# Fungal Endophyte Symbiosis and Plant Diversity in Successional Fields

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**Increasing evidence suggests that microbial interactions are important determinants of plant biodiversity. The hypothesis that fungal endophyte symbiosis reduces diversity in successional fields was tested by manipulating infection of tall fescue, the most abundant perennial grass in the eastern United States. Over a 4-year period, species richness declined and tall fescue dominance increased in infected plots relative to uninfected plots without differences in total productivity. A host-specific endophyte, with negligible biomass, altered plant community structure in this long-term field experiment and may be reducing plant diversity throughout its expanding range.**

Ecologists traditionally have emphasized climate, resource levels, disturbance frequency, and plant-plant and plant-herbivore interactions as major determinants of plant biodiversity (*1*). The potential role of microorganisms—for example, mutualistic symbionts—has been largely ignored despite their ubiquity. Recent research suggests that microbial interactions may play a major role in structuring plant communities by affecting colonization, competition, coexistence, and soil nutrient dynamics (*2–5*). However, this conclusion is based primarily on observations of unmanipulated communities, extrapolations from artificial environments, and extrapolations from field applications of biocides that may have confounding effects on nontarget species. Here we report the results of a 4-year experiment demonstrating that fungal endophyte symbiosis can be a major determinant of species richness in successional fields. The high specificity of grass-endophyte associations, the persistence of infection, and the lack of contagious spread permit large manipulative field experiments.

Many cool-season grasses are infected by systemic, seed-transmitted fungal endophytes (*Neotyphodium* spp.) (*6*). Most research has focused on agronomically important species, especially tall fescue (*Festuca arundinacea*), which was introduced to North America from Europe. The endophyte (*Neotyphodium coenophialum*) grows intercellularly throughout above-ground plant parts (*7*). There is no external manifestation of infection and the fungus spreads only with seeds of infected plants. Infected plants are more vigorous, more toxic to herbivores, and more drought-resistant than uninfected plants (*8*). Tall fescue has been widely planted for forage, turf, and soil conservation, but it is also a tenacious invader of natural communities, where it can displace native plant species and reduce wildlife populations (*9, 10*). Fescue is considered to be the most widespread invasive plant in Indiana, the site of this study (*11*). In the eastern United States there are over $15 \times 10^6$ ha of fescue, two-thirds of which is infected (*9*). The potential effect of the endophyte on communities is therefore large, given the abundance and distribution of its host.

We established eight experimental plots ($20 \times 20$ m) in late summer 1994 at the Indiana University Botany Experimental Field. Vegetation initially consisted of perennial grasses, including tall fescue, and forbs. The site was plowed and disked, and then alternating plots were seeded with endophyte-infected (E+) or uninfected (E−) fescue seed (var. KY-31) at a rate of 45 kg/ha (*12*). After 2 months there were stands of fescue along with many other species germinating from the seed bank. Annual samples have shown consistently high infection in E+ plots and low infection in E− plots (Fig. 1) (*13*). No other treatments (fertilizer, pesticides, mowing) were applied. To quantify changes in plant composition, we harvested 20 quadrats per plot each spring and fall (1995–1997) through spring 1998 (*14*). Samples were returned to the laboratory, sorted by species or into litter, dried, and weighed.

There were no differences among plots for any measure in the first harvest when mean species number per quadrat averaged between 9 and 10 (Fig. 2A). Many winter annuals germinated after plowing but subsequently have been rare. Mean species number has decreased since the first harvest with the greatest decrease in E+ plots (Fig. 2A). Seasonal fluctuations also occurred, with higher species numbers in spring samples (Fig. 2A). Mean species number per quadrat in E+ plots has been about 60% that in E− plots for the past three harvests.

We partitioned variance in species number per quadrat into components attributable to endophyte infection, plots within infection class, and quadrats within plots (Fig. 2B). Initially, all variation in species number occurred among quadrats. Since fall 1995, the component of the variance explained by infection has increased steadily with a concomitant decrease in the component explained by plot (Fig. 2B). At the final harvest, nearly 50% of the variance in species number was explained by endophyte infection of fescue. Thus, plots within an infection class are converging in species number, and E+ and E− plots are diverging.

