Inherited microbial symbionts increase herbivore abundances and alter arthropod diversity on a native grass

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Abstract:

Some microbial symbionts of plants are maternally inherited and thus functionally increase genetic and phenotypic variation within plant populations. This variation, coupled with that of the host plant and environment, may alter abundances, diversity, and trophic structure of associated plant and animal communities. Fungal endophytes in the genus *Neotyphodium* are vertically transmitted, asexual microbial symbionts of grasses that remain asymptomatic and rely upon their hosts for resources and transmission via seeds, often providing benefits to their hosts, including protection against herbivores. Endophyte infections may influence associated arthropod communities in agronomic grasses, but the long-term effects of endophytes and variation in host genotype and resource availability on arthropod communities in native grass populations are unknown. We conducted a long-term field experiment with four maternal genotypes of an infected (E+) native grass (*Festuca arizonica*) from whence the endophyte was experimentally removed (E−) and water availability was controlled, to test the effects of infection, plant genotype, and resources on abundances, biomass, diversity (richness and evenness), and trophic structure of the arthropod community. Generally, E+ grasses harbored more arthropods, including more herbivores, predators, and detritivores, suggesting that the effects of endophytes cascaded upward through trophic levels in terms of abundances, at least in early ontogeny of the host. That E+ plants harbored more herbivorous insects than E− plants suggests that infection does not increase but instead decreases resistance to herbivores, contrary to prevailing concepts of endophytes as defensive mutualists. Infection did not alter overall species richness of the arthropod community or richness of herbivores but reduced natural enemy richness, especially that of parasites, and increased richness of detritivores. Reduced richness and shifts in evenness of natural enemies on E+ plants suggest that endophytes may disproportionately affect diversity at higher trophic levels and may partially explain increases in abundances of herbivorous insects on E+ plants. Biomass of predators, detritivores, and omnivores increased on plants with supplemented water, and arthropod and herbivore biomass

varied by plant genotype. Symbiont-mediated phenotypic variation interacts with variation from plant genotype and environmental factors to alter arthropod abundances and diversity, and these effects shift with ontogeny of the host.

Keywords: Arizona, USA | arthropods | community genetics | community structure | defensive mutualist | diversity | endophyte | evenness | Festuca arizonica | Neotyphodium | species richness | symbiont

Article:

Introduction

Genetic diversity within plant species is increasingly recognized as having important ecological consequences at the community and ecosystem levels (Hughes et al. 2008). For example, recent studies show that genetic diversity in plants can alter abundances and diversity of co-occurring plant (e.g., Lankau and Strauss 2007) and arthropod communities (e.g., Crutsinger et al. 2006, Johnson et al. 2006), resistance to invasion by nonnative plants species (Crutsinger et al. 2008), and ecosystem processes, such as decomposition (e.g., Schweitzer et al. 2005) and nutrient cycling (Hughes and Stachowizc 2004). Many of these studies fall under the broader concept of community genetics, in which heritable variation within individual plant species (or extended phenotypes) at one organizational level, such as the primary producers, alters properties and processes at other ecological organizational levels (e.g., consumer levels) (Whitham et al. 2003, Bangert et al. 2008). Such heritable variation and its phenotypic expression within species may often exceed variation among species and have ecological repercussions that span trophic levels, levels of biological organization, and regions (e.g., Whitham et al. 2003, Bangert et al. 2008).

Genetic diversity in plant populations comes in different forms (e.g., discrete vs. continuous traits) and is measured in different ways and at different scales (Hughes et al. 2008). At the population level, genotypic richness or the number of plant genotypes is commonly used in studies of the effects of plant diversity on consumer community diversity and structure (Hughes et al. 2008). There is, however, another source of plant genotypic and phenotypic variation that contributes to plant genotypic richness that is often overlooked in studies of the effects of plant genetic diversity on community properties. All host plants examined to date harbor some form of microbial symbionts, such as belowground mycorrhizae or aboveground endophytes (e.g., Saikkonen et al. 1998). These microbial symbionts, despite their relatively small biomass, also often cause phenotypic changes in their host plants that reverberate throughout the entire community, paralleling ecological effects of plant genetic diversity. For example, Cahill et al. (2008) recently showed that suppression of arbuscular mycorrhizal fungi (AMF) in a native grassland shifts behavior, abundances, and community structure of pollinating bee species. Likewise, AMF colonization and dependencies can determine plant community composition

structure, dominance, and resistance to invasion by nonnative plant species (e.g., Yao et al. 2008).

