

Non-native grass alters growth of native tree species via leaf and soil microbes

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Summary

1. Non-native plants can alter both native microbe communities and ecosystem processes. These changes may indirectly affect native plant communities. We tested whether an invasive plant influences other plant species through above- and below-ground microbial associations. These factors have received less attention than direct competition for resources in research on plant invasions.

2. Previous work in the mid-western US revealed that the above-ground fungal endophyte (*Neotyphodium coenophialum*) in non-native tall fescue grass (*Lolium arundinaceum*) reduced establishment of native tree species, slowing plant succession from grassland to forest relative to communities lacking the symbiont. Here, we examined potential soil-mediated mechanisms underlying these negative impacts on trees.

3. We challenged nine tree species with soil conditioned by tall fescue grass either with (E+) or without (E–) the symbiotic endophyte. We collected field soil from replicated, experimental E+ and E– field plots, sterilized half to eliminate soil microbes, then conditioned the soil in the greenhouse with either E+ or E– tall fescue. Individual tree seeds received one of five soil treatments: E– live, E– sterile, E+ live, E+ sterile, or no tall fescue conditioning (control). Tree survival and biomass were assessed after approximately 80 days.

4. Soil conditioning by E+ tall fescue reduced the biomass of three tree species (*Elaeagnus umbellata*, *Fraxinus pennsylvanica*, *Platanus occidentalis*) and the survival of *P. occidentalis* relative to conditioning by E– tall fescue. However, decreased biomass occurred only in live soil, suggesting indirect effects of the endophyte via altered soil microbes. In addition, two species (*F. pennsylvanica*, *Prunus serotina*) grew larger in both unconditioned soils and E– live soil relative to all other soil treatments, and two species (*Ailanthus altissima*, *P. occidentalis*) performed poorly in all soil treatments relative to unconditioned soil. Finally, three species did not significantly respond to any soil treatment (*Acer negundo*, *Quercus palustris*, *Cercis canadensis*).

5. *Synthesis.* Our results show that negative impacts of a non-native grass on native trees can depend on both above- and below-ground microbial associations. Because tree species responded divergently to soil conditioning, the endophyte symbiosis has the potential to alter plant succession and the future composition of forest communities.

Key-words: allelopathy, *Elaeagnus umbellata*, endophyte, *Fraxinus pennsylvanica*, invasion, *Lolium arundinaceum*, *Neotyphodium*, plant–soil feedback, *Platanus occidentalis*, succession, symbiosis

Introduction

Invasive species can have strong, negative impacts on native communities through diverse pathways, including competition for resources, habitat alteration and allelopathy (Cronk & Fuller 2001; Levine *et al.* 2003). Accumulating evidence suggests

that invasive, non-native plants can alter both native microbial communities and associated ecosystem processes (Ehrenfeld 2003; Wolfe & Klironomos 2005; van der Putten *et al.* 2007). For example, some non-native knapweed plants (*Centaurea* spp.) not only release allelochemicals into soils (Vivanco *et al.* 2004), but also inhibit mycorrhizal fungi associated with the roots of native plants (Mummey & Rillig 2006) and develop positive associations with local soil biota (Callaway *et al.*

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2004). However, it is less clear how often these changes in ecosystem processes and microbial composition cascade to native plants in the community (Reinhart & Callaway 2006; van der Putten *et al.* 2007). The ability to predict when and where non-native species will have lasting impacts on native communities will depend on understanding the mechanisms through which invaders interact with native species.

Here, we investigate both above- and below-ground microbes as potential factors to explain how invasive plants affect native plant communities. These factors have received less attention than direct competition for resources in studies on invasive plants. Invasive plants can release chemical exudates or leachates that may affect native plants directly or indirectly through changes in the soil microbial community (Inderjit *et al.* 2006; Weir 2007; Zhang *et al.* 2007). For example, invasive garlic mustard (*Alliaria petiolata*) inhibits mycorrhizal colonization of native plants, including tree seedlings (Stinson *et al.* 2006).

Vertically transmitted fungal endophytes infect the above-ground tissues of hosts and are widespread in plants, particularly in C₃ grasses (Clay 1990). Endophytes can produce alkaloids that are toxic to insects and grazing mammals (Clay 1996; Bush *et al.* 1997; Schardl *et al.* 2007) and may also negatively affect other organisms, such as soil nematodes (West *et al.* 1988), pathogens causing soil-borne diseases (Gwinn & Gavin 1992), mycorrhizal fungi (Mack & Rudgers 2008) and decomposers (Lemons *et al.* 2005). Our previous work showed that the presence of a fungal endophyte (*Neotyphodium coenophialum*, Clavicipitaceae) in tall fescue grass (*Lolium arundinaceum*, Poaceae) facilitated the invasion of this species into diverse plant communities (Rudgers *et al.* 2004; Rudgers *et al.* 2005) and slowed the natural progression of succession from grassland to forest by reducing the establishment of trees (Rudgers *et al.* 2007). It has been suggested that endophyte-produced alkaloids and other changes in plant chemistry inhibit microbial activity and alter microbial composition in soil and litter (Franzluebbbers *et al.* 1999; Omacini *et al.* 2004; Jenkins *et al.* 2006). Furthermore, presence of the endophyte in tall fescue is associated with the accumulation of organic carbon and nitrogen in the soil relative to tall fescue lacking the endophyte (Franzluebbbers *et al.* 1999; Franzluebbbers & Hill 2005; Franzluebbbers & Stuedemann 2005).

