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FOREST SUCCESSION SUPPRESSED BY AN INTRODUCED PLANT–FUNGAL SYMBIOSIS

JENNIFER A. RUDGERS,^{1,2,3} JENNY HOLAH,^{2,4} SAMUEL P. ORR,² AND KEITH CLAY²

¹*Department of Ecology and Evolutionary Biology, Rice University, Houston, Texas 77005 USA*

²*Department of Biology, Indiana University, Bloomington, Indiana 47405 USA*

Abstract. Microbial symbionts can affect plant nutrition, defensive chemistry, and biodiversity. Here we test the hypothesis that symbionts alter the speed and direction of plant succession in communities that are shifting from grasslands to forests. A widespread C₃ grass introduced to the United States, *Lolium arundinaceum* (tall fescue), hosts a fungal endophyte that is toxic to herbivores. In replicated experimental grasslands, the presence of the endophyte in tall fescue reduced tree abundance and size, altered tree composition, and slowed plant species turnover. In addition, consumption of tree seedlings by voles (*Microtus* spp.) was 65% higher in plots with the endophyte at the one grassland site where these data were collected. Despite its negligible contribution to community biomass, a microbial symbiont suppressed tree establishment, posing an important constraint on the natural transition from grasslands to forests.

Key words: community structure; endophyte; herbivory; indirect effect; *Lolium arundinaceum*; *Microtus*; *Neotyphodium*.

INTRODUCTION

Succession, or the change in species composition over time, is a fundamental process in ecological communities. Plant–plant interactions (Connell and Slatyer 1977, Tilman 1982), plant–herbivore interactions (Huntly 1991), and plant–environment interactions (particularly disturbance [Pickett et al. 1989]), have traditionally been considered the primary mechanisms regulating plant succession. However, microbial symbioses may also influence successional dynamics. Although rigorous experimental tests are lacking, nitrogen-fixing bacteria and mycorrhizal fungi appear to promote changes in species composition by causing general increases in soil fertility or nutrient acquisition belowground (Janos 1980, Allen and Allen 1984, Vitousek et al. 1987, Chapin et al. 1994, Callaway 1995, Nara 2006a, b, Kardol et al. 2006). While the diffuse nature of these symbioses may advance succession, many symbionts confer benefits exclusively to one host. Mathematical and conceptual models predict that exclusive, pairwise benefits will increase host persistence, stabilize the

community, and ultimately slow the process of succession (Kumar and Freedman 1989, Ringel et al. 1996, Bever 2002, Reynolds et al. 2003).

We tested this prediction in two midwestern U.S. plant communities, taking advantage of the widespread symbiosis between the endophytic fungus, *Neotyphodium coenophialum* (Ascomycetes: Clavicipitaceae), and tall fescue grass, *Lolium arundinaceum* (Poaceae). Tall fescue was introduced to the United States in the late 1800s (Ball et al. 1993) and is now a common member of grassland ecosystems, covering more than 15 000 000 ha in the eastern United States alone (Clay and Holah 1999). In the lower midwest of the United States, where this work was conducted, secondary plant succession typically leads to the replacement of herbaceous vegetation by forests. Conversions between grasslands and forests are also worldwide phenomena with important impacts on global CO₂ levels and climate change (Intergovernmental Panel on Climate Change 2001).

In plants, endophyte symbiosis can enhance competitive ability (Clay et al. 1993), increase abiotic stress tolerance (Rodriguez et al. 2004, Malinowski et al. 2005), and deter herbivores, pathogens, and granivores (Clay 1996, Omacini et al. 2001, Arnold et al. 2003, Rudgers and Clay 2005). Benefits of symbiosis are strong in tall fescue grass, where the endophyte produces a variety of well-characterized, anti-herbivore alkaloids,

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³ E-mail: jrudgers@rice.edu

⁴ Present address: Graduate School of Education, George Mason University, Fairfax, Virginia 22030 USA.

including ergot alkaloids, lolines, and peramines (Clay and Schardl 2002, Clay et al. 2005). In the United States, more than two-thirds of the tall fescue is infected by *N. coenophialum* (Clay and Holah 1999), which grows systemically in aboveground tissues, is vertically transmitted to seeds, and lacks contagious spread (Clay and Holah 1999, Clay and Schardl 2002, Schardl et al. 2004). Prior work demonstrated that the presence of the tall fescue endophyte suppressed the diversity of herbaceous plant species in an early successional grassland (Clay and Holah 1999). Although the mechanism of this effect was not investigated, the endophyte may affect herbaceous plant composition by increasing herbivore attack on more palatable plant neighbors (Clay et al. 2005).

Here, we manipulated endophyte symbiosis and quantified tree colonization, tree growth, and plant species turnover in two independent field experiments spanning a 12-year period. In addition, we tested the hypothesis that seedling predators (voles, *Microtus* spp.) mediated differences in succession between endophyte treatments. By avoiding endophyte-infected plants, herbivores may exert greater grazing pressure on tree seedlings and other neighboring plant species. Our results demonstrate that endophyte symbiosis presents a major impediment to the transition from grassland to forested landscapes, and may explain why some introduced, invasive species, such as tall fescue, are difficult to dislodge from communities.