Although microbial symbionts of plants may cause community-wide effects by changing plant phenotypes, most of these are transmitted horizontally and are acquired during host ontogeny and are thus not heritable. However, some microbial symbionts are vertically and maternally transmitted and thus are heritable components, similar to mitochondria (Clay et al. 2005). Of aboveground microbial symbionts that inhabit plants, by far the best known are vertically transmitted fungal endophytes in the genus *Neotyphodium.* These asexual endophytes live intercellularly, systemically, and asymptomatically within the aboveground tissues of many species of pooid grasses and are vertically transmitted (hyphae grow into developing seeds) (Clay 1990, Saikkonen et al. 1998, Schardl et al. 2004). Thus, as inherited symbionts (Clay et al. 2005), *Neotyphodium* endophytes contribute to both the genotypic and phenotypic diversity of the host. Evolutionary theory predicts that asexual, vertically transmitted symbionts such as *Neotyphodium* should be strong mutualists because host and symbiont fitness are tightly linked (e.g., Clay 1990, Ewald 1994, Schardl and Clay 1997, Clay and Schardl 2002).

Community-wide effects of genetic diversity are directly related to the magnitude of variation in phenotypic traits (Hughes et al. 2008). As sources of host genetic variation, *Neotyphodium* endophytes make excellent candidates for vehicles of community- and ecosystem-wide changes because infections are inherited and often radically alter host grass phenotypes (Cheplick and Faeth 2009). Infections may provide a suite of benefits to the host including increased competitive abilities, resistance to abiotic stresses such as drought, and enhanced nutrient uptake (e.g., Faeth and Bultman 2002, Müller and Krauss 2005). However, the most renowned and oft-cited phenotypic alteration is increased resistance to herbivores via the production of fungal alkaloids (e.g., Clay 1988, Clay and Schardl 2002). *Neotyphodium* may produce four different general types of alkaloids, each with varying biological activity against invertebrate and vertebrate herbivores (Leuchtmann et al. 2000, Schardl et al. 2004). Uninfected host grasses or grasses once infected but with the endophyte removed experimentally produce no alkaloids at all (Faeth and Sullivan 2003). Thus, endophytes are viewed as “acquired defenses” (Cheplick and Clay 1988) or “defensive mutualists” (Clay 1988) of grasses, which often lack their own chemical defenses against herbivores. Reduction of herbivory is assumed to benefit the host grass and concomitantly increase fitness of the vertically transmitted endophyte (Saikkonen et al.1998, Schardl et al. 2004). Increased resistance via endophyte alkaloids has been demonstrated in laboratory bioassays and field tests involving mostly introduced agronomic grass cultivars with mostly agricultural insect pests (e.g., Faeth 2002, Saikkonen et al. 2006).

These endophyte-mediated phenotypic changes may also alter community diversity and structure and ecosystem process. In a recent study of agronomic tall fescue (*Lolium arundinaceum*) in field plots, Rudgers and Clay (2008) found that plots sewn with *Neotyphodium*-infected tall fescue showed reduced arthropod abundances, especially herbivorous insects, and altered arthropod species composition compared to endophyte-free plots. Furthermore, plant species

diversity and biomass were reduced in infected grass plots. Earlier studies, with this grass or other introduced agronomic grasses (Italian ryegrass, *Lolium multiflorum*, and perennial ryegrass, *Lolium perenne*), also showed community-wide effects of infection on: (1) plant abundances and community diversity (Clay and Holah 1999, Rudgers et al. 2007), (2) abundances and diversity of plant-associated animals in plots in old fields (e.g., Clay and Holah 1999) and in experimental potted containers (Omacini et al. 2001), (3) coexistence of competing herbivore and parasitoid species (Härri 2007), (4) diversity and abundances of predator (Finkes et al. 2006) and parasite species (de Sassi et al. 2006, Härri et al. 2008), and (5) composition of detritivore communities (Lemons et al. 2005). Some of these effects, such as reduction in plant diversity, may be resource-based or bottom-up effects, as endophyte infection increases growth, biomass, and competitive abilities of their hosts (Clay and Holah 1999). However, most effects are consumer-based or top-down effects, as endophytic alkaloids are presumed to generally reduce abundances and diversity of herbivores (e.g., Rudgers and Clay 2008), natural enemies (e.g., Bultman et al. 1997, Omacini et al. 2001, de Sassi et al. 2006, Finkes et al. 2006) and detritivores (e.g., Lemons et al. 2005). Furthermore, alkaloids may reduce co-occurring plant abundances and diversity by inhibiting seed germination or soil allelopathic effects (e.g., Matthews and Clay 2001).

Inclusion of vertically transmitted endophytes as sources of genetic and phenotypic variation in host plant populations is made more complicated because host and endophyte genotype can also alter the phenotypic traits of the symbiotic unit (e.g., Cheplick and Faeth 2009). Furthermore, expression of the host phenotype and its endophyte-mediated traits are dependent upon the environmental factors (Cheplick and Faeth 2009). For example, alkaloid production may depend on soil nitrogen content because alkaloids are nitrogen-rich compounds (Faeth 2002, Faeth and Fagan 2002) and relative competitive abilities and resistance to drought of infected hosts depend on the availability of water (e.g., Morse et al. 2002, 2007). Nonetheless, Hughes et al. (2008) argued that it is critical to understand the effects of plant genetic diversity relative to these environmental factors. Likewise, at least for perennial plants, these sources of genetic and environmental variation and their effects at the community level likely vary over the ontogeny of the plant (e.g., Faeth 2009), further complicating how genetic diversity in host plants affects community-wide properties and ecosystem processes.