We evaluated whether tall fescue and its fungal endophyte negatively affect tree seedlings via chemical or biotic changes in the soil. Our previous results showed inhibition of native tree seedlings treated with leachates from endophyte-symbiotic tall fescue grass (Orr *et al.* 2005) and were consistent with the hypothesis that endophyte-symbiotic tall fescue could produce inhibitory allelochemicals (but see, Renne *et al.* 2004). However, we did not test whether the presence of the endophyte contributed to the negative effects of tall fescue leachates on trees. In addition, we did not resolve whether effects were due to shifts in soil chemistry or in the associated soil microbial community (Orr *et al.* 2005).

We combined field and greenhouse experiments to address the following questions: (i) Does the presence of the endophyte during soil conditioning reduce tree performance (survival or

seedling growth) relative to soil conditioned by endophyte-free plants? Based on previous studies that tracked soil microbes and tree succession under field conditions (e.g. Franzluebbbers & Hill 2005; Rudgers *et al.* 2007), we hypothesized that endophyte presence above-ground would have a net negative impact on tree survival and seedling growth. (ii) Are the effects of endophyte presence on trees due to chemical factors (plant production of allelochemicals, changes in soil nutrients) or biotic soil factors (alteration of the soil microbial community)? If governed by chemical mechanisms, then we expected negative effects of the endophyte regardless of the presence of soil biota. Alternatively, if biotic soil mechanisms were operating, then we predicted a stronger endophyte effect in live soil than in sterilized soil. (iii) Do tree species vary in response to soil conditioning by tall fescue? Because the potential for soil-mediated effects of invasive plants are often investigated for a small subset of the native plant community (often a single native species), and because native species may vary in their response to invaders, we tested nine tree species to evaluate the generality of soil-mediated effects. Our work is among the first to examine the mechanisms underlying inhibition of native trees by non-native plant–endophyte symbioses and contributes to a broader understanding of how invasive symbioses alter the composition and successional trajectory of native plant communities.

Methods

INVASIVE GRASS STUDY SYSTEM

Tall fescue (*L. arundinaceum*) is a perennial, cool season grass native to Europe and Northern Africa and is widely planted worldwide for pasture and turf (Ball *et al.* 1993). In North America, tall fescue is also classified as a noxious weed in several regions (Raloff 2003; USDA & NRCS 2008). The endophyte, *N. coenophialum*, can benefit tall fescue by increasing host resistance to herbivory and drought stress, as well as by increasing host competitive ability (Malinowski & Belesky 2000; Clay *et al.* 2005; Rudgers & Clay 2007). Approximately 75–80% of tall fescue in North America is infected by *N. coenophialum*, with infection frequencies ranging from 0% to 100% among populations (Ball *et al.* 1993; Clay & Holah 1999; Clay *et al.* 2005).

TARGET TREE SPECIES

We challenged nine tree species with soils conditioned by tall fescue grass. Black cherry (*Prunus serotina*, Rosaceae), box elder (*Acer negundo*, Aceraceae), green ash (*Fraxinus pennsylvanica*, Oleaceae), pin oak (*Quercus palustris*, Fagaceae), redbud (*Cercis canadensis*, Fabaceae), silver maple (*A. saccharinum*, Aceraceae), and sycamore (*Platanus occidentalis*, Platanaceae) are native in eastern North America and are often found in early successional sites where tall fescue is common. Tree-of-heaven (*Ailanthus altissima*, Simaroubaceae) and autumn olive (*Elaeagnus umbellata*, Eleagnaceae) are native to East Asia and Eurasia, respectively, and are common invaders in the mid-western US. Eight of the nine tree species (excepting pin oak) occurred naturally in woodlands and field edges within 100 m of the tall fescue field plots used in our study. Pin oak was present regionally in areas where tall fescue is grown. Tree seeds were collected from 8 to 11 trees per species in Bloomington,

