Managing plant symbiosis: fungal endophyte genotype alters plant community composition

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Summary

1. Understanding the extent to which plant genotype influences community composition has important conservation and management value. Here, we add a new layer to current research by testing whether genotypes of plant symbionts influence plant community composition.

2. Tall fescue grass *Lolium arundinaceum* is planted worldwide for forage, turf, and soil stabilization, has invaded native communities, and commonly hosts the foliar endophytic fungus, *Neotyphodium coenophialum*. We quantified vegetation development over a 6-year period in replicated field plots of the two most commonly introduced genotypes of the endophyte (KY-31, AR-542) in two tall fescue cultivars (Georgia-5, Jesup). The KY-31 endophyte produces ergot alkaloids toxic to mammalian herbivores, while AR-542 lacks ergots. We expected that the KY-31 endophyte would promote tall fescue dominance by altering mammalian herbivory, that endophyte-free plots would support the highest plant diversity, and that AR-542 endophyte would show intermediate effects.

3. Plots with the KY-31 endophyte supported ∼10% fewer plant species than endophyte-free or AR-542 plots. KY-31 also reduced graminoids and forbs more strongly than AR-542, with the greatest response in Georgia-5. Endophyte genotype did not significantly alter the prevalence of tall fescue; however, absence of the endophyte reduced the biomass of Georgia-5.

4. Both plant and endophyte genetic identity influenced the persistence of endophyte symbiosis. In Georgia-5, the frequency of AR-542 declined more through time than did KY-31; these effects were reversed in Jesup.

5. Endophyte presence reduced herbivory by mammals (voles), but endophyte genotype had no effect, suggesting that voles were not driving endophyte genotype-mediated changes in plant composition, and that other compounds besides ergot alkaloids influenced vole feeding.

6. *Synthesis and applications.* When endophyte-symbiotic plants are desired, the AR-542 endophyte in the Georgia-5 background would achieve higher native plant diversity and reduce tall fescue invasion of nearby areas. Conversely, Jesup is recommended for increased tall fescue persistence, as both biomass and reproduction exceeded Georgia-5. In summary, our results demonstrate that, like plant genotype, symbiont genotype can also alter vegetation dynamics and plant composition and should be considered when managing for conservation, restoration, forage, turf, re-vegetation or soil stabilization.

Key-words: community genetics, *Epichloë*, extended phenotype, fungal endophyte, genetic differentiation, herbivory, *Lolium*, MAXQ, *Microtus*, *Neotyphodium*

Introduction

Understanding how individual genotypes affect communities can enhance conservation and land management by improving our ability to predict the consequences of biodiversity loss and introductions of novel genotypes. Declines in genetic

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diversity within species commonly precede species losses from ecosystems as population sizes decline (Ellstrand & Elam 1993). Likewise, newly invading populations may support low levels of genetic diversity (Dlugosch & Parker 2008). In addition, through advanced breeding techniques and genetic engineering, new genotypes and cultivars are being introduced into ecosystems at unprecedented rates (Snow *et al.* 2005).

Genetic variation within plant species can have strong impacts on communities, both in natural ecosystems (Hughes *et al.* 2008) and in agricultural landscapes (e.g. Root 1973). For example, higher genetic diversity in *Solidago* populations increased arthropod species richness and abundance (Crutsinger *et al.* 2006) and altered the distribution of a keystone insect herbivore (Crawford, Crutsinger & Sanders 2007). Genotype identity can also be important. For instance, tall and short genotypes of a coastal shrub supported divergent herbivore communities (Rudgers & Whitney 2006) and altered plant colonization (Crutsinger *et al.* 2009).

Genetic variation of plant symbionts contributes an additional layer of diversity to communities. Plants commonly host symbiotic microbes, including mycorrhizal fungi and N-fixing bacteria in roots and endophytic fungi in leaves. The application of specific symbiont genotypes, including commercially available inoculants, has become a widespread management practice (Schwartz *et al.* 2006; Heneghan *et al.* 2008). For example, acacia trees in Australia were inoculated with *Bradyrhizobium* bacteria to promote re-establishment in degraded habitats (Thrall *et al.* 2005). However, the ecological consequences of distinct symbiont genotypes remain largely unresolved.

Here we focus on fungal endophytes, which grow asymptomatically in above-ground plant tissues (Clay 1990). In grasses, fungal endophytes can benefit plants by producing alkaloid compounds with anti-herbivore properties (Schardl *et al.* 2007). Fungal endophytes can also alter plant and arthropod composition relative to communities that are endophyte-free (Clay, Holah & Rudgers 2005; Rudgers & Clay 2008). Endophyte genotype varies substantially within and between natural populations of host grasses (e.g. Tintjer & Rudgers 2006; de Jong *et al.* 2008), and endophyte genotypes can differ in alkaloid production (Bouton *et al.* 2002).

Endophyte genotypes are currently being manipulated in host grasses for turf and forage improvement, particularly for the management of herbivores (Panaccione *et al.* 2001; Bouton *et al.* 2002). Whether these genotypes have extended phenotypes (*sensu* Whitham *et al.* 2003) affecting the broader community is largely untested. In one case, endophyte genotypes in perennial ryegrass *Lolium perenne* affected the emergence of parasitoids from Argentine stem weevils grown in the laboratory (Bultman, McNeill & Goldson 2003). Given the frequency with which novel genotypes are being introduced and distributed (e.g. Belesky & Bacon 2009), understanding the community consequences of endophyte genotype will be important for turf and forage management, as well as for the conservation of resident, native plants and animals in agricultural, re-vegetated, and invaded ecosystems.

Here, we evaluate how endophyte genotype and grass cultivar affect plant diversity and composition over 6 years and determine the potential for novel endophytes to spread into new communities by assessing host reproduction and the persistence of the symbiosis. We compared two genetically distinct cultivars of tall fescue grass, *Lolium arundinaceum* (Schreb.) S.J. Darbyshire (Georgia-5 and Jesup), across three endophyte treatments: KY-31, AR-542, or endophyte-free. Because of its ergot alkaloid production, we predicted that the KY-31 endophyte would result in the highest productivity of tall fescue and the greatest suppression of other plant species in the community, in part by deterring mammalian herbivores from tall fescue (Clay *et al.* 2005; Rudgers *et al.* 2007). Plots with endophyte-free tall fescue should support the highest plant diversity, and the ergot-free AR-542 genotype should produce intermediate effects. Specifically, we addressed the following questions: (1) Does plant cultivar or endophyte genotype affect the stability of the endophyte symbiosis through time? (2) Does plant cultivar or endophyte genotype affect plant species diversity and composition? (3) Is the effect of endophyte genotype consistent across plant genetic background, or is there an interaction with plant cultivar? (4) Is herbivory by wild mammals (voles, *Microtus* spp.) consistent with observed plant responses to the endophyte? Tall fescue is invasive in the U.S. and is also common in many unimproved and unmanaged grassland habitats, where it was planted originally for forage or soil conservation and now exists in a diverse plant community with many other plant species. Our goal was to evaluate the effects of novel endophyte-grass combinations on surrounding plant communities, especially native species, to inform management decisions about whether, when, and where these cultivars should be planted.