Differences in mean species number also occurred at the larger scale of a 400-$m^2$ plot (Fig. 2C). We calculated species-area curves at the final harvest by plotting cumulative species number against number of quadrats sampled. Twenty-seven versus 22 species, respectively, occurred at the final harvest for E− and E+ plots. Nonparametric estimators of actual species richness indicated that about four more species occurred in E− than in E+ plots [first-order jackknife estimate of 31.0 and 26.9 species for E− and E+ plots, respectively (*15*)]. We encountered six species only in E− plots, including boxelder (*Acer negundo*) and white clover (*Trifolium re-*

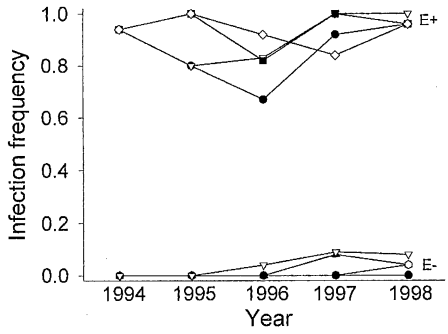


**Fig. 1.** Infection frequency of tall fescue in infected (E+) and uninfected (E−) plots. Each symbol within a plot type represents the infection frequency of one plot. We determined the initial infection rate of the seeds planted in fall 1994 by growing 50 seedlings from each seed type in the greenhouse and checking them microscopically for infection (*13*). Forty-seven of 50 seedlings from E+ seed were infected versus 0 of 50 from E− seeds. In June 1995 we randomly collected five fescue tillers from each plot and examined them for infection. In the following years (1996–1998) 25 tillers per plot were randomly sampled in June and examined. $\chi^2$ tests indicate a significant difference in infection frequency between plot types at every sample date and in total ($P < 0.0001$ for all). Fescue present at the site before seed was sown had a low level of infection (2 of 21 infected) and may account for variation in infection frequencies within plots. Increasing infection in E− plots also may reflect seed dispersal from E+ plots or higher fitness of E+ plants at very low frequencies in E− plots.

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*pens*). One species was found only in E+ plots but only in a single quadrat. Loss of clover from E+ plots could have long-term consequences on soil nitrogen levels and performance of E+ fescue. In New Zealand pastures, clover is more difficult to maintain with E+ perennial ryegrass (*Lolium perenne*) than with E− ryegrass (*16*). The absence of boxelder, an early successional tree, in E+ plots may indicate a slower rate of succession compared with E− plots.

Community biomass ranged from 300 to 800 g/m$^2$ and has declined over the course of the study (Fig. 3A). Mean biomass was marginally higher in E+ plots for six of the seven harvests, but changes in production through time did not depend on infection status (Fig. 3A). Similarly, litter mass increased over time but did not differ between E+ and E− plots. Although species number is often a function of productivity at a local scale (*17*), differences between E+ and E− plots in this study were independent of biomass.

Although standing biomass was similar, its composition differed significantly between E+ and E− plots. Initially the proportion of fescue in the total biomass averaged 80% in both E+ and E− plots, but this proportion has climbed to nearly 90% in E+ plots and has fallen to fewer than 60% in E− plots (Fig. 3B). Simultaneously, the proportion of biomass composed of other grasses [primarily quackgrass (*Elymus repens*) and Kentucky bluegrass (*Poa pratensis*)] increased to over 40% in E− plots compared with fewer than 15% in E+ plots (Fig. 3B). Over the same period, forb biomass declined 97% in E+ plots and 50% in E− plots. Thus, E+ plots became near monocultures of fescue, whereas E− plots became more diverse assemblages of other grasses and forbs.

Variance component analysis of fescue dominance in E+ and E− plots revealed a pattern similar to that found for species number. Infection explained little or no variance in the proportion of fescue in the first 2 years of the study but increased to nearly 50% by the final harvest (Fig. 3C). The component of variance explained by plot within infection class steadily decreased to fewer than 10% at the final harvest.

Although E+ fescue is more dominant and apparently more competitive than E− fescue, total community biomass did not dif-