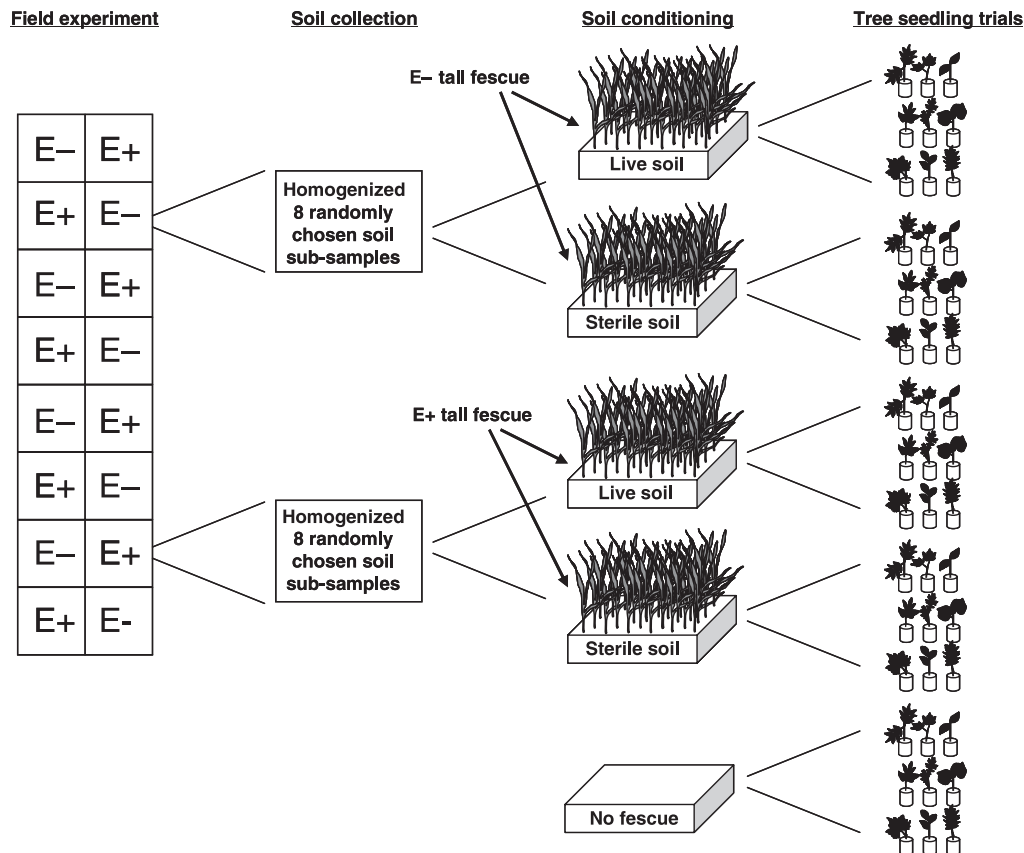


Fig. 1. Schematic of the experimental design, divided into four stages. Field experiment: Plots were seeded with endophyte-symbiotic (E+) or endophyte-free (E-) tall fescue. Soil collection: Soil was collected from every plot in the experiment; subsamples were homogenized within a plot, but plots were maintained separately. Soil conditioning experiment: Inocula from the field were added live or autoclaved (sterile) to sterilized potting soil. In the greenhouse, trays of soil were conditioned by tall fescue plants either with (E+) or without (E-) the endophyte in the greenhouse. The No fescue treatment consisted of only the sterilized potting soil and was not conditioned by tall fescue. Tree seedling trials: Nine tree species were tested.

Indiana, USA during fall 2003 and spring 2004. Pin oak acorns (collected from > 10 trees during fall 2003) were donated by the Indiana Department of Natural Resources nursery in Vallonia, Indiana. Seeds were stored at 3 °C from the time of harvest until planting.

FIELD EXPERIMENT

We manipulated soil inoculum by collecting soil from long-term field plots of tall fescue at the Indiana University Bayles Road Experimental Field (Bloomington, IN, 39°13'9" N, 086°32'29" W). The 16 plots (30 × 30 m each, $n = 8$ plots per endophyte treatment) were initially established in September 2000. Seed stock (endophyte-symbiotic or endophyte-free) for the plots came from fields that freely cross-pollinated for several generations after the original disinfection, allowing for homogenization of plant genotype relative to the endophyte treatment (see further details on this experiment in Rudgers *et al.* 2007). Based on microscopic examination of 544 plant samples taken during 2003–2005, plots seeded with endophyte-free tall fescue (E-) were 0–0.1% infected, and plots sown with endophyte-symbiotic tall fescue (E+) were 92–94% infected.

SOIL COLLECTION

On 12 April 2004, we collected soil samples from eight randomly chosen locations within each of the 16 plots. Soil samples were taken

from the top 15 cm of the soil horizon. We bulked samples within a plot to obtain approximately 1.5 L of soil per plot, but replicate field plots remained separate replicates in the experiment. Tools were sterilized in an antimicrobial wash and 50% bleach dip after sampling each plot.

SOIL CONDITIONING TREATMENT

We used live or sterilized soil samples from the field experiment to inoculate soil conditioning trays planted with tall fescue in the greenhouse (Fig. 1). For the live soil treatment, half of each soil inoculum type (0.75 L) was directly blended into one planting tray (T.O. Plastics Inc. F1020, 25.9 × 52.3 × 6.1 cm, approximately 8 L soil per tray) filled with sterilized potting mix (190 ppm N, 13 ppm P, 188 ppm K, Indiana University Greenhouse bulk soil), producing a total of 16 trays. Each tray corresponded to one field plot. For the sterilized soil treatment, the other half of each soil inoculum was sterilized in an autoclave for 6 h and then added to trays with the sterilized potting mix (Fig. 1). We specifically diluted live and sterile soil inocula to avoid large differences in nutrient availability between treatments. Endophyte-symbiotic (E+) or endophyte-free (E-) tall fescue seeds (var. Jesup) were sown into the trays, with endophyte status matched to the soil inoculum type (e.g. symbiotic seeds were sown only into trays containing soil from endophyte-symbiotic field plots). Plants in the E+ treatment were 94% endophyte-infected