To understand how endophyte infection, plant genotype, and resource availability affect community-level properties of plant-associated arthropods over the ontogeny of the host, we performed a four-year field experiment using clones of four grass genotypes of Arizona fescue, a native and dominant perennial grass in the southwestern United States, from whence the asexual endophyte, *Neotyphodium*, was experimentally removed from one-half of the genets. In addition to controlling plant genotypic diversity, we also controlled soil moisture, a key environmental factor that is usually the limiting resource in these semiarid habitats. To reduce the complexity of the experiment, we used maternal plant genotypes that harbored the same endophyte haplotype. Thus, we examined the contribution of the vertically transmitted endophyte to genetic and

phenotypic diversity of the host in the broad sense (infected or not) and did not include genotypic diversity from variation within the endophyte itself. We then tested the effect of host phenotypic variation caused by the endophyte by determining changes in abundance, biomass, diversity, and trophic structure (herbivores, predators, parasites, omnivores, and detritivores) of the associated arthropod community over three growing seasons.

To our knowledge, there are no previously published studies of how phenotypic variation caused by maternally inherited endophyte infections affects arthropod abundances, diversity, and trophic structure of native grass communities, where plant genotypes, arthropod communities, and environmental factors are much more variable than in introduced, agronomic systems (Faeth and Saikkonen 2007, Cheplick and Faeth 2009). Likewise, we know of no studies of the effects of endophytes on arthropod communities in which plant genotype and environmental factors are simultaneously controlled. Furthermore, since *Neotyphodium* usually inhabits perennial grass hosts (e.g., Clay 1998), it is important to understand how endophyte effects on arthropod abundances, diversity, and trophic structure change over the ontogeny of long-lived grass hosts.

Methods

Experimental design

To test the effects of infection, plant genotype, and varying levels of herbivory and water on associated arthropod trophic and community structure, we designed a long-term, full factorial field experiment using native (wild) Arizona fescue (*Festuca arizonica* Vasey) and its *Neotyphodium* endophyte. Arizona fescue is a widespread, native perennial bunchgrass in semiarid, ponderosa pine–grassland communities above 2000 m elevation in the southwestern United States and reproduces by seed (Kearney and Peebles 1960). *Neotyphodium* infection frequencies are variable but usually high (50–100%) within and among natural populations (Schulthess and Faeth 1998).

Four infected (E+) maternal plants of Arizona fescue from Merritt Draw, a meadow on the Mogollon Rim (elevation 2500 m), Arizona, USA, were randomly selected in 2002 from a pool of ~50 wild E+ plants in the population. Because selected individuals were at least 10 m apart and Arizona fescue outcrosses and reproduces by seed, these individuals are genetically distinct plant genotypes, or minimally, sibs. Infection was determined initially by a modified tissue print immunoassay and later confirmed by staining seeds from each plant after each growing season and examining them for the presence of *Neotyphodium* hyphae (Faeth and Sullivan 2003).

The four plant genotypes harbored the same non-hybrid haplotype of *Neotyphodium* based upon four microsatellite DNA loci and β-tubulin intron sequence (Sullivan and Faeth 2004). Because species designation is unknown (Sullivan and Faeth 2004), we refer to the endophyte as simply *Neotyphodium*.

The E+ maternal plants were split into ramets and treated hydroponically with low levels of the fungicide propiconazole to remove the endophyte (Faeth and Sullivan 2003). Fungicide treatment removes *Neotyphodium* from ~50% of ramets (hereafter, E−); the remaining E+ ramets of one plant genotype were used as fungicide-treated, but infected controls (hereafter, E + F) such that any spurious effects of the fungicide could be tested. Other ramets were treated hydroponically but without fungicide and thus remained E+. After hydroponic treatment, all ramets were planted individually into ~0.5-L cups with native soil and were continually split and repotted as they grew for approximately one year in the greenhouse to provide cloned replicates before transplanting to the field.

A plot at The Arboretum of Flagstaff, Flagstaff, Arizona, USA, was prepared by disking in May 2003 to remove existing vegetation. The original plot was in a natural and previously undisturbed semiarid, ponderosa pine–grassland habitat that harbored native plant species, mostly grasses dominated by Arizona fescue. The entire plot was covered with a weed barrier (Dalen Products, Knoxville, Tennessee, USA) that prevents growth of unwanted plants but is pervious to water and nutrients. The weed barrier was then covered in a layer of pine bark chips to ameliorate any temperature changes caused by the weed barrier. The Arboretum is fenced to prevent disturbance from large vertebrates (elk) but is freely accessible to small vertebrates.