METHODS

Study location

Two sites in southern Indiana previously maintained in the herbaceous state by mowing were plowed, disked, and enriched with tall fescue seeds in alternating plots either with (E+) or without (E-) the endophyte. Tree abundances were initially zero in all plots. The upland site was established at the Indiana University Botany Experimental Field, Bloomington, Indiana, USA (39°10'26.6" N, 86°30'23.2" W), a long-term experimental field site with heavy clay soils (see Plate 1). During September 1994, the site was plowed and disked. Eight alternating plots (20 × 20 m) were enriched with tall fescue seed ($n = 4$ plots per endophyte treatment) at a rate of 45 kg/ha (seed source, germination, and infection rate in Clay and Holah [1999]). By June 1998, tall fescue represented $89\% \pm 3\%$ (mean \pm SE) of total aboveground (live) biomass in E+ plots and $56\% \pm 6\%$ in E- plots (see also Clay and Holah 1999). In September 2000, we established plots at the bottomland site, Bayles Road Experimental Field (39°13'9" N, 86°32'29" W), Bloomington, Indiana, by disking a former agricultural field. This site is prone to flooding, with silt-loam soils. Sixteen plots (30 × 30 m) were arranged in two blocks of eight, with alternating E+ and E- plots ($n = 8$ plots per endophyte treatment). Methods to enrich plots with tall fescue seeds followed the same procedure as the upland site. By June 2004, tall fescue constituted $99\% \pm 0.2\%$ of total aboveground (live) biomass in E+ plots and $97\% \pm 1\%$ in E- plots. No

treatments (e.g., mowing, watering, fertilizing) were conducted at either site following seed additions.

Endophyte treatment

At both sites, the endophyte was originally eliminated via long-term storage of infected seeds at room temperature, which reduced endophyte viability but not seed viability. Seeds used in the experiments were several generations distant from the storage treatment and came from field-grown parents that freely cross-pollinated (tall fescue is self-incompatible), allowing for homogenization of the plant genetic background with respect to the endophyte treatment. Four years post-establishment, 95% of sampled plants were endophyte infected in E+ plots, and 5% in E- plots at the upland site (Clay and Holah 1999), and 94% of sampled plants were endophyte infected in E+ plots, with 0% in E- plots at the bottomland site (endophyte detection following Clark et al. [1983]). Such extremes in endophyte-infection frequency are the norm for tall fescue (Siegel et al. 1984, Shelby and Dalrymple 1987, Spyreas et al. 2001), and reflect alternative management goals, such as forage and pasture (E-) vs. lawn and turf (E+), as well as widespread dispersal into unmanaged habitats, including prairies, old fields, and forest edges (Spyreas et al. 2001, Raloff 2003). In addition, previous research suggests that intermediate infection frequencies may be transitory and increase towards 100% infection (Clay et al. 2005). Thus, the use of nearly 100% E+ vs. E- treatments both was biologically relevant and maximized the potential to detect an effect of the endophyte.

Upland site

Tree succession.—During May of 1998, 1999, and 2002, all woody plants in each plot were counted and identified to species. We also measured the diameter (20 cm aboveground) to the nearest 0.01 cm (1999, 2002) and height to the nearest cm (1999 only), then calculated plot means. The dominant woody species included box elder (*Acer negundo*, 42% of individuals), white mulberry (*Morus alba*, the only non-native woody species, 32%), white ash (*Fraxinus americana*, 16%), sassafras (*Sassafras albidum*, 4%), flowering dogwood (*Cornus florida*, 2%), and persimmon (*Diospyros virginiana*, 2%). Silver maple (*Acer saccharinum*), honey locust (*Gleditsia triacanthos*), and black oak (*Quercus velutina*) occurred at less than 1% frequency. Across the three censuses, we made a total of 562 observations on trees in the plots. For all woody plants and for each the three most abundant species, data were analyzed with repeated-measures ANOVA, including the fixed effect of endophyte and repeated effect of year (SAS Institute 2003). Residuals did not significantly deviate from a normal distribution (Shapiro-Wilks test), and variances were homogeneous (Levene's test).

Plant species turnover.—At the upland site, we also recorded every plant species harvested from 20 randomly located quadrats (0.5 × 0.5 m) per plot from 1995 to 1999

(details in Clay and Holah [1999]). Bi-yearly harvests were conducted in spring (June) and fall (October) because plant composition differed seasonally. Using presence-absence data for all species pooled across the 20 subsamples, we calculated species turnover between two censuses as $(\text{immigrant number per plot} + \text{emigrant number per plot})/2$ following Williamson (1978). From our long-term data set, we could calculate turnover for four pairs of censuses (1995–1996, 1996–1997, 1997–1998, 1998–1999). We then determined the average turnover for each plot across these four pairs. In addition, because of strong differences in species composition between spring and fall floras, we calculated average turnover for spring and fall censuses separately. For each plot, we then had one average turnover estimate for spring and one for fall. Using repeated-measures ANOVA, we tested the effects of the endophyte, season (repeated factor) and endophyte \times season with plot as the unit of replication. The analysis met assumptions of normality and homogeneity (SAS Institute 2003). Because we used destructive harvests, new subplots were sampled during each census. Therefore, turnover estimates accurately quantified differences between treatments, but may have only estimated absolute turnover because subplots could not be resampled.