($n = 278$ plants tested) and plants in the E⁻ treatment were 0% infected ($n = 287$ plants). The greenhouse conditioning phase allowed tall fescue to condition soil under the initial presence or absence of a soil microbial community, producing effects that could not be obtained by sterilizing field soil alone. We also included an additional eight trays of sterile potting soil to serve as unplanted, unconditioned controls (No fescue). In total, forty trays of soil were produced, representing five treatment combinations: E⁺ live, E⁺ sterile, E⁻ live, E⁻ sterile, and No fescue (Fig. 1).

Trays were randomized on benches in the Indiana University greenhouse during 14–21 April 2004 and watered daily with tap water. We placed trays approximately 15 cm apart to prevent leaf overhang and water splash into adjacent trays. After nine weeks (before plants became root-bound), we clipped tall fescue leaves and stems at the soil surface and sifted the soil to remove roots.

TREE SEEDLING TRIALS

For each of the nine tree species, we filled 230 mL Styrofoam cups (DART brand Mason, MI) with 80 mL of soil from one of the five soil treatments (40 replicates per treatment per tree species with five replicates per soil tray, 1800 cups total). Seeds were randomly assigned to treatments, and cups were randomly assigned to locations within the greenhouse, spaced at least 10 cm to reduce contamination. We used intact cups so that microbial communities and putative allelochemicals were not washed out during watering. To facilitate drainage, we added a 2 cm layer of sterile pea gravel to each cup before adding soil. After planting, we watered each cup daily with tap water, taking care not to over-water. We applied 10 mL of fertilizer (Ionic plant nutrient solution diluted 1 : 200 with tap water, 5–2–6 N–P–K, HydroDynamics International, Lansing, MI) to each cup to compensate for potential differences in resource availability among the soil conditioning treatments.

We tested the germination rates and viability of each tree species in a growth chamber (22 °C) before planting the experiment. Because of low germination relative to other tree species, tree-of-heaven, autumn olive and sycamore seeds required a pre-germination treatment on sterile, wet filter paper in sealed plastic containers. We therefore do not have data for the effect of treatments on the germination of these three tree species. In addition, we planted four box elder seeds, three green ash seeds or four redbud seeds (all untreated) per cup and removed surplus seeds after the first seed germinated. Due to higher germination rates, only one untreated seed per cup was necessary for silver maple, pin oak and black cherry.

RESPONSE VARIABLES

Seedling emergence and mortality were monitored every 2 days for the duration of the experiment. We harvested trees approximately 80 days (11.5 weeks) after planting, with the exception of silver maple, which was harvested at 56 days due to rapid growth. All individuals of the same species were harvested within 36 h. We counted leaf number and measured shoot length to the nearest 0.1 cm for each seedling. We dried seedlings to constant mass in a convection oven for a minimum of 72 h at 60 °C. We recorded dry root and shoot (stem + leaves) to the nearest 0.001 g.

STATISTICAL ANALYSIS

To compare the endophyte treatments to the control (No fescue) soil treatment and address question (i) Does the presence of the

endophyte during soil conditioning reduce tree performance relative to soil conditioned by endophyte-free plants?, we used a general linear model that included the independent factors of tree species (nine species), treatment (five levels: E⁺ live, E⁺ sterile, E⁻ live, E⁻ sterile and No fescue), and tree species × treatment (PROC GLM, SAS Institute 2004, v. 9.1, Cary, NC). To compare the five treatment levels, we used *post hoc* Tukey HSD tests. We initially included the plot of origin (each plot inoculated a separate soil tray) as a random factor nested within treatment, but we excluded it from the final analysis because it did not explain significant variation in the data ($0.069 < P < 0.967$ across species). Total biomass was square-root transformed to satisfy assumptions of normality of residuals and homogeneity of variances. Statistical results were qualitatively similar regardless of whether plot of origin was included in the models.

To test for effects of the treatments on seedling emergence from the soil and seedling survival (for the subset of seedlings that emerged), we used log-linear models (PROC GENMOD, SAS Institute 2004, v. 9.1, Cary, NC). We tested each tree species independently because the algorithm failed to converge in the model including all species. Each model included the independent factor of treatment (five levels). Because seeds of three species were pre-germinated prior to the experiment (and these species had 100% emergence), we did not include these in the analysis of seedling emergence. Plot of origin was also not included in the final models because it failed to explain significant variation in the data.

In a second analysis, we specifically addressed question (ii) Are the effects of endophyte presence on trees due to chemical factors or biotic soil factors? This analysis included the fixed factors of tree species identity (nine species), endophyte conditioning treatment (E⁺ vs. E⁻), soil sterilization treatment (live vs. sterile) and all interactions. Unlike the first analysis, this factorial model did not include the 'No fescue' treatment. The effect of plot of origin (nested within endophyte and sterilization) did not explain significant variation in the data and was removed from the final analysis.