Materials and methods

STUDY SYSTEM

Tall fescue is a perennial C_3 grass native to Europe and North Africa. It is planted worldwide for forage, turf, re-vegetation, and erosion control and can reduce wildlife grazing, tree invasion, accumulation of heavy metals and management costs (e.g. Bacon & Hill 1997; Li *et al.* 2000; Barnes 2004). In the USA, tall fescue covers ∼15 million ha (Fribourg & Hannaway pers. comm.; Ball, Pedersen & Lacefield 1993). It has spread from planted areas into native communities and is classified as a noxious weed (USDA & NRCS 2004). Due to widespread propagation of a single cultivar (KY-31), most tall fescue in the US is infected by a single genotype of the endophyte *Neotyphodium coenophialum*, KY-31. The endophyte spreads exclusively through vertical transmission to the seeds via vegetative hyphal growth (Leuchtmann & Clay 1990). Thus, all seed progeny are infected with the same clone, or genotype, of the endophyte. Some genotypes of *N. coenophialum* can increase host resistance to herbivory and drought (Malinowski & Belesky 2000; Rudgers & Clay 2005).

The genotype of *N. coenophialum* in the KY-31 cultivar (hereafter, KY-31 endophyte) produces several types of alkaloids, particularly the ergot alkaloid, ergovaline, that is toxic to mammals (Bacon *et al.* 1977; Bacon & Hill 1997). The KY-31 endophyte reduces livestock weight gain, lowers lactation and fertility, causes gangrene (Paterson *et al.* 1995; Panaccione *et al.* 2001), and negatively affects wild animals (Rudgers & Clay 2007). Endophyte toxicity has generated considerable interest in genotypes that lack ergot alkaloids. In the USA, genotype AR-542 (marketed under the commercial trade name, MaxQ, Pennington Seed, Madison, GA, USA) is the most commonly planted after the KY-31 endophyte. AR-542 was originally discovered in a native Moroccan population of tall fescue, has low to no production of ergot alkaloids (Bouton *et al.* 2002), and can increase cattle weight gain relative to KY-31 (Beck *et al.* 2008).

EXPERIMENTAL DESIGN

The experiment was planted in September 2002 at the Indiana University Research and Teaching Preserve, Bayles Road (N 39°13′9″, W 86°32′29″). The site is a former corn (*Zea mays* spp. *mays*) and soybean (*Glycine max*) field previously maintained by mowing (Lemons, Clay & Rudgers 2005) on flood plain silt loam soils in the Haymond Soil Series. Soils are primarily Wilbur silt loam with smaller areas of Stendal silt loam. Prior to establishment, the field was disked twice. Throughout the experiment, vegetation surrounding the plots was periodically mowed, but no other management was applied.

We established 42 plots (10 × 10 m) in a 2 × 3 factorial design with two tall fescue cultivars (Georgia-5, Jesup), three endophyte treatments [KY-31 endophyte-infected, AR-542 endophyte-infected, endophyte-free (E-)], and seven replicate plots per combination. The cultivars were chosen because only this material was publically available with the AR-542 endophyte. Plots were arranged in a 6 × 7 grid. Each plot was surrounded by a silt fence to prevent seed migration among plots. Endophyte × cultivar combinations were assigned randomly with the stipulation that identical treatments did not occupy adjacent plots. We obtained seeds from commercial field plots, where seeds were propagated for at least one generation following artificial inoculations to introduce the endophyte (Chris Agee, Pennington Seed, Madison, GA, USA). We sowed seeds at a standard rate of 45 kg ha^{-1} and raked the plots after sowing. Most seeds germinated by late September 2002. The silt fence was removed the following April.

ENDOPHYTE FREQUENCY

To estimate endophyte frequency we collected 4 tillers from randomly chosen locations in each plot, during 8–24 September 2003, 12 tillers during 21–30 June 2005, and 10 tillers on 24 May 2006. We stained thin sections of the inner leaf sheath with aniline blue-lactic acid. We also used ELISA immunoblots (Hill *et al.* 2002) (Agrinostics Ltd Co., Watkinsville, GA, USA) on 10 tillers per plot, collected 14 August 2006. We calculated the proportion of tillers infected per plot per census. Results for leaf peels and immunoblots were qualitatively similar, and data were combined in repeated measures MANOVA. The model included the repeated factor of census (four dates) with the fixed factors of endophyte treatment (KY-31, AR-542, or E-), tall fescue cultivar (Georgia-5 or Jesup), and the endophyte × cultivar interaction. Data met assumptions of normality of residuals and homogeneity of variances.

PLANT COMMUNITY COMPOSITION

Vegetation harvests

Harvest was carried out on 23–27 June 2003, 23–27 September 2003, 30 June–1 July 2004, 23–29 September 2004. We also sampled during 23–30 June in 2005 and 2007, but not in September due to lack of labour. No samples were taken in 2006. On each date, we randomly located four, unique 0·5 × 0·25 m quadrats in each plot and harvested all vegetation to the soil surface. No quadrat was harvested twice. On a given census, we harvested 0·5% of total plot area. In each quadrat, we recorded the number of fescue inflorescences (in June during peak flowering), number and identities of all plant species, and total number of woody species. In June 2007, we did not record plant species identity. Harvested vegetation was returned to the laboratory and sorted into five categories: tall fescue, other graminoids, forbs, woody plants, or thatch (i.e., dead leaf litter). Sorted above-ground

plant biomass was dried at 60 °C to constant mass and weighed to the nearest 0·01 g.

Non-destructive census

During May–August 2008, we completed a non-destructive census. In each plot, we placed 0·5 × 0·25 m quadrats every 2 m along transects spaced at 2 m intervals, avoiding the outer 2 m edge (16 quadrats per plot, 2% of plot area). In each quadrat, we recorded the identity of all plant species and counted tall fescue inflorescences.

Analysis

We first tested for effects of the endophyte treatment, fescue cultivar, and the endophyte × cultivar interaction on total above-ground biomass per plot with repeated measures MANOVA for all census dates. Then, we partitioned biomass into four components: % tall fescue, other graminoids, forbs, or thatch. When responses varied through time (significant time × treatment interactions), we examined each census individually to understand temporal patterns. Because woody biomass yielded a large number of zeros on any given date, we summed the number of individual trees per quadrat across census dates and applied ANOVA. We analysed fescue inflorescence production per plot with repeated measures MANOVA. All analyses met assumptions of univariate normality of residuals and homogeneity of variances with the exception of % forbs, which was arcsin square-root transformed, and tree count (square-root transformed).

We determined cumulative plant species richness per plot for each census. We used repeated measures analysis, as described above, including the six census dates for which we recorded individual species identities (June 2003, September 2003, June 2004, September 2004, June 2005, and June 2008). Non-metric multidimensional scaling analysis (McCune & Grace 2002) produced unacceptably high stress, and ANOSIM showed no treatment effects on ordinated plant composition (data not shown). Thus, to examine responses of the ten most abundant species, we applied ANOVA to the fraction of census dates on which each species was observed. *P*-values were corrected for multiple comparisons following Holm (1979).

