

LETTER

An invasive plant–fungal mutualism reduces arthropod diversity

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Abstract

Ecological theory holds that competition and predation are the most important biotic forces affecting the composition of communities. Here, we expand this framework by demonstrating that mutualism can fundamentally alter community and food web structure. In large, replicated field plots, we manipulated the mutualism between a dominant plant (*Lolium arundinaceum*) and symbiotic fungal endophyte (*Neotyphodium coenophialum*). The presence of the mutualism reduced arthropod abundance up to 70%, reduced arthropod diversity nearly 20%, shifted arthropod species composition relative to endophyte-free plots and suppressed the biomass and richness of other plant species in the community. Herbivorous arthropods were more strongly affected than carnivores, and for both herbivores and carnivores, effects of the mutualism appeared to propagate indirectly via organisms occurring more basally in the food web. The influence of the mutualism was as great or greater than previously documented effects of competition and predation on arthropod communities. Our work demonstrates that a keystone mutualism can significantly reduce arthropod biodiversity at a broad community scale.

Keywords

Community structure, endophyte, food web, indirect effects, insect, *Lolium arundinaceum*, *Neotyphodium*, ordination, rarefaction, symbiosis.

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INTRODUCTION

Mutualism, in which two species interact to their mutual benefit, has long been assumed to be less important than competition or predation in shaping biodiversity and community composition (Hacker & Bertness 1996; Bruno *et al.* 2003; Hay *et al.* 2004; Begon *et al.* 2006; Halpern *et al.* 2007). There are several possible explanations for this presumed asymmetry in the importance of positive vs. negative species interactions. First, relative to experiments on competition and predation, experimental tests of the effects of mutualisms on whole communities have been rare (Stachowicz 2001; Bruno *et al.* 2003; Styrsky & Eubanks 2007). Second, early theoretical models predicted that mutualisms would have unstable dynamics, resulting in a historical emphasis on costs and benefits within mutualisms, rather than on the community impacts of mutualism (May 1982). Finally, many mutualisms, like many diseases, involve microbes (e.g. Smith & Read 1997; Chanway 1998; Bacon & White 2000), which have tended to receive less attention from community ecologists and can pose challenges for community-wide experiments in natural ecosystems.

Despite a general lack of attention to mutualism at the community scale (Agrawal *et al.* 2007), most organisms engage in multiple mutualisms during their lifetimes. Plants, for example, host diverse assemblages of microbes – including mycorrhizal fungi, endophytic fungi and bacteria – that can promote fitness through enhanced nutrient uptake, pathogen and herbivore resistance, and tolerance of abiotic stress (Smith & Read 1997; Chanway 1998; Bacon & White 2000; Clay & Schardl 2002; Herre *et al.* 2007; Marquez *et al.* 2007). These mutualisms could additionally affect the diversity of plant-associated food webs (Omacini *et al.* 2001; Faeth & Bultman 2002; Gehring & Whitham 2002; Müller & Krauss 2005; Rudgers & Clay 2007). Herbivores and carnivores in the ecosystem may respond directly to the presence of plant mutualisms or may be influenced indirectly by mutualist-mediated changes in the plant community. For example, both nitrogen-fixing bacteria and mycorrhizal fungi can alter plant diversity and productivity (Vitousek *et al.* 1987; Chapin *et al.* 1994; Klironomos *et al.* 2000; van der Heijden *et al.* 2008), which could indirectly affect associated herbivores and carnivores.

Experimental tests for the effects of mutualisms across multiple trophic levels have received limited investigation at the scale of the community and have mainly involved ant-plant and ant-hemipteran mutualisms (Wimp & Whitham 2001; Mooney 2007; Styrsky & Eubanks 2007). For example, in field plots of cultivated cotton, natural variation in the abundance of the mutualism between fire ants and aphids was negatively correlated with arthropod diversity, and the exclusion of fire ants increased arthropod diversity (Kaplan & Eubanks 2005). Most prior studies, however, have focused on 'component communities' (sensu Root (1973)), particularly those subsets of the community associated with a single target plant species (e.g. see review by Styrsky & Eubanks (2007)). Broader community surveys are needed to evaluate how commonly mutualisms may function as keystone interactions (sensu Paine (1969)), with effects that cascade to many community members.

Predicting how a mutualism will affect the surrounding community may depend on understanding how benefits are exchanged. When benefits from a mutualism are available to multiple community members (e.g. general increases in soil fertility via N-fixing bacteria), the mutualism may enhance community diversity. However, many symbionts confer benefits exclusively to one partner (i.e. benefits are private). Current mathematical and conceptual models predict that pair-wise, private benefits will feed back to increase partner persistence and abundance (Kiers & van der Heijden 2006). These feedbacks may ultimately reduce the diversity of associated species in the community, particularly competitors of the mutualists (Ringel *et al.* 1996; Bever 2003; Reynolds *et al.* 2003).

Mutualisms comprise a great diversity of interaction types that may vary in the strength of their impacts on communities. For example, Janzen (1985) identified four key types of mutualism: dispersal, pollination, nutrition and protection. Because dispersal and pollination influence the reproduction and spatial arrangement of one partner species, community level effects, if any, may occur only indirectly through changes in the abundance of a single, partner species (and its consumers). Community-level effects of nutritional mutualisms, such as mycorrhizal fungi or N-fixing bacteria, may depend on the degree to which benefits are private and whether the mutualism enhances the dominance of a single (or few) species to the detriment of others (e.g. Hartnett & Wilson 1999). Important in this regard is whether or not the mutualism promotes foundational species (e.g. in coral mutualisms), which thereby support a diverse community (Stachowicz 2001; Bruno *et al.* 2003). Finally, protection mutualisms may be more likely to have strong community level effects than other types of mutualism because they are inherently indirect interactions that require the involvement of at least three species, rather than a simple pair. Determining whether these predictions hold across mutualism types

require additional experiments that manipulate each type of mutualism and track community responses.