If the effects of the endophyte occurred via chemical mechanisms (production of allelochemicals and/or altered nutrient availability), then we anticipated a negative effect of endophyte presence on tree performance and no significant interaction between the endophyte and soil sterilization treatments. Alternatively, if the effects of the endophyte occurred via soil biota, then we expected a stronger negative effect of endophyte presence in live soil than in sterile soil. It is also possible that autoclaving the soil improved nutrient availability in ways that could ameliorate tree responses to the endophyte; this could similarly produce a stronger negative impact of endophyte presence in live than in sterile soil. However, we think this alternative hypothesis is unlikely because the soil inoculum was < 10% of total soil volume, the duration of the experiment was short, and we added fertilizer to every tree seedling to reduce nutrient limitation. Finally, autoclaving the soil may have resulted in stronger nutrient releases in soil inocula from E⁺ plots than in soil from E⁻ plots. Prior research has reported larger organic C and N pools in the presence of the endophyte (e.g. Franzluebbers & Stuedemann 2005). This effect would generate a significant interaction between the endophyte and soil sterilization treatments, but with positive effects of E⁺ conditioning on tree performance relative to E⁻ conditioning; this pattern is opposite of our prediction that E⁺ conditioning will negatively affect trees relative to E⁻ conditioning.

Finally, we addressed the question (iii) Do tree species vary in response to soil conditioning by tall fescue? Differential responses of the nine tree species would be indicated by significant interactions between tree species and the soil conditioning treatment. When these interactions were significant, we tested for differences among

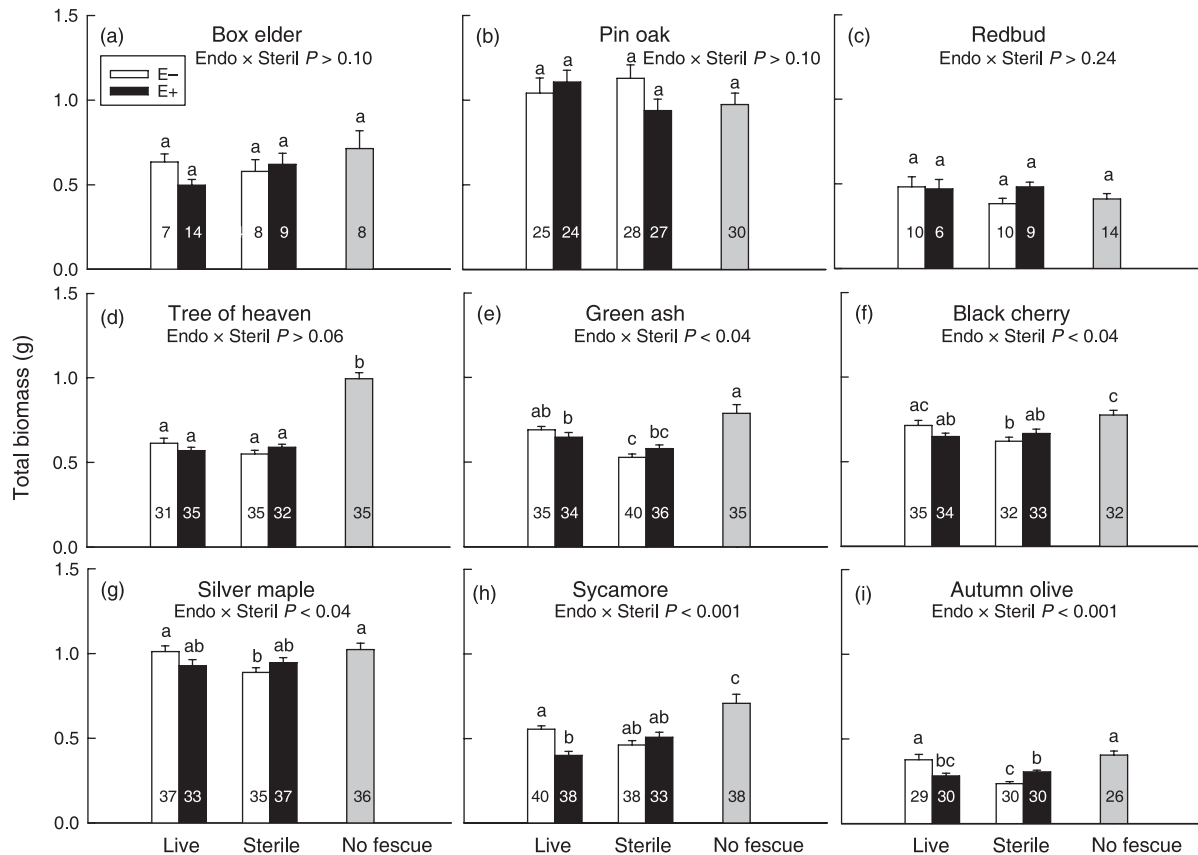


Fig. 2. Mean \pm SEs for the total biomass (g) of nine tree species in the five soil conditioning treatments. Live or sterile soil was conditioned by tall fescue either with (E+, dark bars) or without (E-, open bars) the endophyte. Gray bars show means for plants grown in sterile soil with no tall fescue conditioning (No fescue). Sample sizes are given on each bar. Within a species, significant differences (Tukey HSD) among treatments are indicated by different letters.

treatments within each tree species using Tukey HSD tests. For tree species in which total biomass was significantly affected by the treatments, we applied individual ANOVA to isolate the treatment effects for components of total biomass (i.e. shoot mass, shoot length, leaf number and root mass). Because four responses were measured within each individual, P -values were corrected for multiple comparisons following methods in Holm (1979). For response variables that were significantly affected by the treatment using Holm's method, we assessed differences among treatment levels with Tukey HSD tests.

