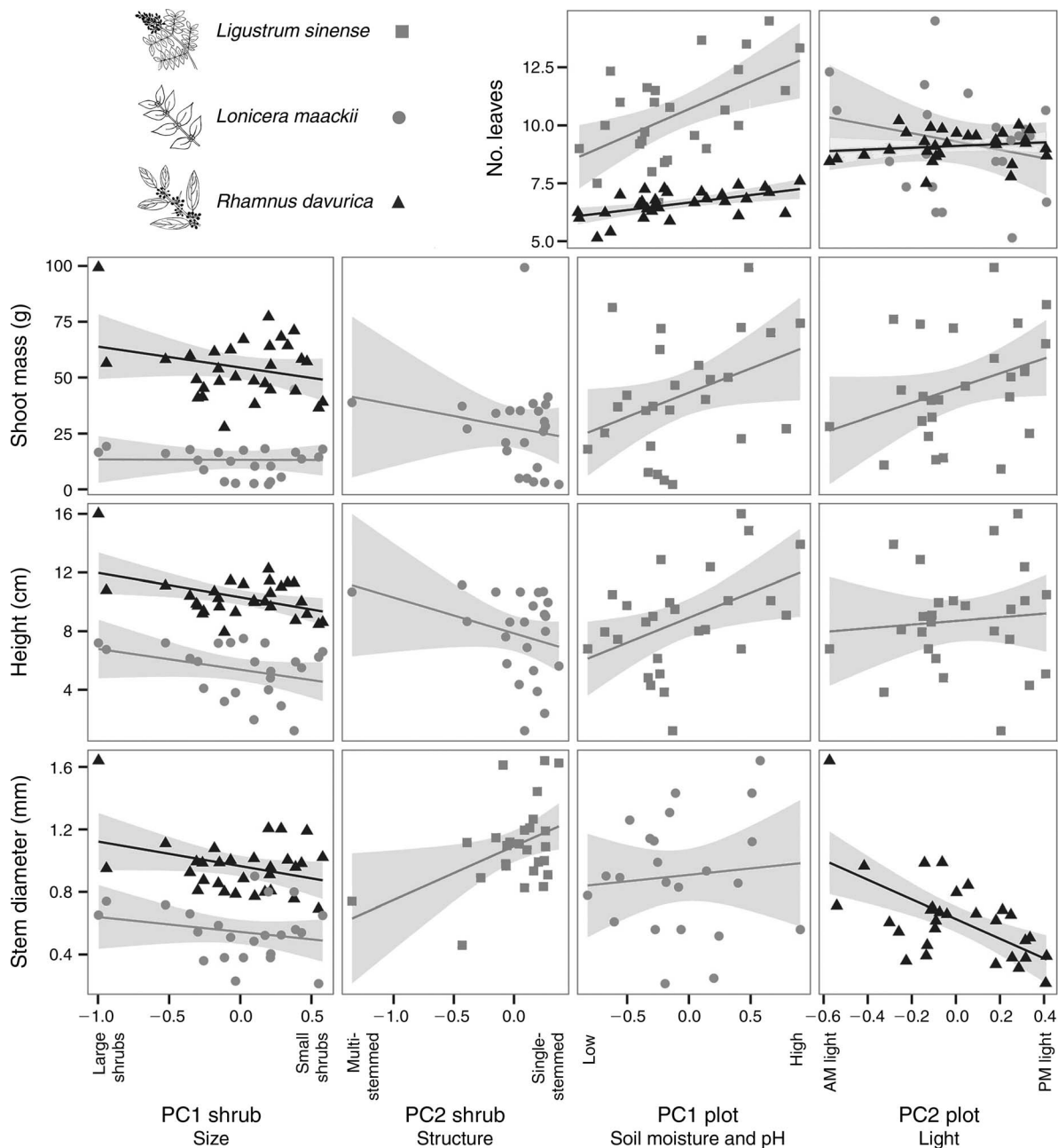† $\alpha < 0.1$; * $\alpha < 0.05$; ** $\alpha < 0.01$; *** $\alpha < 0.001$.