Here, we evaluated the extent to which a private protection mutualism affected community composition and food web structure by manipulating the presence of the endophytic fungus, *Neotyphodium coenophialum* (Ascomycetes: Clavicipitaceae) in its natural host, tall fescue grass (*Lolium arundinaceum*, Poaceae). In plants, fungal endophytes can enhance competitive ability (Clay *et al.* 1993), increase tolerance of abiotic stress (Rodriguez *et al.* 2004; Malinowski *et al.* 2005) and deter individual herbivores, pathogens or seed predators (Clay 1996; Arnold *et al.* 2003; Rudgers & Clay 2005). In tall fescue grass, the endophyte produces a variety of well-characterized, toxic alkaloids, including ergot alkaloids, lolines and peramines, and strong benefits of the endophyte are well-documented (Bush *et al.* 1997; Panaccione *et al.* 2001; Clay & Schardl 2002; Clay *et al.* 2005). Tall fescue is native to Europe and Africa, but has achieved a global distribution and is considered a noxious weed in several regions (Raloff 2003; Barnes 2004; Fribourg & Hannaway 2007). Worldwide, tall fescue is commonly infected by *N. coenophialum*, and in the United States, the endophyte occurs in > 75% of plants; however, endophyte frequencies can vary widely among populations (Ball *et al.* 1993; Clay *et al.* 2005).

In experimental grasslands in the Midwestern US, we manipulated the presence of the mutualism between tall fescue and *N. coenophialum*. The endophyte grows systemically in aboveground plant tissues, is vertically transmitted to seeds, and lacks contagious spread (Ball *et al.* 1993; Clay & Holah 1999; Clay & Schardl 2002). We predicted that because the benefits of this mutualism are exclusively pair-wise (i.e. private), the mutualism would enhance dominance of the host grass and reduce the diversity of co-occurring species in the system. In our prior work, including three experiments in two different habitats, we showed that the presence of the mutualism can reduce plant diversity (Clay & Holah 1999) and slow plant succession from grassland to forest (Rudgers *et al.* 2007). Observations and experiments demonstrated that these negative effects of the mutualism on competing plant species resulted in part because herbivorous animals (voles and insects) consumed more non-tall fescue species in the presence of the mutualism (Clay *et al.* 2005; Rudgers *et al.* 2007), and in part because the endophyte directly altered plant competitive hierarchies (Clay *et al.* 1993). Endophyte-mediated changes in plant composition could indirectly affect arthropods if arthropod diversity responds to plant diversity, as has been shown in other systems (e.g. Siemann *et al.* 1998). Studies by others have additionally found negative direct effects of the endophyte on some insect herbivores, while other insect species were not affected (reviewed by Rudgers & Clay 2007). However, previous research on endophyte mutualisms and arthropods has been conducted on component

communities, usually at the scale of individual plants. It remains unclear whether the mutualism functions as a keystone interaction with effects that cascade through the food web.

Here, we addressed the specific question: does the endophyte mutualism reduce the abundance or diversity of herbivorous and carnivorous arthropods? We predicted reduced community diversity if the availability of plant resources (edible plant biomass) is effectively constrained by the presence of the protection mutualism. This constraint could occur either directly via toxicity of the endophyte, indirectly through behavioural avoidance by arthropods, or indirectly through herbivore-mediated reductions in plant diversity. Alternatively, we predicted no net change in arthropod abundance or diversity if the mutualism alters competition among arthropods such that arthropod taxa insensitive to the mutualism compensate for declines in endophyte-sensitive taxa.

MATERIAL AND METHODS

Field experiment

We planted replicated field plots of tall fescue either with or without the mutualism present and tracked the responses of arthropods. During September, 2000, we established 16 field plots (30 m × 30 m each) at the Indiana University Research and Teaching Preserve, Bayles Road (Bloomington, IN, USA; 39°13'9" N, 86°32'29" W). Plots were created by disking a former agricultural field and enriching with tall fescue seeds at a rate of 45 kg-ha⁻¹. Plots were arranged in two rows of eight plots in a checkerboard pattern with adjacent endophyte present (E+) and absent (E-) treatments. Following seed addition, no further treatments were applied, excepting that we maintained a 2-m wide mowed buffer between plots. Over time, other plant species naturally colonized plots from the seed bank, vegetative fragments and nearby natural areas. The absence of contagious spread of the mutualist allowed us to maintain large-scale and long-term differences in the presence of the mutualism. Based on microscopic examination of 544 plant samples taken during 2003–2005, plots seeded with endophyte-free tall fescue (E-) were 0–0.1% infected, and plots sown with endophyte-infected tall fescue (E+) were 92–94% infected (Finkes *et al.* 2006, Rudgers *et al.* 2007). Rudgers *et al.* (2007) reported results based on the same field plots as used here (in addition to a separate, earlier field experiment), but monitored only the responses of trees.

Endophyte treatment

The endophyte was originally eliminated via long-term storage of infected tall fescue seeds at room temperature,

which reduces endophyte viability but not seed viability. Seeds used in our experiment were several generations distant from the original storage treatment and came from adjacent field plots of plants that freely cross-pollinated (*Lolium arundinaceum* is self-incompatible). This allowed for homogenization of the plant genetic background with respect to the endophyte treatment. The ages of seeds used to establish the experimental plots were the same for both endophyte treatments, *c.* 4 months post-harvest.

Arthropod and plant sampling

We used standard sweep net techniques to sample plant-associated arthropods [38-cm diameter canvas net, 91.5 cm handle, with 200 sweeps per plot (0.22 sweeps-m⁻²)] avoiding the outer 1-m edge of each plot. We collected on 28 September 2002, 11 July 2003, 1 October 2003 and 12 July 2004. Arthropod samples were frozen immediately following collection. In the laboratory, we sorted and keyed all specimens to order and family (Triplehorn & Johnson 2005). Abundant specimens were also identified to genus and species with the assistance of taxonomic experts (see section Acknowledgements). Any potential taxonomic bias in the use of sweep nets was equivalent across the endophyte treatments. In addition, we monitored changes in plant composition by harvesting above-ground biomass from eight randomly placed 0.5 m × 0.5 m quadrants per plot during June 2003 and 2004, and October 2003 and 2005. Mean total above-ground plant biomass per census (± SEM) was E+ = 114 ± 9 g, E- = 110 ± 7 g, *n* = 8 plots ($F_{1,12} = 0.3$, $P = 0.6$). Because the endophyte did not affect total above-ground plant biomass, it is unlikely that the endophyte treatment altered arthropod sampling efficiency. Plant harvests (during 2003–2004) were used to determine the maximum plant species richness per plot as well as non-fescue biomass per plot. These response variables allowed us to test for potential indirect effects of the mutualism (via an altered plant community) on associated arthropods (see section Indirect effects).