Results

SEEDLING EMERGENCE AND SURVIVAL

Tree seedling emergence and survival appeared relatively insensitive to soil conditioned by non-native tall fescue grass. We observed no significant effects of endophyte conditioning or sterilization on the probability that seedlings emerged ($P \geq 0.07$ for all tree species). For the subset of seedlings that emerged, only sycamore showed reduced survival due to tall fescue conditioning, with lower survival in endophyte-present (E+) (89.9% survived) relative to endophyte-free (E-) (97.5% survived) conditions, and no significant effect of sterilization (factorial log-linear model: endophyte $\chi^2 = 6.2$, $P = 0.013$; sterilization $\chi^2 = 2.6$, $P = 0.106$; endophyte \times sterilization

$\chi^2 = 2.2$, $P = 0.141$). For all other tree species, survival was unaffected by the treatments; all $P \geq 0.16$).

SEEDLING BIOMASS

The effects of soil conditioning on growth varied among the nine tree species (ANOVA Model $F_{44,1208} = 40.9$, $P < 0.0001$; Treatment $F_{4,1208} = 21.0$, $P < 0.0001$; Species $F_{8,1208} = 185.5$, $P < 0.0001$; Species \times Treatment $F_{32,1208} = 4.4$, $P < 0.0001$; Fig. 2). Tree species fell into one of four categories: (i) no treatments affected tree performance relative to the unconditioned control, (ii) all treatments reduced tree performance relative to the unconditioned control except for endophyte-free (E-) conditioned live soil, and (iii) all treatments reduced tree performance relative to the unconditioned control except for endophyte-free (E-) conditioned live soil, and (iv) endophyte (E+) conditioned soil reduced tree performance, but only in the initial presence of soil microorganisms.

First, three tree species (box elder, pin oak, redbud) were insensitive to soil conditioning and sterilization (Fig. 2a–c, Table 1). For box elder and redbud biomass, some sample sizes were as low as $n = 6$ or 7 due to low germination or survival (Fig. 2). These particular non-significant results should be interpreted with caution, as trends suggest a possible negative effect of E+ conditioned live soil on box elder biomass.

Table 1. Statistical results for the effects of endophyte presence in tall fescue (*Lolium arundinaceum*) during soil conditioning (Endophyte) and soil sterilization (Sterilization) on the total biomass (g) of nine tree species. In the one-way ANOVA, treatment included five levels (E- live, E- sterile, E+ live, E+ sterile, and No fescue)

Species	One-way ANOVA				Factorial ANOVA				
	Treatment (5 levels)		Endophyte		Sterilization		Endophyte × Sterilization		
	F	P	F	P	F	P	F	P	
(1) Insensitive to soil conditioning and sterilization									
Box elder	1.8	0.157	0.8	0.377	0.3	0.573	2.8	0.106	
Pin oak	1.1	0.351	0.4	0.513	0.3	0.603	2.7	0.101	
Redbud	1.2	0.326	1.0	0.315	0.7	0.404	1.4	0.244	
(2) Reduced by all treatments relative to the no fescue (control) treatment									
Tree of Heaven	52.6	< 0.001	0.0	0.971	0.8	0.361	3.4	0.066	
(3) Reduced by all treatments relative to control except for E- conditioned live soil									
Green ash	16.0	< 0.001	0.0	0.899	26.5	< 0.001	4.6	0.035	
Black cherry	5.1	< 0.001	0.1	0.785	2.0	0.159	4.3	0.040	
Silver maple	2.7	0.033	0.2	0.660	2.4	0.126	4.7	0.033	
(4) Reduced by E+ conditioned live soil									
Sycamore	11.9	< 0.001	4.6	0.033	0.1	0.825	15.0	< 0.001	
Autumn olive	12.9	< 0.001	0.3	0.581	10.4	0.002	21.2	< 0.001	

Table 2. Means ± SEs for statistically significant plant biomass responses to the presence of the endophyte in tall fescue during soil conditioning (Endophyte: E+ or E-) and soil sterilization (Sterilization: Live or Sterile). Significant differences as demonstrated by Tukey HSD tests are indicated by different bold letters within a given response variable