Bottomland site

Tree succession.—At 44 and 68 months after plot establishment (May 2004 and 2006), we counted and measured the height of all woody saplings >1 year old. Species included box elder (69% of individuals), silver maple (12%), red osier dogwood (*Cornus sericea*, 7%), green ash (*Fraxinus pennsylvanica*, 5%), red maple (*Acer rubrum*, 4%), and black cherry (*Prunus serotina*, 1%), with several additional species occurring at <1% frequency. Combined over the two censuses, 2084 trees were observed. Total number and mean height per plot were analyzed with repeated-measures ANOVA, including endophyte treatment (fixed), block (random), and interactions with year (SAS Institute 2003). The same analysis was applied to each of the three most common tree species (both years) and to tree species richness in 2006, by which time, on average, 6.56 ± 0.82 species had colonized the plots ($n = 16$), providing sufficient variation to test whether the endophyte treatment altered tree richness. In addition, we constructed rarefaction curves using EcoSim 7.72 to examine whether endophyte effects on richness remained after equalizing differences between plots in the total number of trees (Gotelli and Colwell 2001, Gotelli and Entsminger 2006). Log-transformation of tree count data satisfied assumptions of normality and homogeneity. Height and richness data did not require transformation except for silver maple height (log-transformed). For both count and height data, endophyte \times block interactions were nonsignificant ($P > 0.05$).

Vole herbivory.—We planted seedlings of four tree species: box elder, silver maple, black cherry, and

sycamore (*Platanus occidentalis*), into each plot on 28 May 2004. Locally collected seeds were grown in sterile soil and watered daily in a glasshouse until seedlings emerged. When seedlings were 10–14 days old, one seedling per tree species was planted into each subplot (0.5×0.5 m), with six to eight subplots chosen at random per plot. In subplots, vegetation was cleared and soil was loosened using a sterilized trowel, which facilitated tracking the seedlings and reduced competition with tall fescue. The four species were planted into each subplot in a randomized order and spaced evenly. It is possible that these seedlings competed, but any effects of competition would be similar across both endophyte treatments. From 2 June–1 October 2004, we recorded seedling survival and predation by voles every 10–14 days. Voles sever the stem of woody seedlings and leave a distinctive, 45° cut. For each plot, we determined percentage seedling survival on the final census and total percentage seedling predation by voles. We applied ANOVA including main effects and interactions among the endophyte treatment and tree species (SAS Institute 2003). Both tests met model assumptions following angular transformation (predation) or log transformation (survival). All block main effects and interactions were non-significant. Black cherry was not included in the analysis of survival because all seedlings died by the final census ($21\% \pm 4\%$ were killed by voles; the remainder by unidentified causes).

To quantify vole abundance, we trapped live voles (6–12 traps per plot; Kness Ketch-All, Albia, Iowa, USA) during peak activity (May–October) from 1 August 2003 to 23 June 2004 (17 trapping dates). Vole number per trap (square-root transformed) was analyzed with repeated measures ANOVA including endophyte treatment, block, the repeated factor of trapping date, and all interactions.

RESULTS AND DISCUSSION

Tree abundance

Endophyte symbiosis in tall fescue significantly reduced the abundance of trees, by 64–74% at the upland site and by 68–82% at the bottomland site (Fig. 1; upland, $F_{1,6} = 8.7$, $P = 0.03$; endophyte \times year, $F_{2,5} = 10.2$, $P = 0.02$, $n = 4$ plots; bottomland, $F_{1,12} = 12.3$, $P = 0.004$; Appendix A, $n = 8$ plots). At both sites, the statistical models for each year explained substantial variation in tree abundance (upland $r^2 = 0.31, 0.45$, and 0.79 in 1998, 1999, and 2002, respectively; bottomland $r^2 = 0.57$ in 2004, 0.59 in 2006).

Tree size

Endophyte symbiosis also reduced tree growth, but only at the bottomland site. At the upland site, the endophyte did not significantly affect tree basal diameter ($F_{1,6} = 0.1$, $P = 0.7$; endophyte \times year, $F_{1,6} = 0.1$, $P = 0.8$) or height (1999 only, $F_{1,6} = 0.4$, $P = 0.6$). However, total tree height was significantly lower in E+ than E– plots at the bottomland site (Fig. 2; $F_{1,12} = 5.3$, $P = 0.04$;