Data analysis

Data were analysed with repeated measures MANOVA following von Ende (2001). Models included the independent effect of the endophyte mutualism treatment (present = E+ or absent = E-) and the repeated effect of time, with plot as the unit of replication (SAS Institute Inc. 2004, version 9.1.4). We applied non-metric multidimensional scaling analysis (McCune & Mefford 1999) to examine shifts in community composition (PC-ORD, McCune & Grace 2002). To examine whether treatment differences in arthropod diversity were driven by differences in arthropod abundance, we constructed rarefaction curves for each

treatment. We used cumulative species abundances as well as abundances on each individual census date (EcoSim 7.72, 10,000 iterations; Gotelli & Colwell 2001; Gotelli & Entsminger 2001). For rarefaction curves constructed for each census date, α was Bonferroni-adjusted for comparisons at each of four time points.

Indirect effects

The mechanisms driving arthropod declines could involve both the direct effects of toxic alkaloids produced by the endophyte as well as indirect effects, for example, mediated through changes in the plant community. In a prior experiment, the presence of the endophyte mutualism in tall fescue reduced plant diversity (Clay & Holah 1999). In other observations from the current experiment, we showed that presence of the endophyte also slows plant succession (Rudgers *et al.* 2007). Mutualist-mediated changes in plant composition could indirectly affect arthropods, if arthropod diversity responds to plant diversity (e.g. Siemann *et al.* 1998).

To compare the strengths of potential direct vs. indirect pathways for arthropods, we contrasted two repeated measures of statistical models (SAS Institute Inc. 2004). The first model included only the endophyte treatment (direct + indirect effects model). The second model included the potential indirect pathway as a covariate, including also the covariate \times endophyte interaction. A significant interaction would indicate that the relationship between the covariate and response variable depends on the presence of the endophyte. For herbivore responses, maximum plant species richness and average non-tall fescue biomass were tested as covariates. For carnivore responses, we used average herbivore species richness per plot per census as a covariate. Plant covariates were log-transformed to achieve normality. If arthropod responses were highly correlated with the indirect pathway (i.e. the covariate), and the endophyte treatment in this covariate model became non-significant relative to the first model, we interpreted this as evidence of an indirect pathway of endophyte effect. It should be noted that these models test for correlation, not causation (e.g. while herbivore abundance may track plant species richness, plant species richness may also reflect past arthropod herbivory). In addition, including a covariate necessarily reduces power in the analysis, and denominator degrees of freedom for the endophyte main effect declined from 14 to 12 when the covariate was included. We used these analyses in an exploratory manner to suggest causal hypotheses that can be tested in future studies.

RESULTS

Effects of the grass–fungus mutualism ramified through the food web and strongly reduced arthropod abundance.

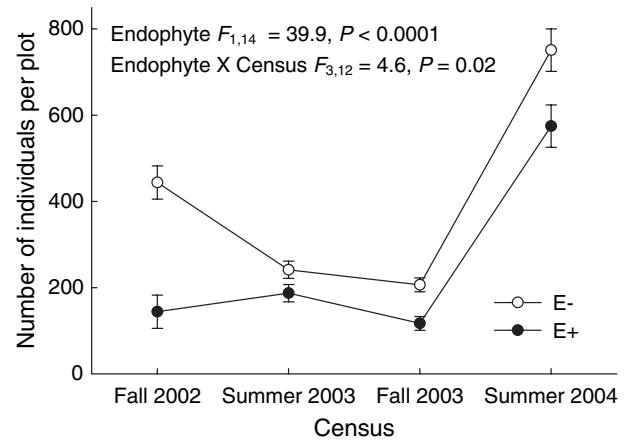


Figure 1 Mean number of arthropod individuals collected per plot (\pm SEM) in response to manipulation of the tall fescue–endophyte mutualism. Symbols show endophyte mutualist-present (E+, filled) and mutualist-absent (E-, open) treatments. Data were analysed with repeated measures ANOVA, which met assumptions of normality of residuals and homogeneity of variances ($n = 8$ plots per treatment per census).

Across census dates, we collected > 30 000 arthropod individuals, spanning 363 morphospecies, 105 families and 14 orders. Averaged over the 2-year study, plots with the mutualism (E+) supported 40% fewer arthropod individuals than plots lacking the mutualism (E-) (Fig. 1). The strongest difference in abundance occurred in fall of 2002 with 70% fewer individuals due to the mutualism (Fig. 1).

Plots with the mutualism also had reduced arthropod diversity, with 17% fewer morphospecies, 12% fewer families and 8% fewer orders than mutualist-free plots (Fig. 2). Species accumulation curves levelled off with increased sampling effort, and rarefaction curves showed that observed differences in cumulative species richness were primarily due to differences in the abundance of individuals between E+ and E- plots (Fig. 3a). However, for one census (Summer 2003), effects of the mutualism on richness persisted even when the samples were rarefied to similar abundances of individuals and statistical significance was Bonferroni-adjusted for multiple census dates (Fig. 3b). The mutualism caused the relative abundances of species to become significantly more even (mean Shannon evenness index $\text{plot}^{-1} \text{ census}^{-1} \pm \text{SEM}$ E+ = 0.72 ± 0.02 , E- = 0.66 ± 0.02 , $F_{1,14} = 6.0$, $P = 0.03$).

Arthropod species composition diverged between the mutualism treatments as demonstrated by non-metric multidimensional scaling analysis and multi-response permutation procedures (Fig. 4). Individual species that were strongly correlated with the axes in the ordination included both specialist and generalist herbivores as well as generalist predators. For example, the abundances of

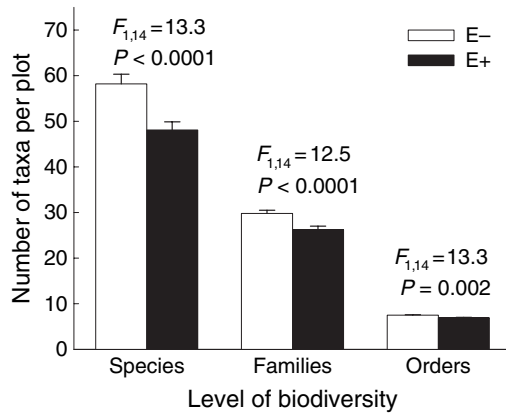


Figure 2 Mean number of arthropod taxa per plot per census date (\pm SEM) in response to manipulation of the tall fescue–endophyte mutualism. Bars show endophyte mutualist-present (E+, filled) and mutualist-absent (E–, open) treatments. Data were analysed with repeated measures ANOVA, and endophyte mutualist treatment \times census interactions were non-significant (all $P > 0.1$). Analyses met assumptions of normality of residuals and homogeneity of variances ($n = 8$ plots per treatment per census).