Species and response	Live		Sterile		Control
	E-	E+	E-	E+	
Green ash					
Root mass (g)	0.33 ± 0.01a	0.28 ± 0.01b	0.25 ± 0.01b	0.27 ± 0.01b	0.38 ± 0.02a
Shoot mass (g)	0.36 ± 0.01a	0.36 ± 0.02ab	0.28 ± 0.01c	0.31 ± 0.01bc	0.40 ± 0.02a
Shoot length (cm)	11.0 ± 0.3ab	11.7 ± 0.4a	10.0 ± 0.2b	10.4 ± 0.2b	10.7 ± 0.3ab
Silver maple					
Root mass (g)	0.27 ± 0.01a	0.26 ± 0.01ab	0.21 ± 0.01b	0.24 ± 0.01ab	0.24 ± 0.01ab
Sycamore					
Root mass (g)	0.18 ± 0.01a	0.13 ± 0.01b	0.15 ± 0.01ab	0.17 ± 0.01a	0.23 ± 0.02a
Shoot mass (g)	0.38 ± 0.01a	0.27 ± 0.02b	0.31 ± 0.02b	0.34 ± 0.02a	0.48 ± 0.04c
Shoot length (cm)	12.5 ± 0.4a	10.2 ± 0.5b	11.4 ± 0.5a	11.8 ± 0.6a	13.5 ± 0.6a
Autumn olive					
Root mass (g)	0.10 ± 0.01a	0.08 ± 0.01b	0.08 ± 0.01b	0.10 ± 0.01a	0.13 ± 0.01a
Shoot mass (g)	0.27 ± 0.03a	0.20 ± 0.01b	0.16 ± 0.01b	0.21 ± 0.01b	0.27 ± 0.02a
Shoot length (cm)	16.0 ± 0.7a	15.4 ± 0.6a	11.9 ± 0.5b	14.3 ± 0.7a	16.4 ± 0.8a
Leaf number	14.8 ± 0.4a	14.8 ± 0.2a	12.5 ± 0.3b	13.8 ± 0.3a	14.3 ± 0.3a

Second, for two tree species (sycamore, tree-of-heaven) any conditioning by tall fescue reduced biomass relative to the unconditioned control treatment (No fescue), suggesting potential allelopathy of tall fescue grass (Fig. 2d,h; Table 1). An alternative hypothesis is that these effects resulted from nutrient depletion during the soil conditioning process.

Third, three native tree species (green ash, black cherry, silver maple) showed generally reduced biomass in all soil treatments except for E- conditioned live soil and the unconditioned control soil (Fig. 2e-g, Table 1). For green ash and black cherry reductions in total biomass were, on average, 26% and 17%, respectively. While silver maple showed similar

trends, only plants in the E- conditioned sterile treatment had statistically significantly lower biomass (by 13%) than the control (Fig. 2g, Table 1). Given these effects on total biomass, we examined individual above- and below-ground responses. For green ash and silver maple, the negative effects of soil conditioning occurred mainly below-ground via reduced root mass (Table 2). For green ash, root mass was, on average, 25% lower in all treatments other than unconditioned soil or E- conditioned live soil (Table 2). In addition, regardless of endophyte presence, sterilization reduced both the shoot mass and shoot length of green ash (Table 2). For silver maple, root mass was 22% lower in E- conditioned sterile soil

than in E⁻ conditioned live soil (Table 2), showing that soil sterilization only affected this species in the absence of conditioning by the endophyte.

Finally, presence of the endophyte during soil conditioning (E⁺) negatively affected two native and one non-native tree species; however, these effects occurred only in live, not sterile, soil. Relative to E⁻ conditioning, E⁺ conditioning reduced sycamore seedling biomass by 28% and that of non-native autumn olive by 25%, but only in live soil (Fig. 2h,i, Table 1). Both tree species had smaller root and shoot mass in E⁺ conditioned live soil, and sycamore was also 18% shorter than in E⁻ conditioned live soil (Table 2). In addition, in the E⁻ conditioned sterile treatment, autumn olive seedlings were significantly shorter and made fewer leaves than seedlings in any other treatment (Table 2). Finally, the root mass of green ash was 15% smaller in E⁺ conditioned live soil than in E⁻ conditioned live soil (Table 2). Thus, three species were negatively affected by the presence of the endophyte through soil microbe-mediated mechanisms.

Discussion

This work uncovers a novel mechanism – endophyte-mediated alteration of soil biota – underlying the impact of an invasive plant on successional dynamics in grassland ecosystems. Here, successional tree species showed divergent responses to soil conditioned by endophyte-symbiotic vs. endophyte-free tall fescue grass. By altering the soil biota, the endophyte in tall fescue indirectly suppressed the growth of three of the nine species tested, reducing the total biomass of sycamore and autumn olive by approximately 25–30% and the root biomass of green ash by 15%. Survival of sycamore declined when the endophyte was present during soil conditioning, regardless of the soil microbial community. In addition to these negative effects of conditioning by endophyte-symbiotic tall fescue, we observed growth reductions (relative to the unconditioned soil) for green ash and black cherry in all treatments except for the endophyte-free conditioned live soil. For these tree species, soil microbes may ameliorate the negative effects of tall fescue, but this occurred only in the absence of the endophyte. For two additional species, sycamore and tree-of-heaven, any conditioning by tall fescue reduced biomass relative to unconditioned soil, which could suggest either release of allelochemicals or general nutrient depletion during tall fescue soil conditioning. The addition of fertilizer to the tree seedlings, the relatively small fraction of soil that was inoculum, and the lack of any soil conditioning effects on three of the tree species tested suggest that the hypothesis of general nutrient depletion may be unlikely. Finally, three species showed no strong effects of any soil treatment. While results for box elder and redbud should be interpreted cautiously due to the low sample sizes for biomass responses, our results suggest that some tree species are insensitive to changes in the soil caused by tall fescue.