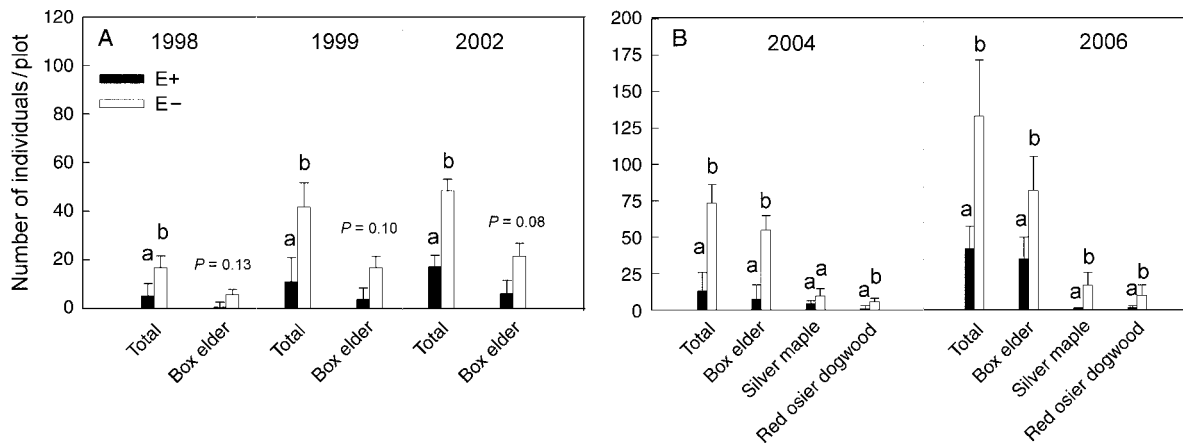


FIG. 1. Abundance of tree saplings in plots enriched with endophyte-infected (E+; solid bars) or endophyte-free (E-; open bars) tall fescue at the (A) upland or (B) bottomland site. Bars represent means + SE, and lowercase letters indicate significant differences between E+ and E- pairs within a year. Statistical results from repeated-measures ANOVA are given in *Results* and in Appendix A (bottomland site).

endophyte × year, $F_{1,12} = 2.7$, $P = 0.1$). In 2004, mean height was 25.0 ± 3.4 cm in E+ plots and 29.5 ± 5.4 cm in E- plots, a 15% reduction; in 2006, trees were 36% smaller in E+ plots (Fig. 2). Both treatments had a high frequency of small seedlings (Fig. 2), probably reflecting seedling recruitment that occurred during the year of the census.

Tree composition

Tree species responded divergently to the endophyte, suggesting that the endophyte may alter future forest composition. In addition to negative impacts on box

elder, endophyte symbiosis caused declines in silver maple, red osier dogwood, and white ash. Silver maple abundance was 94% lower in bottomland E+ plots in 2006 (endophyte × year, $P = 0.003$; Appendix A; Fig. 1B), although height was not affected ($F_{1,12} = 1.1$, $P = 0.3$, endophyte × year, $F_{1,12} = 0.1$, $P = 0.7$). Presence of the endophyte caused an 84–86% decline in red osier dogwood abundance in bottomland plots (Fig. 1B; $P = 0.028$; Appendix A). (Dogwood height data could not be analyzed because too few E+ plots included this species [two plots in 2004, three in 2006]). Although the endophyte did not affect white ash abundance at the

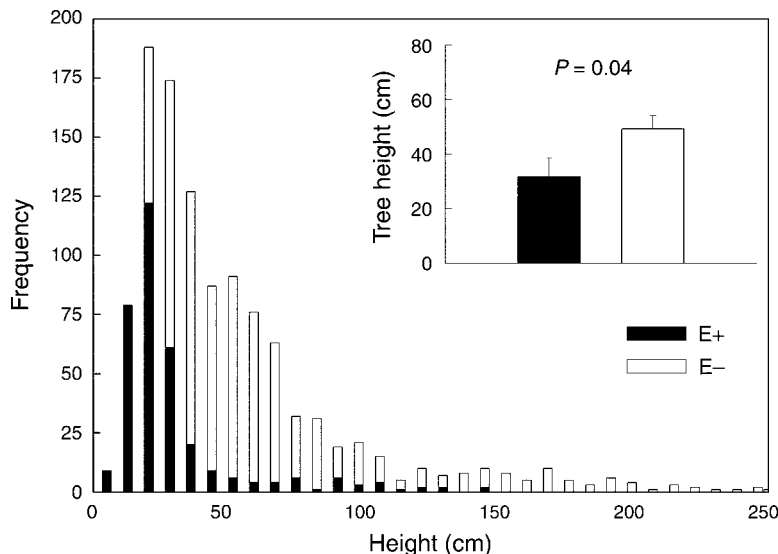


FIG. 2. Histogram of the distribution of total tree height comparing bottomland plots enriched with endophyte-infected (E+; solid bars) or endophyte-free (E-; open bars) tall fescue (data collected in May 2006). Note the disproportionate number of small seedlings in both plots and the longer tail (more large trees) for endophyte-free plots. The inset shows mean bottomland site tree heights (+SE) for E+ and E- treatments in 2006. Additional statistical results are presented in *Results*.