MAMMALIAN HERBIVORY

The dominant mammalian herbivores were *Microtus ochrogaster* (prairie vole) and *Microtus pennsylvanicus* (meadow vole) (Clay *et al.* 2005). For all dates but June 2007, we counted the number of vole-damaged tall fescue tillers (toothpick-sized with a 45° cut at each end) collected during harvest. To correct for differences among plots in the amount of tall fescue, we calculated proportional vole damage by dividing the number of damaged tillers by tall fescue biomass and applied ANOVA. Data satisfied assumptions of normality and homogeneity of variances.

Results

ENDOPHYTE FREQUENCY

For the two endophyte genotypes, the direction of change in endophyte frequency depended on tall fescue cultivar. In Georgia-5, AR-542 frequency declined more over time than did KY-31, while in Jesup, AR-542 was more persistent than KY-31 (Fig. 1; Table 1). Endophyte-free plots did not

remain completely endophyte-free throughout the experiment (Fig. 1).

PLANT COMMUNITY

Vegetation harvest

Total above-ground plant biomass was not affected by tall fescue cultivar or endophyte treatment (Table 2). Mean values ($\pm$SE) for the KY-31 endophyte (74·4 g $\pm$ 2·6), AR-542 endophyte (72·4 g $\pm$ 1·9), and endophyte-free plots (75·3 g $\pm$ 2·0) were statistically equivalent (Table 2).

Dominance of tall fescue (percentage of above-ground biomass) varied with both tall fescue cultivar and endophyte presence, but not with endophyte genotype (Table 2). In Georgia-5, endophyte-symbiotic tall fescue (KY-31 or AR-542) comprised approximately 10% more of the total biomass in the plant community than endophyte-free tall fescue (Fig. 2; Table 2). In Jesup, tall fescue made up ~50% of total biomass, regardless of the endophyte treatment (Fig. 2; Table 2). Tall fescue dominance was positively correlated with endophyte frequency in Georgia-5 (Spearman $r = 0·52$, $P = 0·016$, $n = 21$ plots), but not in Jesup ($r = 0·19$, $P = 0·409$). Overall, prevalence of Jesup tall fescue was 8% higher than Georgia-5 (Table 2). Jesup plants also produced 9·4 $\pm$ 0·6 SE inflorescences per quadrat, whereas Georgia-5 produced 7·2 $\pm$ 0·6 (repeated measures – all dates, Cultivar, $F_{1,36} = 8·4$,
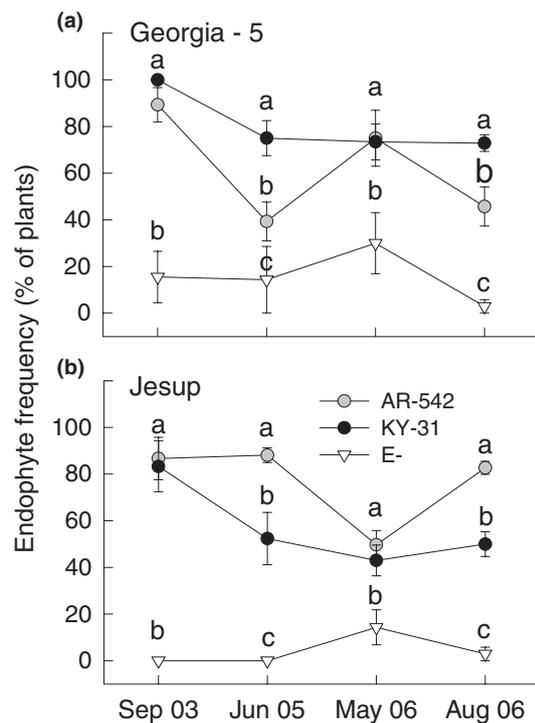

Fig. 1. Endophyte frequency (% of plants with endophyte) in response to tall fescue cultivar (a) Georgia-5 or (b) Jesup and endophyte treatment (KY-31 endophyte, AR-542 endophyte, or endophyte-free (E-)) ($n = 7$ plots). Symbols represent means $\pm$ SE. Different letters indicate significant differences (Tukey HSD) among endophyte treatments within each cultivar.

Table 1. Results from repeated measures MANOVA testing the tall fescue cultivar (Georgia-5 or Jesup) and endophyte treatment (KY-31 endophyte, AR-542 endophyte, or endophyte-free (E-)) on endophyte frequency over four census dates. $n = 7$ plots

Effect	df	F	P
Cultivar	1,36	2·2	0·151
Endophyte	2,36	118	**< 0·001**
Cultivar × Endophyte	2,36	8·5	**< 0·001**
Time	3,34	10·2	**< 0·001**
Time × Cultivar	3,34	5·9	**0·002**
Time × Endophyte	6,70	3·1	**0·011**
Time × Cultivar × Endophyte	6,70	2·4	**0·039**

P-values < 0·05 in bold.

$P = 0·006$). Endophyte effects on inflorescence production interacted with time (repeated measures – all dates, $F_{2,36} = 2·8$, $P = 0·043$). There was no significant effect on inflorescence production through 2007 (Endophyte, $F_{2,36} = 2·8$, $P = 0·072$; Cultivar × Endophyte, $F_{2,36} = 0·6$, $P = 0·533$). However, in 2008, plots with the KY-31 endophyte produced 66% more inflorescences (mean = 6·8 $\pm$ 0·9 SE) than endophyte-free plots (4·1 $\pm$ 0·8) and 100% more than AR-542 (3·3 $\pm$ 0·9) (Endophyte, $F_{2,36} = 4·4$, $P = 0·019$; Cultivar × Endophyte, $F_{2,36} = 0·5$, $P = 0·616$).

Other graminoids comprised a small fraction of above-ground biomass, but nonetheless responded to endophyte presence and genotype. Commonly observed graminoids included *Alopecurus carolinianus*, *Andropogon virginicus*, *Carex* spp., *Hordeum brachyantherum*, *Juncus* spp., *Paspalum laeve*, *Poa annua* (non-native), and *P. pratensis* (see Table S1, Supporting Information). Treatment effects varied temporally (Time × Cultivar × Endophyte was marginally significant, $P = 0·066$, Table 2). During June 2003–June 2004, the KY-31 endophyte significantly reduced other graminoids > 70% relative to the endophyte-free treatment, and AR-542 caused a 50% reduction relative to endophyte-free (Fig. 3a; Cultivar, $F_{1,36} = 0·2$, $P = 0·645$; Endophyte, $F_{2,36} = 5·7$, $P = 0·007$; Cultivar × Endophyte, $F_{2,36} = 0·8$, $P = 0·481$). During September 2004–June 2005, endophyte effects occurred only in Georgia-5, with KY-31 reducing graminoids by 77–80% relative to both endophyte-free and AR-542 (Fig. 3b; Cultivar, $F_{1,36} = 1·4$, $P = 0·252$; Endophyte, $F_{2,36} = 2·4$, $P = 0·109$; Cultivar × Endophyte, $F_{2,36} = 3·2$, $P = 0·050$). In 2007, effects on graminoids were non-significant (data not shown).