The E+ and E− clones of the four maternal plant genotypes, plus the E + F fungicide controls, were randomly assigned positions in the plot and planted 2 m apart in the summer of 2003 into holes cut into the weed barrier. The water treatments were randomly assigned and initiated in the summer of 2004, after the plants had established in the plot and any dead clones had been replaced. This procedure, repotting and growth in native soils in the greenhouse for one year and then growth in the field for one year before experimental treatments began, allowed time for experimental plants to reestablish mycorrhizal infections that were removed during hydroponic growth and fungicide treatment. The three water treatments were ambient water (normal precipitation), supplemented water (drip irrigation, 8 $L \cdot d^{-1} \cdot plant^{-1}$), and reduced water (plastic barriers underneath the weed barrier with inverted funnels to allow soil gas exchange). Soil moisture probes (Theta Probe Soil Moisture Sensor, Dynamax, Houston, Texas, USA) were used at four different time periods for each plant in 2005 to determine whether soil moisture treatments were effective. Repeated-measures ANOVA and Tukey hsd tests were used to test for differences in soil moisture among the three treatments. All E+, E−, and E + F in the water treatments were replicated five times for a total of 135 plants.

Arthropod sampling, identification, and biomass estimates

Arthropods were sampled in May and August 2005, May 2006, and May 2007 from plants with an Insect Vortis Vacuum sampler (Burkhard, Uxbridge, UK). May is the peak of arthropod abundance on the plants. To standardize sampling, a uniform volume of each plant was suctioned for 10 s. This volume (1750 cm^3) was determined by the length and width of the vacuum sampler aperture. Thus, each collection represents a uniform volumetric sample of the arthropod

community from individual plants. Arthropods were preserved in 70% ethyl alcohol (ETOH) and identified to at least family (except for Acari, the mites), and most to genus level (Appendix A) and then each taxon to morphospecies based upon key characteristics for each family or genus. All taxa were assigned to functional guild (herbivore, predator, parasite, omnivore, detritivore) based upon family or genus description. Because mites (Acari) may be omnivores, herbivores, or predators depending upon individual species traits, we excluded mites from analyses of trophic structure. Biomass of arthropods was estimated from length measurements based upon published equations for each taxon (e.g., Sabo et al. 2002).

Alkaloid determination

Levels of the fungal alkaloid peramine, the only alkaloid type found in Arizona fescue, were determined by L. P. Bush, University of Kentucky, Lexington, Kentucky, USA, from leaf tissue samples from each plant removed in 2005 via previously published methods (Faeth et al. 2002).

Statistical analyses: arthropod abundance and biomass

Because the plants were sampled repeatedly four times over the three growing seasons, we used repeated-measures (RM) ANCOVA (Systat 10.0; SPSS, Chicago, Illinois, USA) for statistical analyses of abundances. The RM ANCOVA was first used to test for any spurious effects of the fungicide treatment by comparing E + F and E+ grasses for the same plant genotype for any differences in total abundance and biomass and abundance and biomass of various feeding guilds over the four sampling periods. Vegetative dry biomass was used as a covariate since plant size may influence arthropod abundances (see Faeth 2009). We determined aboveground biomass by hand-cutting, drying, and weighing aboveground biomass from all individual plants at the end of the 2005, 2006, and 2007 growing seasons (Faeth 2009). All assumptions of ANCOVA (homogeneity of variances and normality of residuals) were tested and met.

The RM ANOVA was then used to test for the effects of endophyte infection, plant maternal genotype, and water treatments and their interactions on abundance and biomass of various groups of arthropods, with plant biomass as a covariate as above. We also tested effects of infection and water treatments for differences in dominant herbivores and other functional guilds. We could not use RM ANCOVA to test for differences in herbivore:natural enemy (predators plus parasites) ratios among treatments because of insufficient numbers of plants across all treatments that had at least some herbivores and natural enemies in each of the four sampling periods. Therefore, we used a separate ANCOVA for each sampling period to test the effect of infection, plant genotype, and water treatment on herbivore:natural enemy ratios.

Ascertaining differences in diversity is more problematic since diversity is a function of both species richness and evenness (Gotelli and Colwell 2001). Evenness is an often-overlooked, but important, component of diversity (e.g., Ma 2005). Comparing differences in richness among treatments with ANOVA is invalid because species richness depends upon the number of sampled individuals (Gotelli and Colwell 2001) and individual plants harbored widely different

numbers of arthropod individuals. Therefore we used rarefaction (Gotelli and Entsminger 2007), which uses probability theory to derive expectations and means, variances, and 95% confidence intervals for richness and evenness. For rarefaction analyses, we used number of species for richness and Hulbert's PIE (probability of interspecific encounter) for evenness of arthropods sampled from E+ and E− grasses. Whereas there are other measures of diversity and evenness (e.g., Smith and Wilson 2001), each with their advantages and disadvantages, these two are commonly used. In some cases, differences in richness and evenness for some sampling periods and feeding guilds could not be compared via rarefaction because of relatively low numbers of species and individuals.