Melanoplus grasshopper nymphs and *Conocephalus* katydids were positively correlated with axis 2 (more in plots with the endophyte), while the Chinese mantid (*Tenodera aridifolia sinensis*), a jumping spider (*Marpissa formosa*) and an orb-weaving spider (*Araneus pratensis*) all declined with axis 1 (fewer in plots with the endophyte) (Table S1). Surprisingly, one group of salticid immatures (*Phidippus/Eris* spp.) increased in plots with the endophyte (Table S1). Also, of the leafhoppers (Cicadellidae) that were strongly correlated with the ordination, a xylem-feeder (*Draeculacephala* spp.) increased in the presence of the endophyte, while a phloem feeder (*Agallia constricta*) declined (Table S1).

When classified by position in the food web, herbivores were responsible for a large fraction of the decline in total arthropod abundance. However, both herbivorous and carnivorous arthropods were represented by fewer species in the presence of the mutualism (Fig. 5, see also Table S2). As expected based on our prior experiments, plant responses at the base of the food web were also altered by the mutualism. Both plant species richness and the biomass of non-tall fescue plant species were significantly reduced by the presence of the mutualism ($P < 0.001$ for both, Fig. 6).

Indirect effects appear to contribute to both herbivore and carnivore responses to the mutualism. When statistical models accounted for changes in plant species richness as a covariate for herbivore abundance (plant richness covariate: $P = 0.015$, Table S2) or herbivore richness ($P = 0.063$), the mutualism treatment no longer significantly reduced herbi-

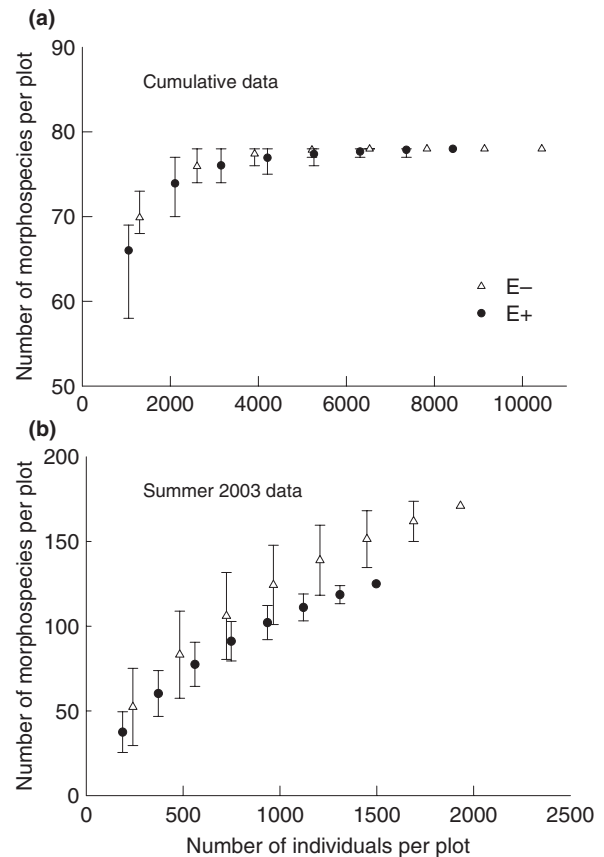


Figure 3 Rarefaction curves plotting the number of morphospecies vs. the number of individuals sampled in response to manipulation of the tall fescue–endophyte mutualism. Filled symbols show mutualist-present (E+) samples, and open symbols show mutualist-free (E–) samples ($n = 8$ plots per treatment). (a) The cumulative number of morphospecies per plot vs. the cumulative number of individuals sampled per plot. Bars represent 95% confidence intervals obtained from 10 000 re-sampling iterations. Bars that overlap the mean for the alternate treatment indicate that treatments were not significantly different ($P > 0.05$). (b) The number of morphospecies per plot vs. the number of individuals per plot for the Summer 2003 census only. Bars represent 99% confidence intervals to reflect Bonferroni correction of α for the four sampling dates examined (i.e. $P < 0.0125$ for statistical significance).

vore abundance ($P > 0.16$, Table S2) or herbivore richness ($P > 0.6$, Table S2) across all census dates. These results reflect the positive correlations between plant species richness and both the average herbivore species richness per census (Spearman $r = 0.60$, $P = 0.01$; $n = 16$ plots) and the average herbivore abundance per census (Spearman $r = 0.58$, $P = 0.02$). Non-tall fescue biomass was a weaker covariate than plant species richness for both herbivore abundance (plant biomass covariate: $P = 0.076$) and herbivore richness ($P = 0.079$). Similar to results for herbivores,

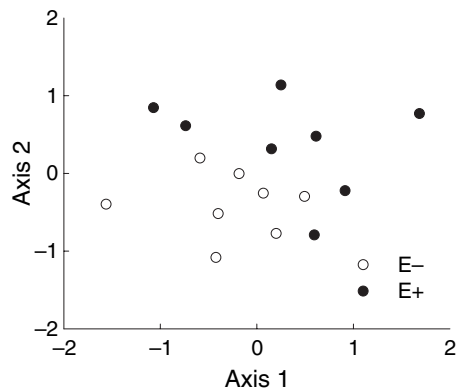


Figure 4 Nonlinear multidimensional scaling ordination of the cumulative abundances of each arthropod morphospecies ($r^2 = 0.9$). Each point represents a plot. Endophyte mutualist-present (E+, filled symbols) and mutualist-absent (E-, open symbols) treatments explained significant clustering in the ordination according to multi-response permutation procedures ($P = 0.015$). The mean stress value ($=7.7$) was significantly lower for real data than randomized runs ($P < 0.02$) and indicates that the ordination had negligible risk of drawing false inferences (Clarke 1993).