Forb responses were also seasonal (Table 2). Commonly observed forbs included *Conyza canadensis*, *Cerastium arvense*, *C. fontanum* (non-native), *Geranium maculatum*, *Glechoma hederacea* (non-native), *Oxalis stricta*, *Plantago lanceolata* (non-native), and *Veronica arvensis* (non-native) (Table S1). From 2003–2004, forb biomass was reduced, on average, 53% in KY-31 plots and 44% in AR-542 relative to endophyte-free plots, but these effects were stronger in Georgia-5 (Fig. 4a; Cultivar, $F_{1,36} = 9·1$, $P = 0·005$; Endophyte, $F_{2,36} = 3·9$, $P = 0·029$; Cultivar × Endophyte, $F_{2,36} = 5·1$, $P = 0·012$). From 2005–2007, treatment effects were no longer significant (Cultivar, $F_{1,36} = 0·1$, $P = 0·753$; Endophyte, $F_{2,36} = 1·0$, $P = 0·371$; Cultivar × Endophyte, $F_{2,36} = 1·0$, $P = 0·373$).

Table 2. Results from repeated measures MANOVA testing tall fescue cultivar (Georgia-5 or Jesup) and endophyte treatment (KY-31 endophyte, AR-542 endophyte, or endophyte-free (E-)) on total above-ground biomass; the percentage of total biomass consisting of tall fescue, other graminoids, forbs, or thatch; and cumulative plant species richness over six census dates. $n = 7$ plots. P-values < 0·05 in bold

| Effect	df	Total bio mass (g)		Tall fescue biomass (% of total)		Graminoid biomass (% of total)		Forb biomass (% of total)		Thatch biomass (% of total)		Plant species richness	
		F	P	F	P	F	P	F	P	F	P	F	P
Cultivar	1,36	1·3	0·260	8·6	**0·006**	3·5	0·071	6·0	**0·019**	1·2	0·290	0·5	0·478
Endophyte	2,36	0·3	0·727	5·0	**0·012**	0·7	0·488	3·7	**0·035**	2·2	0·131	3·7	**0·036**
Cultivar × Endophyte	2,36	0·8	0·476	3·4	**0·045**	1·5	0·239	1·7	0·193	0·6	0·571	2·7	0·082
Time	5,32	30·7	**<0·001**	115·0	**<0·001**	15·6	**<0·001**	100·2	**<0·001**	124·2	**<0·001**	22·0	**<0·001**
Time × Cultivar	5,32	1·4	0·241	1·3	0·308	0·7	0·614	6·1	**<0·001**	1·3	0·279	1·3	0·278
Time × Endophyte	10,66	1·4	0·209	1·3	0·262	1·6	0·120	1·3	0·267	0·4	0·942	1·3	0·274
Time × Cultivar × Endophyte	10,66	0·8	0·622	1·1	0·397	1·9	0·066	2·0	**0·039**	1·0	0·495	1·2	0·305
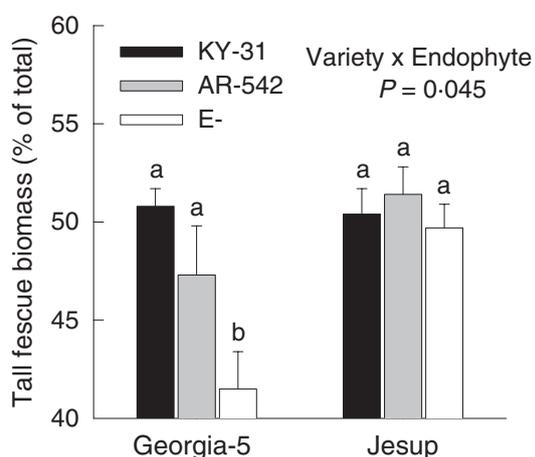
Fig. 2. Prevalence of tall fescue (% of total above-ground biomass) in response to tall fescue cultivar (Georgia-5 or Jesup) and endophyte treatment (KY-31 endophyte, AR-542 endophyte, or endophyte-free (E-)) ($n = 7$ plots). Bars show means ± SE. Different letters indicate significant differences within each cultivar.

Some forb species were especially sensitive to the treatments. Prevalence of *Conyza canadensis* was 25–29% higher in endophyte-free and AR-542 plots than in KY-31 for Georgia-5, but

did not vary in Jesup plots (Fig. 4b, Cultivar × Endophyte $F_{2,36} = 3·4$, $P = 0·044$). *Oxalis stricta* had ~50% higher prevalence in endophyte-free and AR-542 plots than in KY-31 in Georgia-5, but also showed no difference in Jesup (Fig. 4c, Cultivar × Endophyte $F_{2,36} = 4·3$, $P = 0·021$). Finally, thatch represented ~40% of above-ground biomass (mean = 32·0 g ± 0·7 SE), but showed no significant treatment response (Table 2).

Richness and composition

In total, 65 plant species recruited from the seed bank, vegetative fragments, and nearby old fields and woodlands over 6 years (Table S1). There were 10% fewer plant species found in the KY-31 endophyte plots than in AR-542 or endophyte-free plots, regardless of plant cultivar (Fig. 5a, Table 2).

Plant successional trajectory

Plots with AR-542 supported 80% more trees than endophyte-free plots (Fig. 5b, Endophyte, $F_{2,36} = 3·8$, $P = 0·033$). Unexpectedly, KY-31 plots did not differ from endophyte-free plots; however, KY-31 had 46% fewer trees than AR-542. These
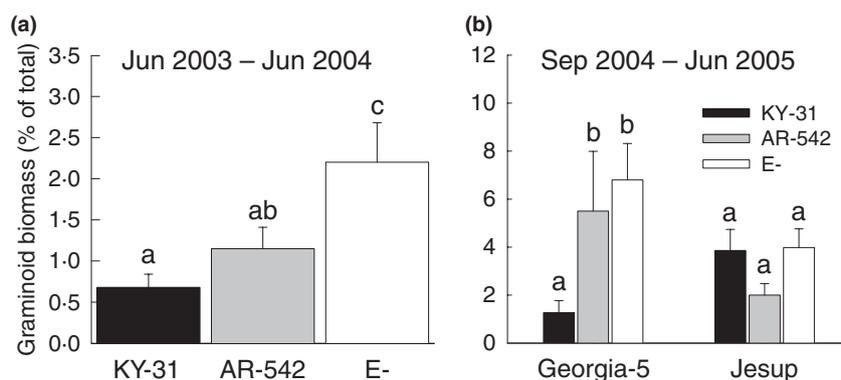