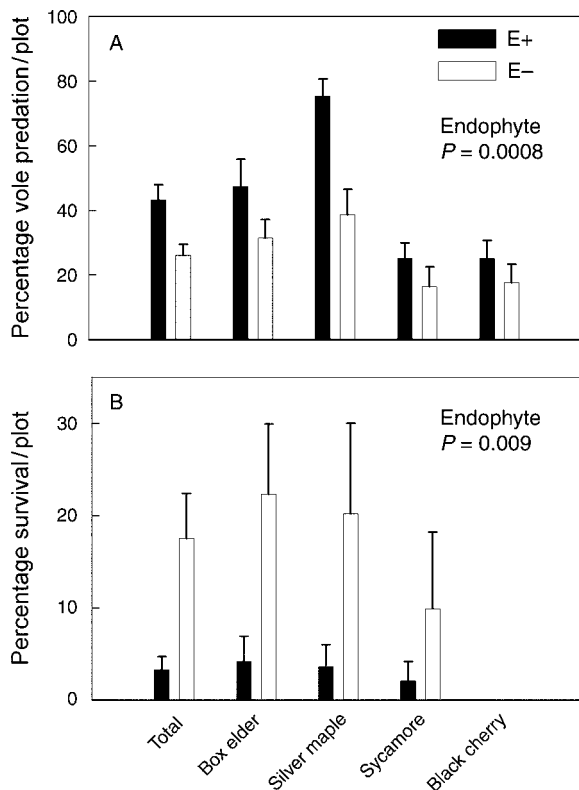


FIG. 3. Fates of tree seedlings planted into bottomland plots enriched with endophyte-infected (E+; solid bars) or endophyte-free (E-; open bars) tall fescue. (A) Percentage of seedling predation by voles per plot (endophyte $F_{1,56} = 12.7$, $P = 0.0008$, $n = 8$ plots), and (B) percentage of survival per plot after four months (endophyte, $F_{1,42} = 7.5$, $P = 0.009$, $n = 8$ plots; black cherry was not included in this analysis because no plants survived). With the exception of black cherry survival, the tree species responded similarly to the endophyte (endophyte \times species, predation, $F_{3,56} = 1.3$, $P = 0.3$; survival, $F_{2,42} = 0.8$, $P = 0.5$). Bars show means + SE.

upland site ($F_{1,4} = 3.5$, $P = 0.1$; endophyte \times year, $F_{1,6} = 0.5$, $P = 0.5$), white ash were 30% smaller in E+ plots in 2002 (mean diameter \pm SE, E+ = 4.5 ± 0.5 cm, E- = 6.4 ± 0.4 cm; $F_{1,4} = 6.1$, $P = 0.07$; endophyte \times year, $F_{1,4} = 14.5$, $P = 0.02$). In contrast to strong effects on the other tree species, the endophyte had no significant influence on the growth or abundance of white mulberry, which was only present at the upland site.

Finally, the most common species at both sites, box elder, experienced both reduced numbers (up to 87% fewer) and height (up to 57% shorter) in the presence of the endophyte. Reductions in box elder abundance were marginal at the upland site (Fig. 1A; $F_{1,6} = 3.9$, $P = 0.09$; endophyte \times year, $F_{2,5} = 1.8$, $P = 0.3$) and significant at the bottomland site (Fig. 1B; $F_{1,12} = 9.6$, $P = 0.009$; Appendix A). It should be noted that the bottomland site yielded higher statistical power than the upland due to a larger sample size ($n = 8$ vs. $n = 4$). In addition, box elder trees were 23–57% shorter in the presence of the endophyte at the bottomland site (mean height \pm SE;

2004 E+ = 20.2 ± 1.2 , 2004 E- = 26.4 ± 1.8 ; 2006 E+ = 21.8 ± 3.1 , 2006 E- = 51.0 ± 6.3 ; endophyte $F_{1,12} = 13.6$, $P = 0.004$), with stronger effects during the later census (endophyte \times year, $F_{1,12} = 13.4$, $P = 0.004$). Box elder age significantly increased with height ($r = 0.56$, $F_{2,25} = 5.9$, $P = 0.008$, $n = 28$ trees collected near the bottomland plots in June 2006 and aged using counts of terminal bud scars and tree rings). Thus, the reduction in box elder height caused by the endophyte may indicate altered age structure, with older trees in endophyte-free plots. These species-specific responses suggest that the endophyte-tall fescue symbiosis may promote the relative dominance of non-native white mulberry at the upland site at the expense of several native tree species (box elder, silver maple, red osier dogwood, and white ash), which were negatively affected by endophyte infection.

Tree species richness

In addition to effects on particular tree species, endophyte symbiosis also caused up to 60% declines in tree species richness (measured at the bottomland site in 2006; $F_{1,12} = 18.3$, $P = 0.001$). Effects were stronger in the block of plots nearer the forest edge (block 1, $F_{1,12} = 3.8$, $P = 0.08$, endophyte \times block $F_{1,12} = 8.9$, $P = 0.01$), perhaps due to greater seed dispersal (mean richness \pm SE, E+ block 1 = $4.0^a \pm 1.1$; block 2 = $5.0^a \pm 0.4$, E- block 1 = $11.0^b \pm 1.5$, block 2 = $6.3^a \pm 0.5$; superscript lowercase letters indicate significance differences from a Tukey hsd test). When the analysis was corrected for the influence of total tree number (more individuals yields a greater probability of sampling a new species), tree richness no longer significantly differed among the treatments (rarefaction curves, Appendix B). This result suggests the endophyte reduced species richness primarily by decreasing total tree abundance.

Plant species turnover

Endophyte symbiosis in tall fescue also significantly reduced temporal fluctuations in the presence of both woody and herbaceous plant species. At the upland site, plant species turnover was significantly lower in the presence of the endophyte ($F_{1,6} = 13.5$, $P = 0.01$; mean turnover \pm SE, Spring E+ = 5.5 ± 0.4 , E- = 6.7 ± 0.7 ; Fall E+ = 4.8 ± 0.1 , E- = 5.1 ± 0.5). Combined across seasons, species turnover declined 12% in E+ plots, with no significant seasonality (season $F_{1,6} = 3.5$, $P = 0.11$, endophyte \times season $F_{1,6} = 0.5$, $P = 0.5$).