We considered arthropods sampled from all E+ grasses as collectively one community and those collected from E− grasses as another community for each sampling period for comparisons. The larger community (always E+ grasses) was rarefied (1000 iterations) to the number of individuals in the smaller community (always the E− grasses) to generate an expected 95% confidence interval. We then determined whether the observed species richness or PIE of the smaller community (E− plants) was greater or less than the 95% confidence interval (Gotelli and Entsminger 2007). If the observed species richness or PIE of the smaller (E− plants) community fell within the expected 95% confidence interval, then the E+ and E− community were not considered different (Gotelli and Entsminger 2007). If the observed species richness or evenness fell below the 95% confidence interval, then the smaller community was considered less rich or even; if above the interval, then more rich or even (Gotelli and Entsminger 2007). We also used similar rarefactions to test for differences in richness and evenness when plants were grouped separately by maternal plant genotype and by water treatment to ascertain whether species richness and evenness varied by these factors.

To gain a larger picture of how arthropod communities changed due to infection and plant genotype across years, we ordinated samples via nonmetric multidimensional scaling (NMDS). In our analysis, we examined how year, genotype, and infection status influenced arthropod abundances and composition. We performed three- and two-dimensional NMDS, with stress of 0.07 and 0.05, respectively. Clarke and Warwick (2001:6) noted that "… a stress of < 0.1 corresponds to a good ordination with no real prospect of misleading interpretation; and a 3- or higher dimensional solution will not add any additional information about the overall structure." Therefore, we present only the results of the two-dimensional NMDS ordination.

Nonmetric multidimensional scaling provides a visual representation of similarity of samples in a small number of dimensions but does not provide a statistical test of differences in similarity between community samples. Therefore, we also used ANOSIM (Community Analysis Package 4.0; Pisces Conservation, New Milton, Hants, UK) to test for differences among genotypes, years, and by infection status. ANOSIM uses randomization tests (here 1000 permutations) of the Bray-Curtis measure of similarity to determine whether two groups are significantly different in similarity of community composition Clarke (1993). We first tested maternal genotype groups

nested within years and then infection status nested within genotype over all years. Finally, we tested for differences in similarity by year, genotype, and infection status separately.

Results

Treatment efficacy

Supplemented water significantly increased soil moisture relative to the other two treatments. Soil moisture in the ambient soil moisture treatment was greater than in the reduced water treatment early in the season but not different later in the season. Details of the differences among the water treatments are reported in Faeth (2009).

Alkaloids

The E+ plants averaged 11.05 ± 0.40 ppm (mean ± SE) peramine alkaloid levels, the only alkaloid found in infected Arizona fescue. Plants with their endophytes removed produced no peramine, as expected. Levels of alkaloid peramine did not vary among plant genotypes ($F_{3,86}$ = 1.34, P = 0.27), by soil moisture ($F_{2,86}$ = 0.50, P = 0.60), or by the interaction of these factors (P > 0.20). The E + F plants (fungicide-treated controls) did not differ from their E+ plant genotypic counterparts ($F_{1,40}$ = 0.00, P = 0.98).

Arthropod abundances and biomass

A total of 16650, 3423, 17782, and 3230 arthropods were collected and identified in May and August 2005, May 2006, and May 2007, respectively. The difference in total abundance among years is likely related to regional variation in precipitation. The year 2005 was a relatively wet year interposed among 13 years of drought (Faeth 2009). An RM ANCOVA showed that E + F control plants did not differ from E+ plants for abundance or biomass of arthropods collectively or for any group of arthropods (P > 0.13 for all tests), indicating no spurious effects of fungicide treatment on arthropod abundances.

Mean total arthropod abundances on E+ plants were higher than on E− plants over the four sampling periods (Table 1, Fig. 1). These differences were particularly evident in the first three sampling periods, during which abundances on E+ plants were more than twice that of E− plants. Likewise, total arthropod biomass was greater on E+ than E− plants (Table 2) and showed the same trends over time (data not shown) as arthropod abundances. Neither plant genotype nor water treatments affected total arthropod abundances (Table 1) but total arthropod biomass varied by plant genotype (Table 2). There were no significant two- or three-way interactions among infection status, plant maternal genotype, and water for total arthropod abundances (Tables 1 and 2). The only significant two- or three-way interaction for total arthropod biomass was the interaction between infection status and plant genotype, indicating that total arthropod biomass did not respond in the same way to all plant maternal genotype and infection combinations. All four plant genotypes showed similar trends of increased abundances on E+

plants over time, but one genotype (E) had increased abundances only in the first sampling period (see Appendix B).