Fig. 3. Other graminoid responses to tall fescue cultivar (Georgia-5 or Jesup) and endophyte treatment (KY-31 endophyte, AR-542 endophyte, or endophyte-free (E-)) ($n = 7$ plots). (a) June 2003–June 2004 average prevalence of other graminoids (Endophyte, $F_{2,36} = 5·7$, $P = 0·007$; all other factors $P > 0·1$). (b) September 2005–June 2005 average prevalence of other graminoids (Cultivar × Endophyte, $F_{2,36} = 3·2$, $P = 0·05$; all other factors $P > 0·07$). Bars show means ± SE. Different letters indicate significant differences.
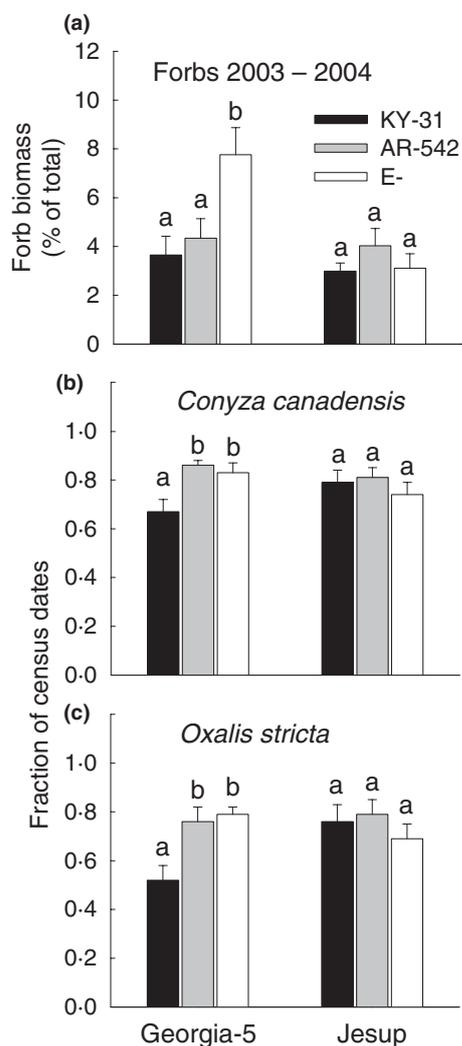
Fig. 4. Forb r AUTHOR: Please check the part labels given in the caption of Figure 4esponses to tall fescue cultivar (Georgia-5 or Jesup) and endophyte treatment (KY-31 endophyte, AR-542 endophyte, or endophyte-free (E-)) (*n* = 7 plots). (a) 2003–2004 prevalence of forbs (% of total above-ground biomass), (b) Fraction of all census dates on which *Conyza canadensis* was observed per plot, (d) Fraction of all census dates on which *Oxalis stricta* was observed per plot. Bars show means ± SE. Different letters indicate significant differences.

effects were mainly driven by the abundances of box elder *Acer negundo* and silver maple *Acer saccharinum* (data not shown). Tall fescue cultivar did not significantly affect tree abundance (Cultivar $F_{1,36} = 3.9$, $P = 0.055$; Cultivar × Endophyte $F_{2,36} = 1.5$, $P = 0.244$).

MAMMALIAN HERBIVORY

We expected that vole herbivory would be reduced more in KY-31 than in AR-542 or endophyte-free, due to ergot alkaloid production by KY-31. As expected, endophyte presence reduced vole feeding. However, vole herbivory (adjusted for tall fescue biomass) was 35% lower in both KY-31 and AR-542 than in endophyte-free (Fig. 5c,d, Endophyte,

$F_{2,36} = 4.0$, $P = 0.028$). Tall fescue cultivar had no effect (Cultivar $F_{1,36} = 0.02$, $P = 0.877$; Cultivar × Endophyte $F_{2,36} = 0.09$, $P = 0.916$).

Discussion

This work is among the first to examine impacts of plant symbiont genotypes on community dynamics and has applications for conserving wild and unmanaged grassland habitats and for managing agronomic systems to reduce the spread of invasive plants. We found that the AR-542 endophyte in two tall fescue cultivars resulted in plant communities with higher plant species richness compared to the KY-31 endophyte, but not to endophyte-free cultivars. Our results suggest that management efforts aimed to encourage plant species other than tall fescue (e.g. soil erosion and stabilization, re-vegetation projects, some forage systems) could benefit from Georgia-5/AR-542 over other endophyte-symbiotic material because: (i) reductions in other graminoid species and in native forbs were weakest in the Georgia-5/AR-542 plots; and (ii) tall fescue inflorescence production was reduced relative to other endophyte-symbiotic treatments, which should slow its spread into novel habitats. Conversely, management to promote monocultures of tall fescue, would benefit from endophyte-symbiotic Jesup cultivars, which had higher dominance than Georgia-5, lower levels of forbs and graminoids, and greater reproduction.

(1) Does plant cultivar or endophyte genotype affect the stability of the endophyte symbiosis through time? The persistence of endophyte symbiosis declined during the experiment and depended on endophyte genotype and plant cultivar. In the Georgia-5 cultivar, the decline in frequency was greater for the AR-542 endophyte than for KY-31. In Jesup, these differences were reversed. We do not currently know the mechanism underlying these differences in endophyte persistence. However, our data support other evidence that endophyte effects are contingent on plant genotype (e.g. Cheplick 2007). Under field conditions, the most vigorous plant/endophyte genotype combinations should predominate over time. Plant genotypes will be recombined each generation because tall fescue is self-incompatible and highly heterogeneous, but endophyte genotypes, which are asexual, will not recombine. Our results contrast with to past experiments on the KY-31 cultivar of tall fescue infected with the KY-31 endophyte, where endophyte frequencies remained very high or increased through time, with no evidence of decline (e.g. Clay *et al.* 2005; Rudgers *et al.* 2007). Although results here cannot be compared directly with experiments on the KY-31 cultivar, the current experiment was conducted within 200 m of our prior KY-31 plots and occurred over much of the same time period (Rudgers *et al.* 2007; Rudgers & Clay 2008). Because the endophyte does not spread contagiously among plants, this temporal variability could result only from a loss of the endophyte from infected plants (Afkhami & Rudgers 2008) or from invasion by endophyte-free seeds.