Because tree species responded divergently, the symbiosis between tall fescue and the *Neotyphodium* endophyte has the potential to alter plant succession and future forest

composition. Thus far, tree species-specific effects appear to be idiosyncratic: there were no patterns in the direction of soil conditioning effects on early vs. later successional tree species, on closely vs. distantly related species, or on native vs. non-native species. Future studies spanning a greater breadth of tree species and including more representatives of non-native trees would yield more statistical power to detect such trends.

Our prior field studies showed that endophyte-symbiotic tall fescue can inhibit neighbouring plants, including tree seedlings, relative to endophyte-free tall fescue (Orr *et al.* 2005; Rudgers & Clay 2007). There, reduced tree colonization was explained, in part, by higher rates of vole seedling predation in plots with the endophyte (Rudgers *et al.* 2007). However, direct competition with endophyte-symbiotic tall fescue (e.g. Clay *et al.* 1993) may account for additional variation in tree performance. Results here suggest that endophyte-mediated changes in soil microbial communities may also underlie reductions in tree establishment, because effects of soil conditioning by endophyte-symbiotic fescue were only observed in live, not sterile, treatments. Our data were consistent with chemically-mediated mechanisms only for sycamore, in which survival was greater in soil conditioned by E⁻ tall fescue than by E⁺ tall fescue regardless of the sterilization treatment. In contrast, earlier work has shown that tall fescue can have direct allelopathic potential (Peters & Luu 1985; Lipinska & Harkot 2007), including against trees (Larson & Schwarz 1980; Preece *et al.* 1991). However, these prior studies, which largely used extracts from leaves or leachates, may have exposed target plants to unnaturally high levels of potential allelochemicals, relative to the levels present in our conditioning experiment. Although these earlier studies did not report endophyte status, it is likely that tall fescue plants were endophyte-symbiotic, given the high rates of infection in the US (Ball *et al.* 1993).

Our experiment took a black box approach to the soil community through the use of experimental sterilization. Results suggest that different soil microbial communities developed under endophyte-symbiotic vs. endophyte-free tall fescue because of the observed declines in tree seedling growth. At this stage, we can only speculate on potential responses of particular components of the soil microbial assemblage. However, prior work has shown that the presence of the endophyte in tall fescue suppresses mycorrhizal colonization of tall fescue roots and reduces densities of mycorrhizal spores in soil (Chu-chou *et al.* 1992; Mack & Rudgers 2008; see also Omacini *et al.* 2006). Other C₃ grasses similarly reduce mycorrhizal fungi, possibly through allelochemicals (Javaid 2008). All tree species in our study (or their close relatives) have been documented to form mycorrhizae, suggesting reduced mycorrhizae as one possible mechanism. Consistent with this hypothesis, black walnut (*Juglans nigra*) seedlings treated with leaf leachates from tall fescue showed reduced mycorrhizal colonization and lower phosphorous concentrations (Ponder 1986), although tall fescue endophyte status was not reported. In addition, it is possible that pathogens of the native trees (e.g., Packer & Clay 2000) accumulated

in endophyte conditioned soil. For example, invasive *Tamarix* appears to elevate levels of pathogenic *Fusarium* in the soil (Rekah *et al.* 2001). Finally, inhibition of bacteria poses another potential mechanism (Larson & Schwarz 1980; Jenkins *et al.* 2006). For example, both autumn olive and redbud associate with nitrogen-fixing bacteria; however, these species showed divergent responses to our soil treatments.

More information on the timing and sequence of changes in above- and below-ground diversity would yield greater insight into the dynamics of tall fescue invasion (see also Matthews & Clay 2001). It would be useful to tease apart the degree to which changes in the soil community depend on the direct effect of endophyte presence vs. the associated indirect changes in the plant community, such as declines in plant diversity and tree abundance (Rudgers *et al.* 2007). Our experiments used field soil from plots that had experienced endophyte treatments for > 3 years. Thus, any differences in soil microbial composition would include both direct and indirect effects of endophyte presence. Future work could monitor temporal changes in both plant and microbe composition as tall fescue plants establish.

While prior research has revealed negative direct and indirect effects of non-native species on native plants, few studies have considered the role of non-native symbionts in promoting species invasions (but see Vitousek *et al.* 1987; Richardson *et al.* 2000; Wolfe & Klironomos 2005; van der Putten *et al.*, 2007). Here, we showed that a non-native endosymbiont can mediate the negative impacts of an invasive plant on native (and non-native) trees. Because these effects varied strongly among tree species, the presence of the symbiosis has the potential to alter plant succession and the future composition of forest communities.

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