Tree seedling predation

Endophyte symbiosis in tall fescue indirectly affected woody plants, in part, by increasing consumption of tree seedlings by voles. The presence of the endophyte significantly increased vole seedling predation by 65% (Fig. 3A). Further, the percentage of planted seedlings that survived to the study's end was 81% lower in endophyte-infected compared to endophyte-free plots (Fig. 3B), closely corresponding with the 82% reduction

in the abundance of naturally colonizing trees at this site during the same year (2004). Voles exhibited preference among tree species ($F_{3,56} = 13.0$, $P < 0.0001$), with higher overall predation on silver maple ($57\% \pm 7\%$) and box elder ($39\% \pm 5\%$) than sycamore ($21\% \pm 4\%$) or black cherry ($21\% \pm 4\%$; significantly different by Tukey hsd test). However, the endophyte had similar effects on seedling predation across all four focal tree species, as indicated by non-significant endophyte \times species interactions (Fig. 3). Of the seedlings that died, $38\% \pm 3\%$ were killed by voles. Thus, stronger competition for resources (Clay et al. 1993), increased insect herbivory (Clay 1996), reduced mycorrhizal fungi (Chu-chou et al. 1992), elevated allelopathy (Orr et al. 2005), suppression by dead leaf litter (Lemons et al. 2005) or other factors likely also contribute to the endophyte's impact on tree establishment. Further experiments, including manipulations of voles, are required to estimate the relative importance of each of these competing mechanisms. Prior work has shown that voles can strongly influence plant community structure and succession through consumption (Gill and Marks 1991, Ostfeld and Canham 1993, Ostfeld et al. 1997, Howe et al. 2006); however, endophyte symbiosis was not previously known to mediate these effects.

We propose that vole predation on woody species was more severe in endophyte-infected plots because the dominant plant (endophyte-infected tall fescue) was unpalatable and, as a result, voles fed more heavily on other plant species. Data thus far are consistent with this hypothesis. In a previous study, experimental manipulation of vole abundance in small (5×5 m) plots at the bottomland site revealed that these herbivores can increase the biomass of endophyte-infected tall fescue 48% relative to plots where voles have been removed (Clay et al. 2005). Further, herbaceous forb biomass was significantly reduced in plots with voles compared to plots with voles excluded (Clay et al. 2005). In addition, prior work revealed that meadow voles (*Microtus pennsylvanicus*) avoided consuming endophyte-infected tall fescue, and that the endophyte negatively affected the growth or reproduction of both prairie voles (*M. ochrogaster*) and meadow voles (Conover 1998, Fortier et al. 2000). In the current study, vole abundance (estimated via live trapping) at the bottomland site was similar in both endophyte-free and endophyte-infected plots during 2003–2004 (mean voles per trap [95% CI], $E^- = 0.6$ [0.5–0.7], $E^+ = 0.5$ [0.4–0.6]; $F_{1,192} = 1.1$, $P = 0.3$), suggesting that differential consumption by voles, rather than changes in vole abundance, was an underlying mechanism of vegetational change. Although the endophyte did not affect vole abundance, the two blocks differed significantly (mean vole number/trap/plot [95% CI], block 1 = 0.60 [0.51–0.69]; block 2 = 0.45 [0.37–0.54]; $F_{1,192} = 7.1$, $P = 0.008$), showing the analysis had sufficient power. Because plot size (400–900 m²) was considerably larger than a typical vole territory, voles may not be choosing territories based on palatability of

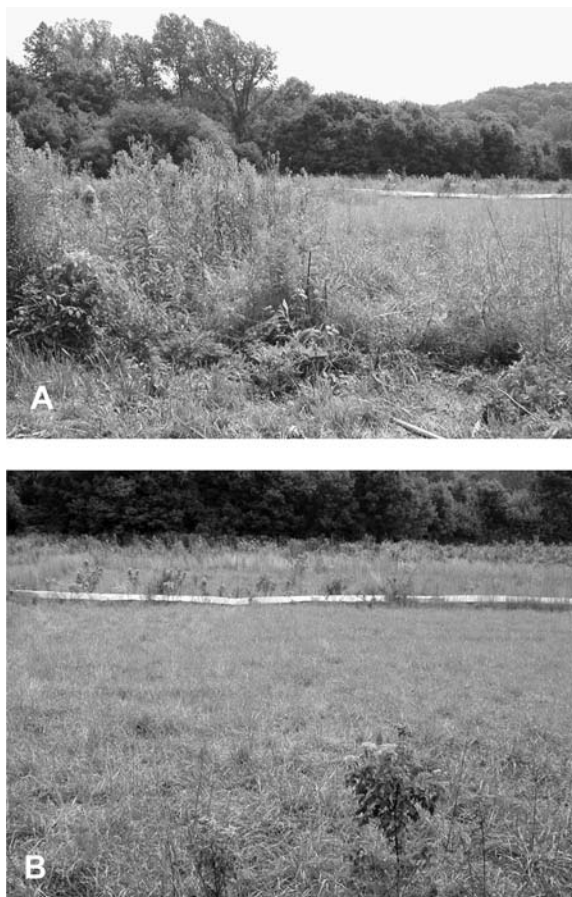


PLATE 1. Example of tall fescue (*Lolium arundinaceum*) plots from the Bottomland site in Bloomington, Indiana (USA): (A) endophyte-free plot; (B) endophyte-infected by *Neotyphodium coenophialum*. Note the difference in cover of trees and tall forbs. The fence (aluminum flashing) divides E^+ and E^- plots and shows clearly the transition in vegetation in (B). Photo credit: K. Clay.

the dominant vegetation. In a prior study at the upland site using radio-collared voles, the average home range size of female voles was 113 m² in E^+ plots and 102 m² in E^- plots ($P = 0.7$ for differences between treatments) (Fortier et al. 2001).