Our results are consistent with prior studies showing that endophyte-free plots exhibited a gradual increase in infection frequency (Shelby & Dalrymple 1993; Clay & Holah 1999;
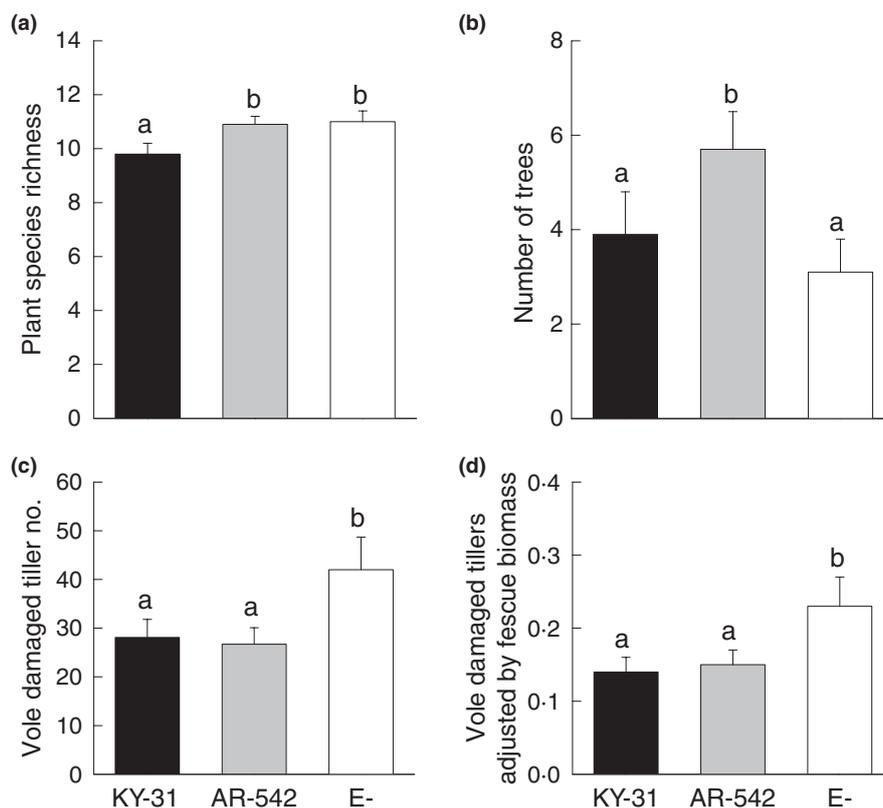
Fig. 5. Community responses to the endophyte treatment (KY-31 endophyte, AR-542 endophyte, or endophyte-free (E-)) ($n = 14$ plots). (a) Cumulative plant species richness per plot per census over six census dates, (b) Number of trees per plot summed over six census dates, (c) Cumulative number of vole damaged tall fescue tillers per plot for six census dates, (d) Vole damaged tillers adjusted for tall fescue above-ground biomass per plot. Bars show means $\pm$ SE. Different letters indicate significant differences.

Clay *et al.* 2005). This increase may represent seed dispersal or colonization from the seed bank, combined with possible competitive superiority of symbiotic plants. The increase also suggests that some contamination of genotypes could have occurred, but we did not test for genotype identity when scoring for endophyte presence. Any contamination that may have resulted would serve to make the effects of endophyte genotype observed here more conservative. These findings have management implications for endophyte persistence, as the AR-542 endophyte appears to be more stable in the Jesup genetic background than in Georgia-5. In addition, because grasses are wind pollinated, mixed or nearby plantings of different tall fescue cultivars could result in plant hybridization, producing plant genotypes that may be more or less compatible with particular endophyte genotypes present in the maternal lineage.

(2) *Does plant cultivar or endophyte genotype affect plant species diversity or composition?* Due to their difference in alkaloid profiles, we predicted the KY-31 and AR-542 endophyte genotypes would have divergent effects on the plant community. As predicted, plant composition varied with endophyte genotype. The KY-31 endophyte more strongly suppressed plant species richness than AR-542 across both fescue cultivars. Additionally, in Georgia-5, the KY-31 endophyte reduced other graminoids up to 80% and reduced the prevalence of two common, native forbs relative to the endophyte-

free treatment, whereas the effect of the AR-542 genotype was weaker, and not significantly different from endophyte-free plots, on later census dates.

The natural course of plant succession in our old field community is toward a forested ecosystem. Earlier work showed that endophyte presence in KY-31 tall fescue can slow succession by reducing tree establishment (Rudgers *et al.* 2007). Here, we detected an effect of endophyte genotype on tree abundance: Communities with AR-542 had 80% more trees than either the KY-31 or endophyte-free treatments. This result is inconsistent with our prior work in that, here, we observed no differences between KY-31 and endophyte-free plots. We suspect three factors may lead to these differences. First, endophyte-mediated suppression of trees may depend on plant genetic background, with larger effects of the KY-31 endophyte when in the KY-31 cultivar of fescue. Second, plots in this study were 100 m^2, whereas our previous work used plots that were nine times larger (900 m^2) and encompassed the territories of several individual voles. In a previous study conducted at a nearby site with radio-collared voles, average home range size of female voles was 113 m^2 in E+ plots and 102 m^2 in E- plots ($P = 0.7$ for differences between treatments) (Fortier *et al.* 2001). If vole territories span multiple endophyte treatments, altered vegetation dynamics, including tree seedling predation, may be less pronounced. However, this does not explain why trees appear to benefit

from association with AR-542. Third, the KY-31 endophyte in KY-31 tall fescue can have allelopathic effects on trees (Orr, Rudgers & Clay 2005; Rudgers & Orr 2009) and reduce root colonization by mycorrhizal fungi (Mack & Rudgers 2008). If such effects were absent or reversed in AR-542, this could benefit tree establishment.

In contrast to our initial predictions, the dominance of tall fescue did not depend on endophyte genotype during our 6-year study. In Georgia-5, tall fescue with either endophyte genotype was ~20% more prevalent than endophyte-free tall fescue. This result corresponded with differences among plots in vole herbivory, which was reduced in both KY-31 and AR-542 plots relative to endophyte-free plots. In contrast, Jesup tall fescue remained at ~50% of total plant biomass regardless of the endophyte treatment (including endophyte-free plots), even with significant differences in vole activity. Management strategies should consider that Georgia-5 and Jesup tall fescue do not appear as well adapted to the Midwestern climatic region compared to the KY-31 cultivar, which often comprises > 90% of above-ground plant biomass when endophyte-symbiotic (Clay & Holah 1999; Rudgers & Clay 2005, 2008). In contrast with our results, in Georgia field trials, Bouton *et al.* (2002) found higher persistence of both AR-542 and KY-31 Jesup and Georgia-5 relative to endophyte-free. We did find effects of endophyte genotype on host reproduction, which suggests greater dispersal of the KY-31 than AR-542 endophyte. This result is important for predicting tall fescue invasions.

Because endophyte genotype did not affect tall fescue dominance, our results suggest that endophyte genotype-driven changes in plant composition occur through indirect mechanisms, rather than through increased competitive ability. For example, shifts in the composition of the insect herbivore community (Rudgers & Clay 2008), in soil microbial composition (Matthews & Clay 2001; Rudgers & Orr 2009), or in allelopathic compounds (Orr *et al.* 2005) caused by endophyte genotypes could alter changes in plant composition. In support of an insect herbivore-mediated indirect effect, two growth chamber studies showed differences in aphid preference and population growth among genotypes of *N. coenophialum* in tall fescue (Hunt & Newman 2005; Bultman *et al.* 2006).

(3) *Is the effect of endophyte genotype consistent across plant genetic background?* For some forb and graminoid responses, the effect of endophyte genotype was only observed in the Georgia-5 cultivar. Two common native forbs, *Conyza canadensis* and *Oxalis stricta,* were especially sensitive to endophyte genotype, with 25–50% higher prevalence in AR-542 plots than in KY-31, but only in the Georgia-5 cultivar. In addition, graminoid biomass during 2004-2005 significantly differed between the endophyte genotypes only in Georgia-5. From a management perspective, these results suggest Georgia-5 infected with AR-542 may have fewer negative impacts on native plant communities than the more widespread KY-31 endophyte. However, the persistence of the endophyte in this cultivar declined over the course of the experiment and could result in a trade-off between maintaining fescue productivity and maintaining plant diversity. It remains unclear why

endophyte genotype differences were more apparent in Georgia-5, but this may relate to the lower persistence of AR-542 in the Georgia cultivar (Fig. 1).