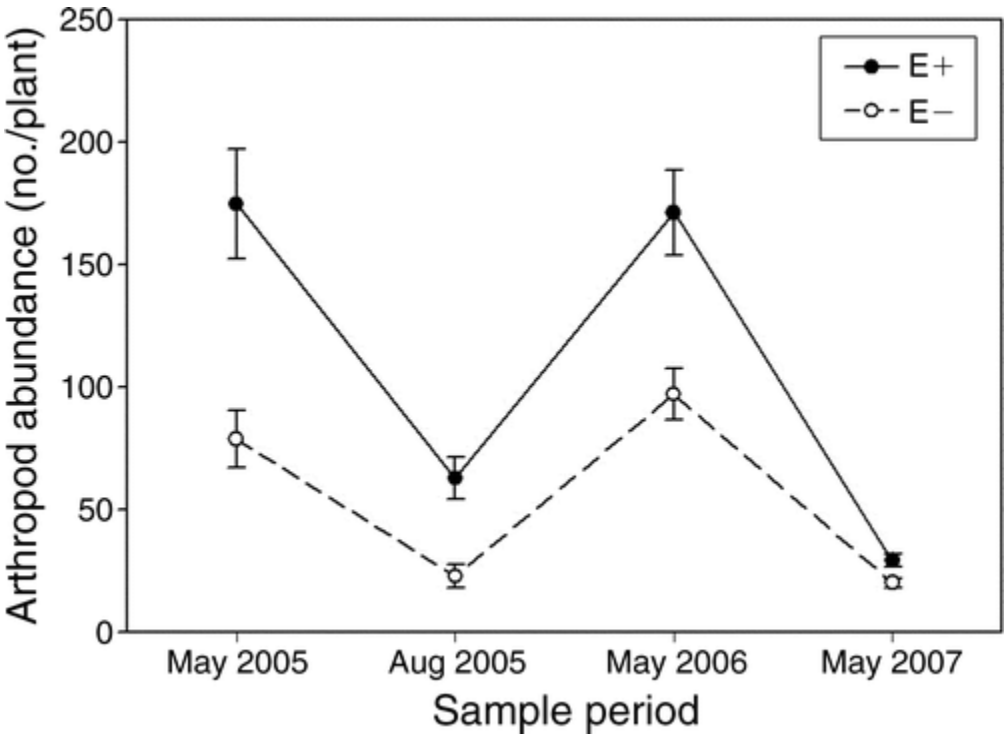

Fig. 1. Total arthropod individuals per unit volume (1750 cm^3) (mean ± SE) on *Neotyphodium* endophyte-infected (E+) and uninfected (E−) native grass (*Festuca arizonica*) over the four sampling periods. The study was conducted at The Arboretum of Flagstaff, Flagstaff, Arizona, USA.

Table 1. Summary of P values from repeated-measures ANOVAs of the effects of endophyte infection, maternal plant genotype, and water treatments and their interactions on arthropod abundances, with plant biomass as the covariate.

Effect	df	Total arthropods	Herbivores	Predators	Parasites	Natural enemies	Omnivores	Detritivores
Between subjects								
Endophyte (E)	1, 90	**<0.001**	**<0.001**	**0.02**	0.80	0.34	0.70	**0.06**
Plant genotype (G)	3, 90	0.24	0.27	0.63	0.46	0.42	**<0.01**	0.47
Water (W)	2, 90	0.46	0.80	0.03	0.75	0.37	0.88	**<0.001**
E x G	3, 90	0.32	0.52	0.84	0.93	0.96	0.22	0.23
E x W	2, 90	0.24	0.35	0.52	0.34	0.45	**0.02**	0.16

G x W	6, 90	0.20	0.18	**0.04**	0.16	**0.08**	0.28	0.99
E x G x W	6, 90	0.60	0.92	0.85	0.91	0.81	0.24	0.34
Within subjects								
Time (T)	3, 270	<**0.001**	<**0.001**	0.12	0.26	0.07	0.77	0.34
T x E	3, 270	<**0.003**	<**0.001**	0.11	**0.05**	**0.01**	**0.07**	0.65
T x G	9, 270	0.67	0.35	0.66	0.43	0.68	0.19	0.50
T x W	6, 270	0.69	0.56	0.58	0.70	0.64	0.95	<**0.001**
T x E x G	9, 270	0.76	0.88	0.74	0.21	0.40	0.47	0.65
T x E x W	6, 270	0.34	0.11	0.65	0.14	0.42	0.50	0.56
T x G x W	18, 270	0.70	0.67	0.69	0.81	0.76	0.90	0.98
T x E x G x W	18, 270	0.54	0.30	0.54	0.44	0.24	0.99	0.92

Notes: Significant ($P < 0.05$) or marginally significant ($0.05 < P < 0.10$) values appear in boldface. The study was conducted at The Arboretum of Flagstaff, Flagstaff, Arizona, USA.

Table 2. Summary of P values from repeated-measures ANOVAs of the effects of endophyte infection, maternal plant genotype, and water treatments and their interactions on arthropod biomass, with plant biomass as the covariate

Effect	df	Total arthropods	Herbivores	Predators	Parasites	Natural enemies	Omnivores	Detritivores
Between subjects								
Endophyte (E)	1, 90	<**0.001**	<**0.001**	**0.06**	0.30	**0.09**	0.58	**0.05**
Plant genotype (G)	3, 90	0.01	0.06	0.12	0.99	0.13	<**0.001**	0.25
Water (W)	2, 90	0.87	0.22	0.19	0.35	0.17	0.76	<**0.001**
E x G	3, 90	**0.07**	0.33	0.24	0.19	0.16	0.15	0.21
E x W	2, 90	0.73	0.88	0.14	0.70	0.12	0.11	0.14
G x W	6, 90	0.13	0.25	0.01	0.40	<**0.01**	0.90	0.97
E x G x W	6, 90	0.75	0.89	0.56	0.58	0.50	0.76	0.27
Within subjects								
Time (T)	3, 270	.24	0.11	0.32	0.38	0.30	0.57	0.48