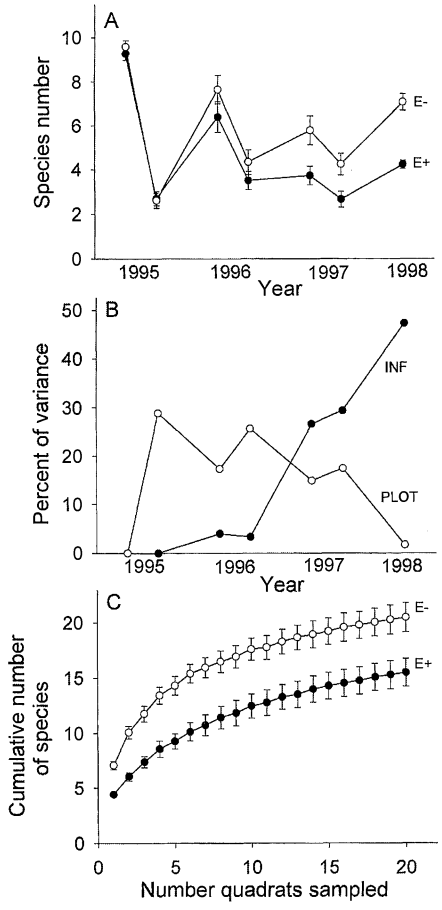


**Fig. 2.** Plant diversity in E+ and E− plots. (**A**) Mean species number in E+ and E− plots (±SE among plots) per 0.25-m$^2$ quadrat sample. We conducted repeated measures analysis of variance (ANOVA) with plot means to determine whether species number changed over time and whether treatments differed over time following the methods in (*28*). A first-degree polynomial contrast for time indicates species number changing linearly through time ($F_{1,6}$ = 100.53; $P$ = 0.0001) and that this change was significantly different depending on the infection status of the plot ($F_{1,6}$ = 24.15; $P$ = 0.0027). This indicates that species number has declined over time in both types of plots but that the decline is greater in E+ plots. We examined other measures of diversity (Shannon–Weaver index, evenness) and found them to be significantly correlated with species number. At the final harvest $H'$ = 0.345 ± 0.029 and $J$ = 0.248 ± 0.020 for E+ quadrats compared with $H'$ = 0.793 ± 0.040 and $J$ = 0.419 ± 0.021 for E− quadrats. (**B**) Species number variance components. Total variance in species number was partitioned into components due to differences among infected versus uninfected plots (INF), differences among plots within an infection class (PLOT), and differences among quadrats within a plot. These components add to 100% so the within-plot component can be calculated from the INF and PLOT effect. We analyzed data with nested ANOVA with 1, 6, and 152 degrees of freedom. (**C**) Species-area curve for 1998 harvest. We selected one quadrat from each plot at random and determined species number; then we selected a second quadrat at random and determined the cumulative number of species and so forth through 20 quadrats. Given the numerous possible permutations of quadrat sequence, this subsampling procedure was repeated 500 times for each plot using PC-ORD (B. McCune, M. J. Mefford, 1995, MjM Software Design, Gleneden Beach, Oregon). Mean cumulative species number per plot (±SE among plots) is also shown.

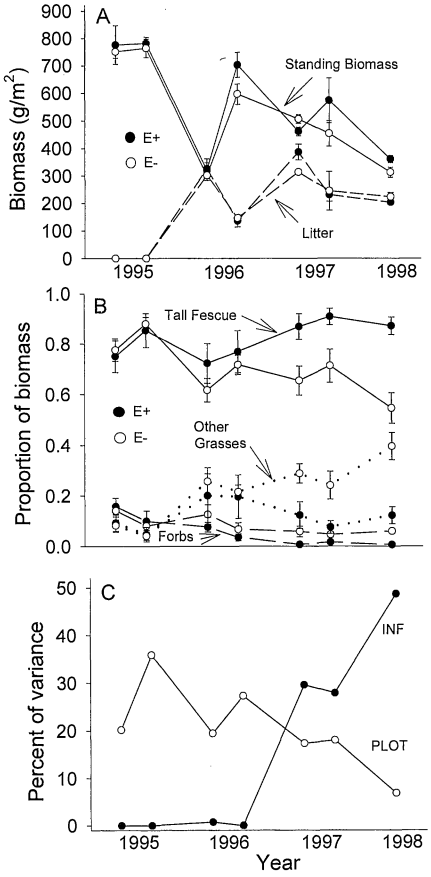


**Fig. 3.** Aboveground biomass in E+ and E− plots. (**A**) Mean standing biomass and litter in E+ and E− plots (±SE among plots). A record snowfall in early spring 1996 matted down all standing vegetation, delaying the onset of plant growth for several weeks. Repeated measures ANOVA using plot means and a first-degree polynomial contrast for time indicates an overall decrease in biomass over all harvests ($F_{1,6}$ = 170.17; $P$ = 0.0001), but this change did not depend on the infection status of the plots ($F_{1,6}$ = 0.95; $P$ = 0.37). Analysis of litter biomass revealed that there was a significant increase through time ($F_{1,6}$ = 308.01; $P$ = 0.0001), but it did not depend on infection status ($F_{1,6}$ = 0.24; $P$ = 0.64). (**B**) Biomass composition. We divided total standing biomass into three proportions: tall fescue, other grasses, and forbs. Both the proportion of tall fescue and proportion of grasses other than fescue changed linearly over time depending on the infection status of the plot ($F_{1,6}$ = 11.51, $P$ = 0.015; $F_{1,6}$ = 13.16, $P$ = 0.011 for fescue and other grasses, respectively). The proportion of forb species did not change linearly through time depending on infection status ($F_{1,6}$ = 4.41; $P$ = 0.081) but has significantly decreased generally through time ($F_{1,6}$ = 48.87; $P$ = 0.0004). Statistics are the results of a repeated measures ANOVA using plot means and a first-degree polynomial contrast for time. (**C**) Proportion of tall fescue variance components. Total variance in the proportion of total biomass composed of tall fescue was partitioned into components due to differences among infected versus uninfected plots (INF), differences among plots within an infection class (PLOT), and differences among quadrats within a plot. See Fig. 2B.