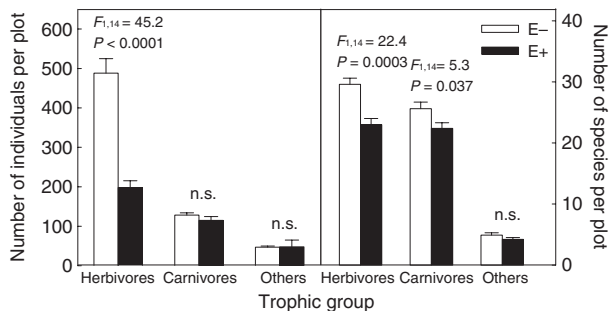


Figure 5 Mean number of arthropod individuals or morphospecies per plot per census (\pm SEM) in response to manipulation of the tall fescue–endophyte mutualism. Bars show endophyte mutualist-present (E+, filled) and mutualist-absent (E-, open) treatments and are ordered by trophic group. Data were analysed with repeated measures ANOVA (Table S2), and met assumptions of normality of residuals and homogeneity of variances ($n = 8$ plots per treatment per census).

the influence of the endophyte mutualism on carnivore richness became non-significant in the analysis that accounted for changes in herbivore richness ($P > 0.07$, Table S2). As a covariate, herbivore richness explained significant variation in carnivore species richness ($P = 0.006$, Table S2), and across plots there was a positive correlation between average carnivore richness and average herbivore richness per census (Spearman $r = 0.68$, $P = 0.004$, $n = 16$ plots). Thus, effects of the mutualism

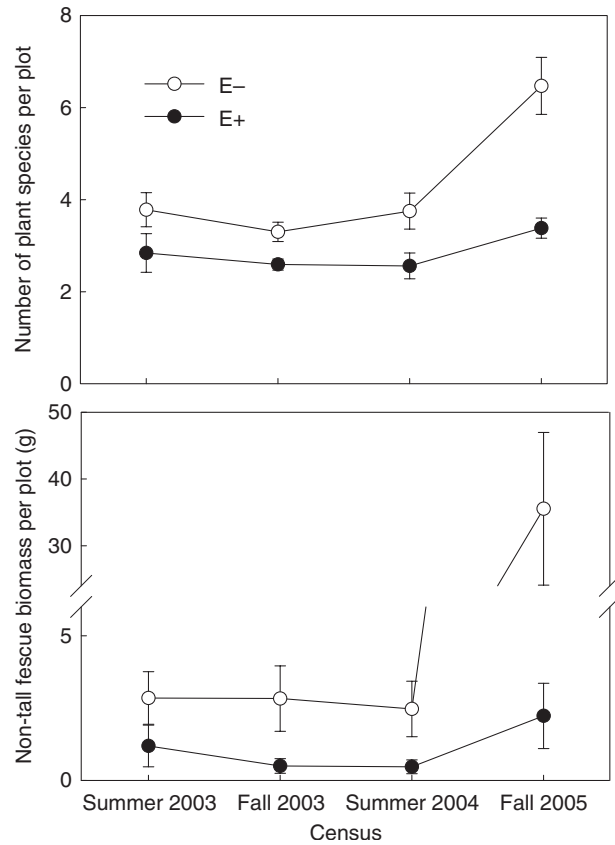


Figure 6 Plant responses to the presence of the endophyte mutualism in tall fescue grass. Symbols show endophyte mutualist-present (E+, filled) and mutualist-absent (E-, open) treatments. $n = 8$ plots per treatment per census. (a) Mean plant species richness (\pm SEM) per plot per census. Repeated measures ANOVA: endophyte $F_{1,14} = 31.4$, $P < 0.001$; endophyte \times census $F_{3,12} = 1.7$, $P = 0.2$. (b) Mean biomass of all non-tall fescue plants combined (\pm SEM) per plot per census. Repeated measures ANOVA endophyte $F_{1,14} = 38.9$, $P < 0.001$; endophyte \times census $F_{3,12} = 3.8$, $P = 0.039$.

on the community may propagate indirectly via the responses of organisms occurring more basally in the food web.

For herbivore abundance and carnivore richness, there were no significant interactions between the covariate and the endophyte treatment. This pattern suggests that the basic relationship between plant species richness and herbivore abundance (or herbivore richness and carnivore richness) was unaffected by the presence of the endophyte. For the response of herbivore richness, however, the relationship with plant species richness did vary with the endophyte treatment and time (endophyte \times covariate \times census, $P = 0.027$, Table S2). When decomposed by each census, there was a marginally more positive relationship in the absence of the mutualism than in its presence,

but only for the first census date (first census: endophyte \times covariate, $P = 0.09$).

DISCUSSION

We demonstrate that the effects of a plant–fungal mutualism cascade through a food web, affecting both plants and arthropods. Prior work has shown that grass–endophyte mutualisms can negatively affect specialized parasitoids of weevils and aphids (Omacini *et al.* 2001; Bultman *et al.* 2003), as well as generalist ladybird beetles (de Sassi *et al.* 2006) and spiders (Finkes *et al.* 2006). Here, we found that the endophyte reduced arthropod abundance and diversity across many generalist and specialist taxa, with effects that reach far beyond the component communities that are closely associated with the endophyte host plant. This is some of the first experimental evidence from any mutualism to show broad, community-wide effects across trophic levels. Our results suggest that the endophyte mutualism acts as a keystone interaction in this grassland ecosystem.

Our prior work has shown that the private protection mutualism between the endophyte and tall fescue grass alters herbivory by voles, which in turn suppress the richness and biomass of other plant species in the community (Clay & Holah 1999; Clay *et al.* 2005). In the present study, effects of the endophyte on plant diversity were strong and repeatable in a new experiment and location that differed in soils, topography and land-use history. The presence of the endophyte mutualism also reduced the abundance and richness of herbivorous arthropods and the richness of carnivorous arthropods at the scale of the whole community. It is possible that herbivorous arthropods responded to the endophyte indirectly through changes in the plant community, rather than directly to the toxic effects of endophyte alkaloids. Positive covariance between plant species richness and herbivore responses in our study were consistent with this hypothesis. While our covariate analysis cannot assign causality to these indirect pathways, our previous research suggested that insects alone cannot drive changes in plant composition in this system. In plots sown with 50 : 50 mixtures of E+ and E– tall fescue, manipulation of insects (via insecticides) had no effect on the biomass of non-fescue plants relative to control plots. However, excluding voles significantly increased the biomass of non-fescue plants, suggesting that voles are a key driver of the plant community response (Clay *et al.* 2005). Like herbivores, carnivores also appear responsive to indirect effects rather than direct effects of the endophyte mutualism because carnivorous arthropod richness strongly covaried with the richness of herbivorous arthropods. Direct experimental manipulations of plant species richness (and herbivore richness) are required to confirm these indirect, causal mechanisms.

The construction of detailed food webs, while beyond the scope of this study, could also help untangle the complex direct and indirect responses in this system. For example, some leafhopper and grasshopper species (e.g. xylem feeders, melanoplinae) appear insensitive to, and even increase with, the presence of the endophyte. Predators specific to these groups may track the response of their prey. In contrast, some generalist predators (salticid and araneid spiders, mantids) may respond to the overall reduction in prey availability due to the mutualism, resulting in their decline (see also Finkes *et al.* 2006).