T x E	3, 270	**0.002**	<**0.01**	**0.08**	**0.02**	**0.05**	0.51	0.62
T x G	9, 270	0.48	0.62	0.42	**0.09**	0.40	<**0.01**	0.51
T 3 W	6, 270	0.94	0.64	0.40	0.80	0.35	0.79	<**0.001**
T x E x G	9, 270	0.76	0.88	0.37	0.51	0.38	0.39	0.66
T x E x W	6, 270	0.92	0.97	0.41	0.33	0.37	0.79	0.52
T x G x W	18, 270	0.71	0.56	0.51	**0.06**	0.78	0.99	0.99
T x E 3 G x W	18, 270	0.99	0.99	0.29	0.85	0.39	0.54	0.96

Note: Significant (P < 0.05) or marginally significant (0.05 < P , 0.10) values are in boldface.

Herbivorous insects followed the same pattern as total arthropods (Table 1), with greater numbers of herbivores on E+ than E− plants (Fig. 2A). Likewise, biomass of herbivorous insects was greater on E+ plants than E− plants (Table 2) and biomass but not abundances (Table 1) varied by plant genotype. Both total arthropod and herbivore abundances also varied by infection status over time (significant time × endophyte interaction; Tables 1 and 2). The abundance of dominant herbivore families also varied by endophyte infection. The E+ grasses had significantly greater numbers ($F_{1,90}$ = 13.93, P < 0.001) and biomass ($F_{1,90}$ = 11.54, P = 0.001) of thrips (Thysanoptera). Likewise, the E+ plants had marginally greater numbers ($F_{1,90}$ = 3.74, P = 0.06) and significantly more biomass ($F_{1,90}$ = 5.06, P = 0.03) of planthoppers (Delphacidae) than the E− grasses. Like total arthropod abundances, water treatments did not affect overall herbivore abundance or biomass, and there were no significant two- or three-way interactions among infection status, plant maternal genotype, and water (Tables 1 and 2).

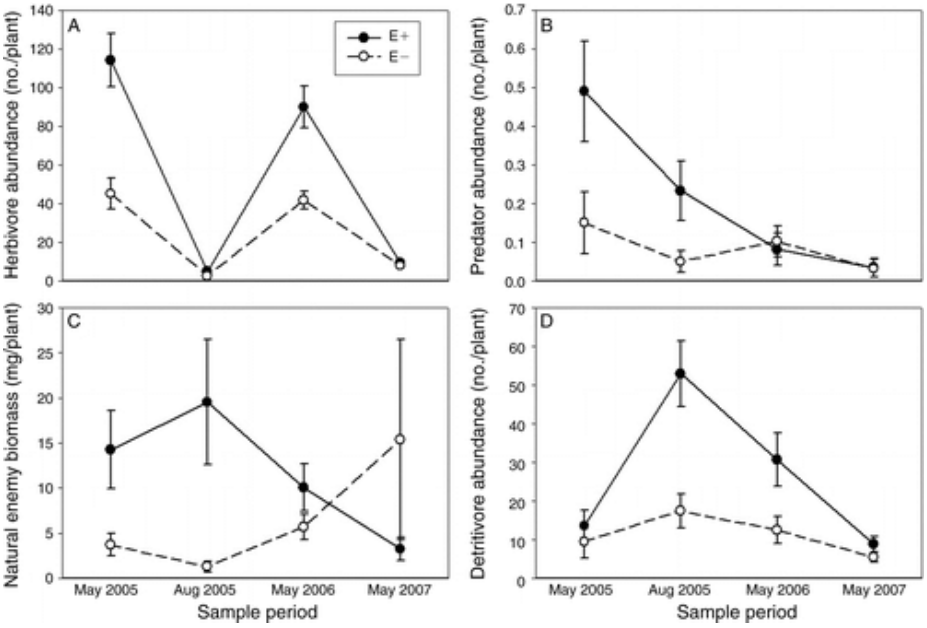

Fig. 2. Number of (A) herbivore and (B) predator individuals, (C) biomass of natural enemies, and (D) number of detritivore individuals on endophyte-infected (E+) and endophyte-uninfected (E−) grasses over the four sampling periods. Values are means ± SE.

The number (Table 1, Fig. 2B) and biomass (Table 2) of predators was greater on the E+ plants than the E− plants. This difference was driven by greater numbers on the E+ plants in the first two sampling periods (Fig. 2B). Water treatments also affected the number (Table 1) but not biomass of predators, with more predators on the plants with supplemented water than the other two water treatments in the first two sampling periods (see Appendix C). Unlike predators, numbers and biomass of parasites were not different on E+ and E− plants (Tables 1 and 2). When predators and parasites are considered collectively as natural enemies, biomass (Table 2, Fig. 2C), but not number (Table 1), of natural enemies was greater on E+ plants than E− plants.