Conclusion

Mutualistic endophyte symbiosis suppressed plant succession by reducing tree establishment, tree growth, and plant species turnover, and thereby produced the more persistent communities predicted by current theory (Kumar and Freedman 1989, Ringel et al. 1996, Reynolds et al. 2003). In addition, the tall-fescue-endophyte symbiosis altered tree composition; favoring nonnative white mulberry over native trees at the upland site, and reducing tree species richness at the bottomland site. Effects were robust across two plant communities that varied in plant composition, land use history, topogra-

phy, soil type, and the time frame of the experiment. The endophyte appears to affect plant succession at least in part indirectly by altering patterns of seedling predation by voles. Here, voles consumed 65% more tree seedlings in the presence than the absence of the endophyte. The suppression of the natural transition from grassland to forest may have significant ecosystem consequences due to differences in standing biomass and CO₂ uptake between grassland and forest communities. Our results highlight symbiosis as a key mechanism affecting the process of succession, and thereby challenge traditional ideas about factors governing plant succession. Long-term effects of a dominant, introduced grass on the direction and rate of forest succession depended on symbiosis with a fungal endophyte.

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LITERATURE CITED

- Allen, E. B., and M. F. Allen. 1984. Competition between plants of different successional stages: mycorrhizae as regulators. *Canadian Journal of Botany* 62:2625–2629.
- Arnold, A. E., L. C. Mejia, D. Kylo, E. I. Rojas, Z. Maynard, N. Robbins, and E. A. Herre. 2003. Fungal endophytes limit pathogen damage in a tropical tree. *Proceedings of the National Academy of Sciences (USA)* 100:15649–15654.
- Ball, D. M., J. F. Pedersen, and G. D. Lacefield. 1993. The tall fescue endophyte. *American Scientist* 81:370–379.
- Bever, J. D. 2002. Negative feedback within a mutualism: host-specific growth of mycorrhizal fungi reduces plant benefit. *Proceedings of the Royal Society of London Series B-Biological Sciences* 269:2595–2601.
- Callaway, R. 1995. Positive interactions among plants. *Botanical Review* 61:306–349.
- Chapin, F. S., III, L. R. Walker, C. L. Fastie, and L. C. Sharmar. 1994. Mechanisms of primary succession following deglaciation at Glacier Bay, Alaska. *Ecological Monographs* 64:149–175.
- Chu-chou, M., G. Guo, Z. Q. An, J. W. Hendrix, R. S. Ferris, M. R. Siegel, C. T. Dougherty, and P. B. Burrus. 1992. Suppression of mycorrhizal fungi in fescue by the *Acremonium coenophialum* endophyte. *Soil Biology and Biochemistry* 24:633–637.
- Clark, E. M., J. F. White, and R. M. Patterson. 1983. Improved histochemical techniques for the detection of *Acremonium coenophialum* in tall fescue and methods of in vitro culture of the fungus. *Journal of Microbial Methods* 1:149–155.
- Clay, K. 1996. Interactions among fungal endophytes, grasses and herbivores. *Researches on Population Ecology* 38:191–201.
- Clay, K., and J. Holah. 1999. Fungal endophyte symbiosis and plant diversity in successional fields. *Science* 285:1742–1744.
- Clay, K., J. Holah, and J. A. Rudgers. 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.
- Clay, K., S. Marks, and G. P. Cheplick. 1993. Effects of insect herbivory and fungal endophyte infection on competitive interactions among grasses. *Ecology* 74:1767–1777.
- Clay, K., and C. Schardl. 2002. Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. *American Naturalist* 160:S99–S127.
- Connell, J. H., and R. O. Slatyer. 1977. Mechanisms of succession in natural communities and their role in community stability and organization. *American Naturalist* 111:1119–1144.
- Conover, M. R. 1998. Impact of consuming tall fescue leaves with the endophytic fungus, *Acremonium coenophialum* on meadow voles. *Journal of Mammalogy* 79:457–463.
- Fortier, G. M., N. Bard, M. Jansen, and K. Clay. 2000. Effects of tall fescue endophyte infection and population density on growth and reproduction in prairie voles. *Journal of Wildlife Management* 64:122–128.
- Fortier, G. M., M. A. Osmon, M. Roach, and K. Clay. 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.
- Gill, D. S., and P. L. Marks. 1991. Tree and shrub seedling colonization of old fields in central New York. *Ecological Monographs* 61:183–206.
- Gotelli, N. J., and R. K. Colwell. 2001. Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecology Letters* 4:379–391.
- Gotelli, N. J., and G. L. Entsminger. 2006. EcoSim: Null models software for ecology. Version 7. Acquired Intelligence, Inc. and Kesey-Bear. Jericho, Vermont, USA.
- Howe, H. F., B. Zorn-Arnold, A. Sullivan, and J. S. Brown. 2006. Massive and distinctive effects of meadow voles on grassland vegetation. *Ecology* 87:3007–3013.
- Huntly, N. 1991. Herbivores and the dynamics of communities and ecosystems. *Annual Review of Ecology and Systematics* 22:477–503.
- Intergovernmental Panel on Climate Change. 2001. Climate change 2001: synthesis report. A contribution of working groups I, II, and III to the Third Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.
- Janos, D. P. 1980. Mycorrhizae influence tropical succession. *Biotropica* 12:56–64.
- Kardol, P., T. Martijn Bezemer, and W. H. van der Putten. 2006. Temporal variation in plant–soil feedback controls succession. *Ecology Letters* 9:1080–1088.
- Kumar, R., and H. I. Freedman. 1989. A mathematical model of facultative mutualism with populations interacting in a food chain. *Mathematical Biosciences* 97:235–261.
- Lemons, A., K. Clay, and J. A. Rudgers. 2005. Connecting plant–microbial interactions above and belowground: a fungal endophyte affects decomposition. *Oecologia* 145:595–604.
- Malinowski, D. P., D. P. Belesky, and G. C. Lewis. 2005. Abiotic stresses in endophytic grasses. Pages 187–199 in C. A. Roberts, C. P. West, and D. E. Spiers, editors. *Neotyphodium in cool-season grasses*. Blackwell Publishing, Ames, Iowa, USA.
- Nara, K. 2006a. Ectomycorrhizal networks and seedling establishment during early primary succession. *New Phytologist* 169:169–178.
- Nara, K. 2006b. Pioneer dwarf willow may facilitate tree succession by providing late colonizers with compatible ectomycorrhizal fungi in a primary successional volcanic desert. *New Phytologist* 171:187–198.
- Omacini, M., E. J. Chaneton, C. M. Ghersa, and C. B. Muller. 2001. Symbiotic fungal endophytes control insect host–parasite interaction webs. *Nature* 409:78–81.
- Orr, S. P., J. A. Rudgers, and K. Clay. 2005. Invasive plants can inhibit native tree seedlings: testing potential allelopathic mechanisms. *Plant Ecology* 181:153–165.