(4) *Is herbivory by voles consistent with observed plant community responses to the endophyte?* Our prior work on the KY-31 tall fescue cultivar showed that mammalian herbivores (voles) and insect herbivores collectively drive increases in KY-31 endophyte frequency through time, with concomitant shifts in plant composition (Clay *et al.* 2005; Rudgers *et al.* 2007). Here, we predicted that levels of vole herbivory would be similar between the AR-542 endophyte treatment and endophyte-free plots, because only the KY-31 endophyte produces high levels of ergot alkaloids, the primary mammal-deterring compounds (Bouton *et al.* 2002). Unexpectedly, levels of vole herbivory were reduced by 35% in both the KY-31 and AR-542 plots relative to the endophyte-free treatment. This result suggests that voles are responding to endophyte traits other than ergot alkaloids. For example, recent work in perennial ryegrass *Lolium perenne* has revealed diverse changes in plant chemical profiles in response to endophyte presence, including higher nitrogen levels in endophyte-free plants (Rasmussen *et al.* 2008). Despite the known toxicity of ergot alkaloids to domesticated animals, and the increased performance of both cattle (Beck *et al.* 2008) and sheep (Parish *et al.* 2003) on tall fescue with AR-542 relative to tall fescue with the KY-31 endophyte, a recent study suggested that neither goats, sheep, nor cattle preferred AR-542 over the KY-31 endophyte in feeding trials with hay (Fisher & Burns 2008). Our results suggest, similarly, that voles do not alter feeding preferences in response to endophyte genotype. Thus, voles are unlikely to be causing differences between the endophyte genotypes in endophyte persistence or plant composition. Due to the production of insect-deterrent and insecticidal alkaloids by both endophyte genotypes, differential herbivory by insects may be a key, alternative, mechanism underlying the effects of endophyte genotype on plant composition.

Conclusions

Our results suggest that particular endophyte genotype and plant cultivar combinations can reduce the negative impacts of non-native tall fescue on other plant species. Specifically, of all symbiotic treatments, the combination of the AR-542 endophyte and the Georgia-5 cultivar had the lowest impact on plant species richness, graminoids, and two native forbs. Unexpectedly, neither altered vole herbivory nor competitive dominance of tall fescue explained the differences in plant composition between endophyte genotypes, suggesting other, more indirect species interactions may be important factors. More generally, our results show that genotypic differences among plant symbionts can have cascading effects on the composition of plant communities.

Acknowledgments

References

Afkhami, M.E. & Rudgers, J.A. (2008) Symbiosis lost: imperfect vertical transmission of fungal endophytes in grasses. *American Naturalist*, **172**, 405–416.

Bacon, C.W. & Hill, N.S. (1997) *Neotyphodium/Grass Interactions*. Plenum Press, New York.

Bacon, C.W., Porter, J.K., Robbins, J.D. & Luttrell, E.S. (1977) *Epichloë typhina* from toxic tall fescue grasses. *Applied and Environmental Microbiology*, **34**, 576–581.

Ball, D.M., Pedersen, J.F. & Lacefield, G.D. (1993) The tall fescue endophyte. *American Scientist*, **81**, 370–379.

Barnes, T.G. (2004) Strategies to convert exotic grass pastures to tall grass prairie communities. *Weed Technology*, **18**, 1364–1370.

Beck, P.A., Gunter, S.A., Lusby, K.S., West, C.P., Watkins, K.B. & Hubbell, D.S. (2008) Animal performance and economic comparison of novel and toxic endophyte tall fescues to cool-season annuals. *Journal of Animal Science*, **86**, 2043–2055.

Belesky, D.P. & Bacon, C.W. (2009) Tall fescue and associated mutualistic toxic fungal endophytes in agroecosystems. *Toxin Reviews*, **28**, 102–117.

Bouton, J.H., Latch, G.C.M., Hill, N.S., Hoveland, C.S., McCann, M.A., Watson, R.H., Parish, J.A., Hawkins, L.L. & Thompson, F.N. (2002) Reinfection of tall fescue cultivars with non-ergot alkaloid-producing endophytes. *Agronomy Journal*, **94**, 567–574.

Bultman, T.L., McNeill, M.R. & Goldson, S.L. (2003) Isolate-dependent impacts of fungal endophytes in a multitrophic interaction. *Oikos*, **102**, 491–496.

Bultman, T.L., Pulas, C., Grant, L., Bell, G. & Sullivan, T.J. (2006) Effects of fungal endophyte isolate on performance and preference of bird cherry-oat aphid. *Environmental Entomology*, **35**, 1690–1695.

Cheplick, G.P. (2007) Costs of fungal endophyte infection in *Lolium perenne* genotypes from Eurasia and North Africa under extreme resource limitation. *Environmental and Experimental Botany*, **60**, 202–210.

Clay, K. (1990) Fungal endophytes of grasses. *Annual Review of Ecology and Systematics*, **21**, 275–297.

Clay, K. & Holah, J. (1999) Fungal endophyte symbiosis and plant diversity in successional fields. *Science*, **285**, 1742–1744.

Clay, K., Holah, J. & Rudgers, J.A. (2005) Herbivores cause a rapid increase in hereditary symbiosis and alter plant community composition. *Proceedings of the National Academy of Sciences, USA*, **102**, 12465–12470.

Crawford, K.M., Crutsinger, G.M. & Sanders, N.J. (2007) Host-plant genotypic diversity mediates the distribution of an ecosystem engineer. *Ecology*, **88**, 2114–2120.

Crutsinger, G.M., Collins, M.D., Fordyce, J.A., Gompert, Z., Nice, C.C. & Sanders, N.J. (2006) Plant genotypic diversity predicts community structure and governs an ecosystem process. *Science*, **313**, 966–968.

Dlugosch, K.M. & Parker, I.M. (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology*, **17**, 431–449.

Ellstrand, N.C. & Elam, D.R. (1993) Population genetic consequences of small population size—implications for plant conservation. *Annual Review of Ecology and Systematics*, **24**, 217–242.

Fisher, D.S. & Burns, J.C. (2008) Testing for variation in animal preference for Jesup tall fescue hays with wild-type, novel, or no fungal endophyte. *Crop Science*, **48**, 2026–2032.

Fortier, G.M., Osmon, M.A., Roach, M. & Clay, K. (2001) Are female voles food limited? Effects of endophyte-infected tall fescue on home range size in female prairie voles (Microtus ochrogaster) *American Midland Naturalist*, **146**, 63–71.

Heneghan, L., Miller, S.P., Baer, S., Callaham, M.A., Montgomery, J., Pavao-Zuckerman, M., Rhoades, C.C. & Richardson, S. (2008) Integrating soil ecological knowledge into restoration management. *Restoration Ecology*, **16**, 608–617.

Hill, N.S., Hiatt, E.E., Bouton, J.H. & Tapper, B. (2002) Strain-specific monoclonal antibodies to a nontoxic tall fescue endophyte. *Crop Science*, **42**, 1627–1630.