The impact of the tall fescue–endophyte mutualism was comparable in magnitude to other factors important to arthropod community structure. In our work, the effect size [log response ratio = $\ln(\text{control}/\text{treatment})$ (Hedges *et al.* 1999)] for the mutualism was 0.49 for total arthropod abundance and 0.08 for arthropod species richness. Predation can similarly alter arthropod abundance and diversity, and effect sizes in other systems ranged from 0.03 to 0.44 [calculated from data and figures in (Holmes *et al.* 1979; Marquis & Whelan 1994; Spiller & Schoener 1998; Dyer & Letourneau 1999; Romero & Vasconcellos-Neto 2004; Gruner & Taylor 2006)]. Experiments manipulating competition between vertebrates and arthropods have produced comparable effect sizes ranging from 0.09 to 1.27 (Rambo & Faeth 1999; Bailey & Whitham 2002; Kruess & Tschamtker 2002; Lill & Marquis 2003). Finally, experiments altering plant genetic or species diversity have shown effect sizes of 0.07–0.18 for arthropod richness (Siemann *et al.* 1998; Crutsinger *et al.* 2006; Johnson *et al.* 2006). Clearly, the private, protection mutualism examined here can rival or exceed the antagonistic interactions of predation and competition as well as the ‘bottom-up’ effects of plant diversity. Experiments that manipulate other types of mutualism, such as nutritional symbiosis, pollination or dispersal, will be very useful for understanding how the type of mutualism and the specificity of benefits exchanged may influence the magnitude of impacts on the community. In addition, factorial experiments that test for the combined effects of mutualism and other ecological interactions could evaluate the relative importance of positive vs. negative species interactions and may uncover novel, non-additive effects (Mooney 2007; Morris *et al.* 2007).

Our results have important practical, as well as conceptual, implications. Although it is native to Europe and Africa, tall fescue covers significant land area in the United States and is also widespread in Asia, Australia, New Zealand and South America (Fribourg & Hannaway 2007). Tall fescue is commonly planted for pasture, turf and soil conservation, has invaded unmanaged ecosystems in many regions, and is frequently endophyte-infected (Ball *et al.* 1993; Raloff 2003; Barnes 2004). Our prior work revealed that the presence of the endophyte mutualism can facilitate

the invasion of tall fescue into diverse plant communities (Rudgers *et al.* 2005). Results presented here predict that invasions of endophyte-infected tall fescue will cause widespread suppression of plant and arthropod diversity and significantly alter the composition of the arthropod community. Because arthropods are consumed by birds, reptiles, small mammals and other carnivores (e.g. Holmes *et al.* 1979; Marquis & Whelan 1994; Nakano & Murakami 2001), the mutualism may constrain energy flow even at higher levels in the food web.

It remains unclear whether the strong community-level impacts of the tall fescue–endophyte mutualism are related to the non-native status of both the host grass and its mutualist. For example, a recent meta-analysis suggested that grass–endophyte symbiosis in native habitats have smaller interaction strengths than symbiosis in introduced, agronomic grasses (Saikkonen *et al.* 2006); this could lead to correspondingly weaker community effects. However, too few native grass–endophyte systems have been investigated to draw firm conclusions. Future studies on native grass–endophyte interactions, as well as on other types of mutualisms, could help resolve the importance of coevolution with the native community in affecting the magnitude of community-level effects.

Arthropods constitute the most diverse and abundant macro-organisms on Earth (Triplehorn & Johnson 2005). Understanding the factors that determine their composition and abundance is critical to the preservation of global biodiversity and to the functioning of terrestrial ecosystems (Losey & Vaughan 2006). Our results show that a plant–microbe mutualism can dramatically alter the composition of arthropods, producing effects on biodiversity that rival those of competition and predation. We suspect that large-scale experimental manipulations of mutualisms in other systems may uncover similarly strong impacts on communities and overturn the long-held view that competition and predation are the primary interactions structuring ecological communities. Given the strength of its effects, mutualism should be better incorporated into empirical and theoretical studies of forces governing the structure of ecological communities.

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REFERENCES

- Agrawal, A.A., Ackerly, D.D., Adler, F., Arnold, A.E., Caceres, C., Doak, D.F. *et al.* (2007). Filling key gaps in population and community ecology. *Front. Ecol. Environ.*, 5, 145–152.
- Arnold, A.E., Mejia, L.C., Kylo, D., Rojas, E.I., Maynard, Z., Robbins, N. *et al.* (2003). Fungal endophytes limit pathogen damage in a tropical tree. *Proc. Natl. Acad. Sci. U.S.A.*, 100, 15649–15654.
- Bacon, C.W. & White, J.F. (2000). *Microbial Endophytes*. Marcel Dekker, Inc., New York, NY.
- Bailey, J.K. & Whitham, T.G. (2002). Interactions among fire, aspen, and elk affect insect diversity: reversal of a community response. *Ecology*, 83, 1701–1712.
- Ball, D.M., Pedersen, J.F. & Laceyfield, G.D. (1993). The tall fescue endophyte. *Am. Sci.*, 81, 370–379.
- Barnes, T.G. (2004). Strategies to convert exotic grass pastures to tall grass prairie communities. *Weed Technol.*, 18, 1364–1370.
- Begon, M., Townsend, C.R. & Harper, J.L. (2006). *Ecology: from Individuals to Ecosystems*. Blackwell, Malden, MA.
- Bever, J.D. (2003). Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. *New Phytol.*, 157, 465–473.
- Bruno, J.F., Stachowicz, J.J. & Bertness, M.D. (2003). Inclusion of facilitation into ecological theory. *Trends Ecol. Evol.*, 18, 119–125.
- Bultman, T.L., McNeill, M.R. & Goldson, S.L. (2003). Isolate-dependent impacts of fungal endophytes in a multitrophic interaction. *Oikos*, 102, 491–496.
- Bush, L.P., Wilkinson, H.H. & Schardl, C.L. (1997). Bioprotective alkaloids of grass–fungal endophyte symbiosis. *Plant Physiol.*, 114, 1–7.
- Chanway, C.P. (1998). Bacterial endophytes: ecological and practical implications. *Sydowia*, 50, 149–170.
- Chapin, F.S., III, Walker, L.R., Fastie, C.L. & Sharman, L.C. (1994). Mechanisms of primary succession following deglaciation at Glacier Bay, Alaska. *Ecol. Monogr.*, 64, 149–175.
- Clarke, K.R. (1993). Nonparametric multivariate analyses of changes in community structure. *Aust. J. Ecol.*, 18, 117–143.
- Clay, K. (1996). Interactions among fungal endophytes, grasses and herbivores. *Res. Popul. Ecol.*, 38, 191–201.
- Clay, K. & Holah, J. (1999). Fungal endophyte symbiosis and plant diversity in successional fields. *Science*, 285, 1742–1744.
- Clay, K. & Schardl, C. (2002). Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. *Am. Nat.*, 160, S99–S127.
- Clay, K., Marks, S. & Cheplick, G.P. (1993). Effects of insect herbivory and fungal endophyte infection on competitive interactions among grasses. *Ecology*, 74, 1767–1777.
- Clay, K., Holah, J. & Rudgers, J.A. (2005). Herbivores cause a rapid increase in hereditary symbiosis and alter plant community composition. *Proc. Natl. Acad. Sci. U.S.A.*, 102, 12465–12470.
- 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.