Fig. 4. Growth of nonnative shrub seedlings planted in an invaded forest stand underneath the canopies of mature nonnative shrubs was influenced by variation in plot characteristics (plot soil moisture, soil pH, and light availability) and variation in parent shrub features (shrub size and structure). The performance of each nonnative shrub in the field was modeled separately with mixed-effect models. Gray bands represent 95% confidence intervals.

Plant–soil feedbacks may regulate temporal succession among these nonnative shrubs (Kardol et al. 2006) by promoting persistence of *L. sinense* and invasion by *R. davurica*. Although the community feedback model predicts indefinite coexistence between the codominant nonnatives, *L. sinense* and *L. maackii*, under current feedback conditions, the assumption that feedbacks will remain stable through time is likely unrealistic (Kardol et al. 2006, Diez et al. 2010, Lankau 2012). While this does not negate the importance of the feedbacks in regulating current populations of these shrubs, it does suggest that plant–soil feedbacks could be an important regulating mechanism for "succession" of nonnatives in invaded ecosystems that are now following an alterna-

tive stable state trajectory (Kulmatiski 2006, Firn et al. 2010).

A potential mechanism that could favor the invasion of additional nonnatives into sites already invaded by a nonnative shrub is an increase in the abundance of generalist soil fungi that benefit other nonnatives (Moora et al. 2011). In the greenhouse, the performance of all nonnative shrubs was stunted in sterilized soils compared to live soils, and AMF root colonization for *L. sinense* and *L. maackii* was significantly higher in live soils compared to sterile soils. This indicates that beneficial soil microbes are essential for the optimal growth of these nonnative shrubs. However, we found that AMF and pathogen colonization rates did not differ between live soil treatments, which may indicate that nonbiotic mechanisms, such as invasive-induced changes in soil chemistry, could also be important for promoting the growth of other nonnative shrubs. In addition to the promotion of *L. sinense* by *L. maackii*, we also found that the regionally rare nonnative shrub *R. davurica* experienced positive feedbacks in invaded soils relative to uninvaded soils while the common native shrub *Lindera benzoin* had no growth differences between these soil treatments. This result is consistent with the finding that these codominant shrubs harbor double the number of subdominant nonnatives compared to areas lacking either shrub (Kuebbing et al. 2014). If dominant nonnative species are creating sites that are more favorable for other nonnative species through changes in soil biotic communities or abiotic characteristics, then plant–soil feedbacks could provide an explanation for why the control of a dominant invasive species can lead to reinvasion of the site by subdominant nonnatives (Firn et al. 2010).

Finally, it is of note that we found stronger feedbacks in the field than in the greenhouse. The field experiment took place underneath a shaded forest canopy while our greenhouse experiment was in a high-light environment. Research in temperate forests suggests that feedbacks for woody species increase in lower light levels and that some species experience feedbacks only at low light levels, which may be caused by higher pathogen levels in shaded soils (McCarthy-Neumann and Ibáñez 2013). Although feedbacks are expected to be stronger in controlled greenhouse settings compared to field settings, these expectations arise from feedback studies on herbaceous and graminoid plants from grassland ecosystems (Kulmatiski et al. 2008). The light gradient difference between our field and greenhouse experiment may have influenced feedbacks for these forest understory shrubs.

To predict what sites are likely to have higher levels of invasion or the suite of nonnative species that might be more likely to co-occur, we need to understand the underlying mechanisms that influence patterns of nonnative plant co-occurrence. If nonnative invasive plants are promoting the occurrence of other invasive

species, then removal of a dominant or codominant invader might lead to "invasion treadmills" (Thomas and Reid 2007) or "secondary invasions" (Pearson et al. 2009) by other nonnatives, which may mitigate any benefits of nonnative species management. The invasion by shade-tolerant, long-lived woody shrubs into forests poses a unique challenge to the management and restoration of these communities because of the wideranging and long-term impacts of forest invaders (Martin et al. 2009). Thus, understanding interactions among co-occurring nonnatives is particularly important in forested ecosystems.

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SUPPLEMENTAL MATERIAL

Ecological Archives

The Appendix is available online: http://dx.doi.org/10.1890/14-2006.1.sm