- Ostfeld, R. S., and C. D. Canham. 1993. Effects of meadow vole population density on tree seedling survival in old fields. *Ecology* 74:1792–1801.
- Ostfeld, R. S., R. H. Manson, and C. D. Canham. 1997. Effects of rodents on survival of tree seeds and seedlings invading old fields. *Ecology* 78:1531–1542.
- Pickett, S. T. A., J. Kolassa, J. J. Armesto, and S. L. Collins. 1989. The ecological concept of disturbance and its expression at various hierarchical levels. *Oikos* 54:129–136.
- Raloff, J. 2003. Cultivating weeds: is your yard a menace to parks and wild lands? *Science News* 163:232.
- Reynolds, H. L., A. Packer, J. D. Bever, and K. Clay. 2003. Grassroots ecology: Plant–microbe–soil interactions as drivers of plant community structure and dynamics. *Ecology* 84:2281–2291.
- Ringel, M. S., H. H. Hu, and G. Anderson. 1996. The stability and persistence of mutualisms embedded in community interactions. *Theoretical Population Biology* 50:281–297.
- Rodriguez, R. J., R. S. Redman, and J. M. Henson. 2004. The role of fungal symbioses in the adaptation of plants to high stress environments. *Mitigation and Adaptation Strategies for Global Change* 9:261–272.
- Rudgers, J. A., and K. Clay. 2005. Fungal endophytes in terrestrial communities and ecosystems. Pages 423–442 in E. J. Dighton, P. Oudemans, and J. F. J. White, editors. *The fungal community*. M. Dekker, New York, New York, USA.
- SAS Institute. 2003. SAS version 9.1. SAS Institute, Inc., Cary, North Carolina, USA.
- Schardl, C. L., A. Leuchtman, and M. J. Spiering. 2004. Symbioses of grasses with seedborne fungal endophytes. *Annual Review of Plant Biology* 55:315–340.
- Shelby, R. A., and L. W. Dalrymple. 1987. Incidence and distribution of the tall fescue endophyte in the USA. *Plant Disease* 71:783–786.
- Siegel, M. R., M. C. Johnson, D. R. Varney, W. C. Nesmith, R. C. Buckner, L. P. Bush, P. B. Burrus II, T. A. Jones, and J. A. Boling. 1984. A fungal endophyte in tall fescue [*Festuca arundinacea*]: Incidence and dissemination. *Phytopathology* 74:932–937.
- Spyreas, G., D. J. Gibson, and M. Basinger. 2001. Endophyte infection levels of native and naturalized fescues in Illinois and England. *Journal of the Torrey Botanical Society* 128:25–34.
- Tilman, D. 1982. *Resource competition and community structure*. Princeton University Press, Princeton, New Jersey, USA.
- Vitousek, P. M., L. R. Walker, L. D. Whiteaker, D. Muellerdombois, and P. A. Matson. 1987. Biological invasion by *Myrica faya* alters ecosystem development in Hawaii. *Science* 238:802–804.
- Williamson, G. B. 1978. A comment on equilibrium turnover rates for islands. *American Naturalist* 112:241–243.

APPENDIX A

Statistical results for the effects of endophyte treatment on tree abundance at the bottomland site (*Ecological Archives* E088-001-A1).

APPENDIX B

Rarefaction curve for tree species richness at the bottomland site (*Ecological Archives* E088-001-A2).