- Dyer, L.A. & Letourneau, D.K. (1999). Trophic cascades in a complex terrestrial community. *Proc. Natl Acad. Sci. U.S.A.*, 96, 5072–5076.
- von Ende, C.N. (2001). Repeated measures analysis: growth and other time dependent measures. In: *Design and Analysis of Ecological Experiments* (eds Scheiner, S.M. & Gurevitch, J.). Oxford University Press, New York, pp. 134–157.
- Faeth, S.H. & Bultman, T.L. (2002). Endophytic fungi and interactions among host plants, herbivores, and natural enemies. In: *Multitrophic Level Interactions* (eds Tscharntke, T. & Hawkins, B.A.). Cambridge University Press, Cambridge, U.K, pp. 89–123.
- Finkes, L.K., Cady, A.B., Mulroy, J.C., Clay, K. & Rudgers, J.A. (2006). Plant–fungus mutualism affects spider composition in successional fields. *Ecol. Lett.*, 9, 347–356.
- Fribourg, H.A. & Hannaway, D.B. (2007). *Tall Fescue On-line Monograph, Forage Information System*. <http://forages.oregon-state.edu/is/tf/is/monograph.html>
- Gehring, C.A. & Whitham, T.G. (2002). Mycorrhizae–herbivore interactions: population and community consequences. In: *Mycorrhizal Ecology* (eds van der Heijden, M.G.A. & Sanders, I.R.). Springer-Verlag, Heidelberg, Germany, pp. 295–320.
- Gotelli, N.J. & Colwell, R.K. (2001). Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecol. Lett.*, 4, 379–391.
- Gotelli, N.J. & Entsminger, G.L. (2001). *EcoSim: Null Models Software for Ecology Version 7.0*. Acquired Intelligence Inc. & Keesey-Bear. <http://homepages.together.net/~gentsmin/ecosim.htm>.
- Gruner, D.S. & Taylor, A.D. (2006). Richness and species composition of arboreal arthropods affected by nutrients and predators: a press experiment. *Oecologia*, 147, 714–724.
- Hacker, S.D. & Bertness, M.D. (1996). Trophic consequences of a positive plant interaction. *Am. Nat.*, 148, 559–575.
- Halpern, B.S., Silliman, B.R., Olden, J.D., Bruno, J.P. & Bertness, M.D. (2007). Incorporating positive interactions in aquatic restoration and conservation. *Front. Ecol. Environ.*, 5, 153–160.
- Hartnett, D.C. & Wilson, G.W.T. (1999). Mycorrhizae influence plant community structure and diversity in tallgrass prairie. *Ecology*, 80, 1187–1195.
- Hay, M.E., Parker, J.D., Burkepile, D.E., Caudill, C.C., Wilson, A.E., Hallinan, Z.P. *et al.* (2004). Mutualisms and aquatic community structure: the enemy of my enemy is my friend. *Annu. Rev. Ecol. Evol. Syst.*, 35, 175–197.
- Hedges, L.V., Gurevitch, J. & Curtis, P.S. (1999). The meta-analysis of response ratios in experimental ecology. *Ecology*, 80, 1150–1156.
- van der Heijden, M.G.A., Bardgett, R.D. & van Straalen, N.M. (2008). The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol. Lett.*, 11, 296–310.
- Herre, E.A., Mejia, L.C., Kylo, D.A., Rojas, E., Maynard, Z., Butler, A. *et al.* (2007). Ecological implications of anti-pathogen effects of tropical fungal endophytes and mycorrhizae. *Ecology*, 88, 550–558.
- Holmes, R.T., Schultz, J.C. & Nothnagle, P. (1979). Bird predation on forest insects: an enclosure experiment. *Science*, 206, 462–463.
- Janzen, D.H. (1985). The natural history of mutualisms. In: *The Biology of Mutualism* (ed. Boucher, D.H.). Oxford University Press, New York, NY, pp. 40–99.
- Johnson, M.T.J., Lajeunesse, M.J. & Agrawal, A.A. (2006). Additive and interactive effects of plant genotypic diversity on arthropod communities and plant fitness. *Ecol. Lett.*, 9, 24–34.
- Kaplan, I. & Eubanks, M.D. (2005). Aphids alter the community-wide impact of fire ants. *Ecology*, 86, 1640–1649.
- Kiers, E.T. & van der Heijden, M.G.A. (2006). Mutualistic stability in the arbuscular mycorrhizal symbiosis: exploring hypotheses of evolutionary cooperation. *Ecology*, 87, 1627–1636.
- Klironomos, J.N., McCune, J., Hart, M. & Neville, J. (2000). The influence of arbuscular mycorrhizae on the relationship between plant diversity and productivity. *Ecol. Lett.*, 3, 137–141.
- Kruess, A. & Tscharntke, T. (2002). Contrasting responses of plant and insect diversity to variation in grazing intensity. *Biol. Conserv.*, 106, 293–302.
- Lill, J.T. & Marquis, R.J. (2003). Ecosystem engineering by caterpillars increases insect herbivore diversity on white oak. *Ecology*, 84, 682–690.
- Losey, J.E. & Vaughan, M. (2006). The economic value of ecological services provided by insects. *Bioscience*, 56, 311–323.
- Malinowski, D.P., Belesky, D.P. & Lewis, G.C. (2005). Abiotic stresses in endophytic grasses. In: *Neotyphodium in Cool-season Grasses* (eds Roberts, C.A., West, C.P. & Spiers, D.E.). Blackwell Publishing, Ames, Iowa, pp. 187–199.
- Marquez, L.M., Redman, R.S., Rodriguez, R.J. & Roossinck, M.J. (2007). A virus in a fungus in a plant: three-way symbiosis required for thermal tolerance. *Science*, 315, 513–515.
- Marquis, R.J. & Whelan, C.J. (1994). Insectivorous birds increase growth of white oak through consumption of leaf-chewing insects. *Ecology*, 75, 2007–2014.
- May, R.M. (1982). Mutualistic interactions among species. *Nature*, 296, 803–804.
- McCune, B. & Grace, J.B. (2002). *Analysis of Ecological Communities*. MjM Software Design, Gleneden Beach, OR, USA.
- McCune, B. & Mefford, M.J. (1999). *PC-ORD. Multivariate Analysis of Ecological Data. Version 4*. MjM Software Design, Gleneden Beach, OR, USA.
- Mooney, K.A. (2007). Tritrophic effects of birds and ants on a canopy food web, tree growth, and phytochemistry. *Ecology*, 88, 2005–2014.
- Morris, W.F., Hufbauer, R.A., Agrawal, A.A., Bever, J.D., Borowicz, V.A., Gilbert, G.S. *et al.* (2007). Direct and interactive effects of enemies and mutualists on plant performance: a meta-analysis. *Ecology*, 88, 1021–1029.
- Müller, C.B. & Krauss, J. (2005). Symbiosis between grasses and asexual fungal endophytes. *Curr. Opin. Plant Biol.*, 8, 450–456.
- Nakano, S. & Murakami, M. (2001). Reciprocal subsidies: dynamic interdependence between terrestrial and aquatic food webs. *Proc. Natl Acad. Sci. U.S.A.*, 98, 166–170.
- Omacini, M., Chaneton, E.J., Ghersa, C.M. & Müller, C.B. (2001). Symbiotic fungal endophytes control insect host–parasite interaction webs. *Nature*, 409, 78–81.
- Paine, R.T. (1969). A note on trophic complexity and community stability. *Am. Nat.*, 103, 91–93.
- Panaccione, D.G., Johnson, R.D., Wang, J., Young, C.A., Damrongkool, P., Scott, B. *et al.* (2001). Elimination of ergovaline from a grass–*Neotyphodium* endophyte symbiosis by genetic modification of the endophyte. *Proc. Natl Acad. Sci. U.S.A.*, 98, 12820–12825.
- Raloff, J. (2003). Cultivating weeds: is your yard a menace to parks and wild lands? *Science News*, 163, 232.