fer between plot types. Therefore, reduced diversity in E+ plots cannot be explained by a diversity–productivity relationship. Endophyte symbiosis could affect the community by several other mechanisms. The endophyte produces toxic alkaloids (*18*), which may have altered feeding patterns of small mammalian herbivores, birds, and insects that were abundant in our plots. Endophyte infection can also suppress mycorrhizal associations and disease prevalence (*19*). Decreased plant diversity may be expected with an impoverished mycorrhizal community (*4*). Independent of biotic interactions, endophyte infection enhances drought tolerance of fescue (*20*); there are often extended periods without rain during summers in southern Indiana. Finally, E+ plants are more productive than E− plants in the absence of biotic or abiotic stresses (*21*). Any or all of these factors may promote the dominance of E+ fescue in mixed vegetation.

Our results demonstrate that fungal endophyte symbiosis reduces plant diversity and enhances fescue dominance in successional fields. Although other studies suggest that mycorrhizal associations are also important determinants of plant community structure (*2–4*), mycorrhizal and endophyte symbioses are quite different. In most communities, mycorrhizal fungi colonize many hosts and they may transfer nutrients among hosts [(*3, 4*) but see (*22*)]. In contrast, seed-transmitted endophytes are limited to cool-season grasses and are host-specific, and the benefits of infection are restricted to individual hosts (*21*). Shared symbioses may equalize competitive abilities among plants and promote diversity, whereas "private" symbioses may increase competitive differentials and decrease diversity.

Traditional views of trophic levels (producer, consumer, predator) assume that the effect of one trophic level on the one below or above it is a function of consumption. The effects of microbial symbionts are often ignored, even though their effects on plants and plant communities are larger than their biomass or consumption of host tissues suggest (*23*). Here *N. coenophialum* protein comprises fewer than 0.1% of aboveground tall biomass (*24*). The fact that endophytes or other plant symbionts exert significant control over the structure of communities belies both bottom-up and top-down hypotheses and instead suggests that inside-out mechanisms of community control may occur.

Our results have implications for conservation because loss of plant diversity is likely where fescue is common and where it is highly infected. Independent evidence suggests that infection frequencies increase in mixtures over time so that sites with low infection can become highly infected (*25*). Although fescue and some of the other species found in this study are not native, they are typical of open habitats throughout much of the eastern United States. The success of both fescue and native E+ grasses may be facilitated by endophyte symbiosis (*26*), but there are other E+ grasses, native and nonnative, that are not widespread or ecologically dominant (*27*). One possible explanation for the great success of some E+ grasses is their high concentrations of alkaloid toxins combined with strong grazing pressure. Seed transmission ensures that, unlike mycorrhizal fungi or rhizobia, the endophyte is dispersed with the host during the colonization process. Given its widespread distribution and high infection frequency (*9*), tall fescue may threaten the persistence of native plant species and associated organisms, affect successional dynamics, and modify food webs in plant communities.

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12. The cultivar KY-31 was originally collected from a stand of tall fescue located in Menifee County, Kentucky, in the 1930s. Tall fescue either was deliberately brought from Europe for forage sometime in the 1800s, or it was an accidental introduction. KY-31 was released in 1943 and soon was widely planted in the eastern United States. The presence of an endophyte in tall fescue in the United States was not known until 1977 [C. W. Bacon, J. K. Porter, J. D. Robbins, E. S. Luttrell, *Appl. Environ. Microbiol.* **34**, 576 (1977)]. Seed used in this study was produced at the University of Kentucky in early summer 1994 and was planted 3 months later. Seed was kindly provided by T. Phillips (Agronomy Department, University of Kentucky). E+ and E− seed fields were established from material several generations removed from the original Menifee County collection. To propagate E− plants, E− seed was obtained from long-term storage of E+ seed. Endophyte viability in seed gradually declines over time. Infected and uninfected stocks are maintained as field-grown plants in alternating blocks where cross-pollination could occur. Because tall fescue is self-incompatible and obligately outcrossing, it is genetically heterogeneous and E+ and E− fescue do not differ except for the presence of the endophyte.
13. Infection was detected by making a thin freehand section of the inner leaf sheath of flowering stems. The tissue sample was stained with lactophenol cotton blue and then examined microscopically at ×100 after 24 hours. The endophyte appeared as long convoluted hyphae running parallel to the long axis of the plant cells. Hyphae are intercellular and lack any haustorial structures. See photos 1 to 6 in (*7*).
14. Each plot was divided into a grid of 400 squares (1 × 1 m) and a table of random numbers was used to select 20 squares for each sample period, with the constraint that none was sampled twice. Each quadrat (0.5 × 0.5 m) was located in the center of the square (1 × 1 m). Samples were harvested by cutting off all vegetation at ground level within the quadrat with a serrated knife. Litter was then collected and quadrats were examined for any remaining biomass.
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