Holm, S. (1979) A simple sequential rejective multiple test procedure. *Scandinavian Journal of Statistics*, **6**, 65–70.

Hughes, A.R., Inouye, B.D., Johnson, M.T.J., Underwood, N. & Vellend, M. (2008) Ecological consequences of genetic diversity. *Ecology Letters*, **11**, 609–623.

Hunt, M.G. & Newman, J.A. (2005) Reduced herbivore resistance from a novel grass-endophyte association. *Journal of Applied Ecology*, **42**, 762–769.

de Jong, E.V., Dobrowolski, M.P., Bannan, N.R., Stewart, A.V., Smith, K.F., Spangenberg, G.C. & Forster, J.W. (2008) Global genetic diversity of the perennial ryegrass fungal endophyte *Neotyphodium lolii*. *Crop Science*, **48**, 1487–1501.

Lemons, A., Clay, K. & Rudgers, J.A. (2005) Connecting plant-microbial interactions above and belowground: a fungal endophyte affects decomposition. *Oecologia*, **145**, 595–604.

Leuchtmann, A. & Clay, K. (1990) Isozyme variation in the *Acremonium/Epichloë* fungal endophyte complex. *Phytopathology*, **80**, 1133–1139.

Li, Y.M., Chaney, R.L., Siebielec, G. & Kerschner, B.A. (2000) Response of four turfgrass cultivars to limestone and biosolids-compost amendment of a zinc and cadmium contaminated soil at Palmerton, Pennsylvania. *Journal of Environmental Quality*, **29**, 1440–1447.

Mack, K.M.L. & Rudgers, J.A. (2008) Balancing multiple mutualists: asymmetric interactions among plants, arbuscular mycorrhizal fungi, and fungal endophytes. *Oikos*, **117**, 310–320.

Malinowski, D.P. & Belesky, D.P. (2000) Adaptations of endophyte-infected cool-season grasses to environmental stresses: mechanisms of drought and mineral stress tolerance. *Crop Science*, **40**, 923–940.

Matthews, J.W. & Clay, K. (2001) Influence of fungal endophyte infection on plant-soil feedback and community interactions. *Ecology*, **82**, 500–509.

McCune, B. & Grace, J.B. (2002) *Analysis of Ecological Communities*. MjM Software Design, Gleneden Beach, OR.

Orr, S.P., Rudgers, J.A. & Clay, K. (2005) Invasive plants can inhibit native tree seedlings: testing potential allelopathic mechanisms. *Plant Ecology*, **181**, 153–165.

Panaccione, D.G., Johnson, R.D., Wang, J., Young, C.A., Damrongkool, P., Scott, B. & Schardl, C.L. (2001) Elimination of ergovaline from a grass-*Neotyphodium* endophyte symbiosis by genetic modification of the endophyte. *Proceedings of the National Academy of Sciences, USA*, **98**, 12820–12825.

Parish, J.A., McCann, M.A., Watson, R.H., Hoveland, C.S., Hawkins, L.L., Hill, N.S. & Bouton, J.H. (2003) Use of nonergot alkaloid-producing endophytes for alleviating tall fescue toxicosis in sheep. *Journal of Animal Science*, **81**, 1316–1322

Paterson, J., Forcherio, C., Larson, B., Samford, M. & Kerley, M. (1995) The effects of fescue toxicosis on beef-cattle productivity. *Journal of Animal Science*, **73**, 889–898.

Rasmussen, S., Parsons, A.J., Fraser, K., Xue, H. & Newman, J.A. (2008) Metabolic profiles of *Lolium perenne* are differentially affected by nitrogen supply, carbohydrate content, and fungal endophyte infection. *Plant Physiology*, **146**, 1440–1453.

Root, R.B. (1973) Organization of a plant-arthropod association in simple and diverse habitats—fauna of collards (*Brassica oleracea*). *Ecological Monographs*, **43**, 95–120.

Rudgers, J.A. & Clay, K. (2005) Fungal endophytes in terrestrial communities and ecosystems. *The Fungal Community* (eds E.J. Dighton, P. Oudemans & J.F.J. White), pp. 423–442. Mercel Dekker, New York, NY.

Rudgers, J.A. & Clay, K. (2007) Endophyte symbiosis with tall fescue: How strong are the impacts on communities and ecosystems? *Fungal Biology Reviews*, **21**, 107–124.

Rudgers, J.A. & Clay, K. (2008) An invasive plant-fungal mutualism reduces arthropod diversity. *Ecology Letters*, **11**, 831–840.

Rudgers, J.A. & Orr, S.P. (2009) Non-native grass alters growth of native tree species via leaf and soil microbes. *Journal of Ecology*, **97**, 247–255.

Rudgers, J.A. & Whitney, K.D. (2006) Interactions between insect herbivores and a plant architectural dimorphism. *Journal of Ecology*, **94**, 1249–1260.

Rudgers, J.A., Holah, J., Orr, S.P. & Clay, K. (2007) Forest succession suppressed by an introduced plant-fungal symbiosis. *Ecology*, **88**, 18–25.

Schardl, C.L., Grossman, R.B., Nagabhyru, P., Faulkner, J.R. & Mallik, U.P. (2007) Loline alkaloids: Currencies of mutualism. *Phytochemistry*, **68**, 980–996.

Schwartz, M.W., Hoeksema, J.D., Gehring, C.A., Johnson, N.C., Klironomos, J.N., Abbott, L.K. & Pringle, A. (2006) The promise and the potential consequences of the global transport of mycorrhizal fungal inoculum. *Ecology Letters*, **9**, 501–515.

Shelby, R.A. & Dalrymple, L.W. (1993) Long-term changes of endophyte infection in tall fescue stands. *Grass and Forage Science*, **48**, 356–361.

Snow, A.A., Andow, D.A., Gepts, P., Hallerman, E.M., Power, A., Tiedje, J.M. & Wolfenbarger, L.L. (2005) Genetically engineered organisms and the environment: current status and recommendations. *Ecological Applications*, **15**, 377–404.

Thrall, P.H., Millsom, D.A., Jeavons, A.C., Waayers, M., Harvey, G.R., Bagnall, D.J. & Brockwell, J. (2005) Seed inoculation with effective root-nodule bacteria enhances revegetation success. *Journal of Applied Ecology*, **42**, 740–751.

Tintjer, T. & Rudgers, J.A. (2006) Grass-herbivore interactions altered by strains of a native endophyte. *New Phytologist*, **170**, 513–521.

USDA & NRCS (2004) The PLANTS Database, Version 3.5 (http://plants.usda.gov). National Plant Data Center, Baton Rouge, LA, USA. Accessed 31 December 2008.

Whitham, T.G., Young, W.P., Martinsen, G.D., Gehring, C.A., Schweitzer, J.A., Shuster, S.M., Wimp, G.M., Fischer, D.G., Bailey, J.K., Lindroth, R.L., Woolbright, S. & Kuske, C.R. (2003) Community and ecosystem genetics: a consequence of the extended phenotype. *Ecology*, **84**, 559–573.

Supporting Information

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

Table S1. Plant species occurrence data.

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