- Rambo, J.L. & Faeth, S.H. (1999). Effect of vertebrate grazing on plant and insect community structure. *Conserv. Biol.*, 13, 1047–1054.
- Reynolds, H.L., Packer, A., Bever, J.D. & Clay, K. (2003). Grassroots ecology: plant–microbe–soil interactions as drivers of plant community structure and dynamics. *Ecology*, 84, 2281–2291.
- Ringel, M.S., Hu, H.H. & Anderson, G. (1996). The stability and persistence of mutualisms embedded in community interactions. *Theor. Popul. Biol.*, 50, 281–297.
- Rodriguez, R.J., Redman, R.S. & Henson, J.M. (2004). The role of fungal symbiosis in the adaptation of plants to high stress environments. *Mitig. Adapt. Strat. Glob. Change*, 9, 261–272.
- Romero, G.Q. & Vasconcelos-Neto, J. (2004). Beneficial effects of flower-dwelling predators on their host plant. *Ecology*, 85, 446–457.
- Root, R.B. (1973). Organization of a plant–arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). *Ecol. Monogr.*, 43, 95–124.
- Rudgers, J.A. & Clay, K. (2005). Fungal endophytes in terrestrial communities and ecosystems. In: *The Fungal Community* (eds Dighton, E.J., Oudemans, P. & White, J.F.J.). M. Dekker, New York, New York, pp. 423–442.
- Rudgers, J.A. & Clay, K. (2007). Endophyte symbiosis with tall fescue: how strong are the impacts on communities and ecosystems? *Fungal Biol. Rev.*, 21, 107–124.
- Rudgers, J.A., Mattingly, W.B. & Koslow, J.M. (2005). Mutualistic fungus promotes plant invasion into diverse communities. *Oecologia*, 144, 463–471.
- Rudgers, J.A., Holah, J., Orr, S.P. & Clay, K. (2007). Forest succession suppressed by an introduced plant–fungal symbiosis. *Ecology*, 88, 18–25.
- Saikkonen, K., Lehtonen, P., Helander, M., Koricheva, J. & Faeth, S.H. (2006). Model systems in ecology: dissecting the endophyte–grass literature. *Trends Plant Sci.*, 11, 428–433.
- SAS Institute Inc. (2004). *SAS version 9.1.4*. SAS Institute, Cary, North Carolina, USA.
- de Sassi, C., Müller, C.B. & Krauss, J. (2006). Fungal plant endosymbionts alter life history and reproductive success of aphid predators. *Proc. R. Soc. B-Biol. Sci.*, 273, 1301–1306.
- Siemann, E., Tilman, D., Haartstad, J. & Ritchie, M. (1998). Experimental tests of the dependence of arthropod diversity on plant diversity. *Am. Nat.*, 152, 738–750.
- Smith, S.E. & Read, D.J. (1997). *Mycorrhizal Symbiosis*, 2nd edn. Academic Press, San Diego.
- Spiller, D.A. & Schoener, T.W. (1998). Lizards reduce spider species richness by excluding rare species. *Ecology*, 79, 503–516.
- Stachowicz, J.J. (2001). Mutualism, facilitation, and the structure of ecological communities. *Bioscience*, 51, 235–246.
- Styrsky, J.D. & Eubanks, M.D. (2007). Ecological consequences of interactions between ants and honeydew-producing insects. *Proc. R. Soc. B-Biol. Sci.*, 274, 151–164.
- Triplehorn, C.A. & Johnson, N.F. (2005). *Borror and DeLong's Introduction to the Study of Insects*, 7th edn. Thomson Brooks/Cole, Belmont, CA.
- Vitousek, P.M., Walker, L.R., Whiteaker, L.D., Muellerdombois, D. & Matson, P.A. (1987). Biological invasion by *Myrica faya* alters ecosystem development in Hawaii. *Science*, 238, 802–804.
- Wimp, G.M. & Whitham, T.G. (2001). Biodiversity consequences of predation host plant hybridization on an aphid–ant mutualism. *Ecology*, 82, 440–452.

SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article:

Table S1 Arthropod species most strongly correlated with the partitioning of plots in NMS ordination.

Table S2 Results for repeated measures statistical models comparing potential direct vs. direct + indirect effects of the endophyte treatment on arthropod response variables.

This material is available as part of the online article from: <http://www.blackwell-synergy.com/doi/full/10.1111/j.1461-0248.2008.01201.x>

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