The ratio of herbivore to natural enemies (predators and parasites collectively) was significantly greater on E+ plants than E− plants in May 2006 ($F_{1,58} = 9.66$, $P < 0.01$) but not in other sampling periods (see Appendix D). Thus, at least in May 2006, E+ plants harbored proportionately fewer natural enemies per herbivore than E− plants.

Neither abundance nor biomass of omnivorous insects varied by infection status (Tables 1 and 2). However, both abundance and biomass varied by plant maternal genotype. The abundance (Table 1, Fig. 2D) and biomass (Table 2) of detritivorous arthropods was greater on E+ than on E− plants. Most of the detritivores were springtails (Collembola), and both abundance ($F_{1,89} = 3.55$, $P = 0.06$) and biomass ($F_{1,90} = 3.68$, $P = 0.05$) of springtails were greater on E+ plants. As expected, the abundances and biomass of detritivores varied with water treatment (Tables 1 and 2), with the supplemental water treatment supporting the greatest abundances of detritivores (see Appendix C).

Arthropod richness

Rarefaction of the arthropod community on the E+ and E− plants collectively showed that for total arthropod species richness, the E+ and the E− plants were equivalent (Table 3), except in May 2006 when E+ plants had lower richness than the E− plants. Herbivore richness was equivalent in all sampling periods. For parasite species richness, the E+ plants had significantly fewer species than the E− plant in three of four sampling periods, while predator species richness, with lower richness overall, varied by year and E+ and E− plants. When parasites and predators were grouped into natural enemies, the results mirrored those for parasites: lower richness on E+ plants in three of four sampling periods, undoubtedly because parasites comprised the bulk of both species numbers and abundances of natural enemies. Detritivore species richness was greater on the E+ than on the E− plants in two sampling periods and equivalent in the other two.

Table 3. Results of rarefaction analyses of total arthropod species richness and individual feeding guild richness

	May 2005						August 2005					
Guild	E+	n(E+)	E-	n(E-)	Significance	95% CI	E+	n(E+)	E-	n(E-)	Significance	95% CI
Total species	73	12 128	59	4660	E+= E-	47–60	45	2373	31	1068	E+= E-	27–37
Herbivore species	30	8096	26	2708	E+= E-	21–27	14	612	11	253	E+= E	8–13
Parasite species	19	112	15	41	**E+< E-**	8–14	12	44	9	19	**E+ < E-**	4–8
Predator species	6	246	3	167	E+ < E-	2–5	5	4		ISS		
Natural enemy species	27	142	20	48	**E+< E**	11–18	17	59	11	22	E+= E-	6–12
Omnivore species	6	19	6	10	E+= E-	4–6	5		2		ISS	
Detritivore species	12	1223	9	576	E+= E-	8–12	9	1175	7	569	**E+< E-**	8–9

Notes: Inequalities (in boldface) indicate that E+ (endophyte-infected) differed (based upon 95% CI) from E- (endophyte uninfected) after rarefaction of the larger community (based upon number of individuals sampled, n) to that of the smaller community. In all cases, the number of individuals sampled from the E+ plant community was larger than that sampled from the E- plant community. Thus, for all cases, the number of observed species for E- plants was compared to the 95% CI generated by rarefaction of the E+ community. Plots of significant rarefaction curves (in boldface) may be found in Appendix E. The abbreviation ISS indicates insufficient sample size for meaningful rarefaction analysis.

When plants were grouped by maternal plant genotype (each maternal genotype tested against remaining community as a whole, with the whole community rarefied to smaller, single genotype community), there were no significant differences in species richness or evenness of total arthropods or any arthropod feeding guild. Likewise, there were no significant differences in species richness or evenness when plants were grouped by water treatments (each water treatment tested against the remaining two water treatments combined, with the whole community rarefied to the smaller, single water treatment community). Rarefaction curves of significant relationships are found in Appendix E.

Arthropod evenness

Evenness, or how individuals are distributed among species in a community, of total arthropods varied by infection status and changed throughout the course of the study. On young plants, arthropod communities on the E+ plants collectively were less even than those on the E− plants

collectively (Fig. 3A). However, as plants matured, evenness became consistently greater on E− plants.

Evenness of insect herbivore communities varied even more so than the total arthropod community (Fig. 3B). Initially, insect herbivores were more evenly distributed on the E+ plants, equivalent in August 2005, then less even in May 2006. However, in May 2007, insect herbivores on the E+ plants were again more evenly distributed than on the E− plants.

Natural enemy evenness showed more consistency than herbivore evenness (Fig. 3C). Natural enemies were always less even on the E+ than the E− plants throughout the ontogeny of the host and the duration of the experiment (Fig. 3C).

When plants were grouped by maternal plant genotype (each maternal genotype tested against the remaining community as a whole), there were no significant differences in evenness of total arthropods or any arthropod feeding guild. Likewise, there were no significant differences in evenness when plants were grouped by water treatments.

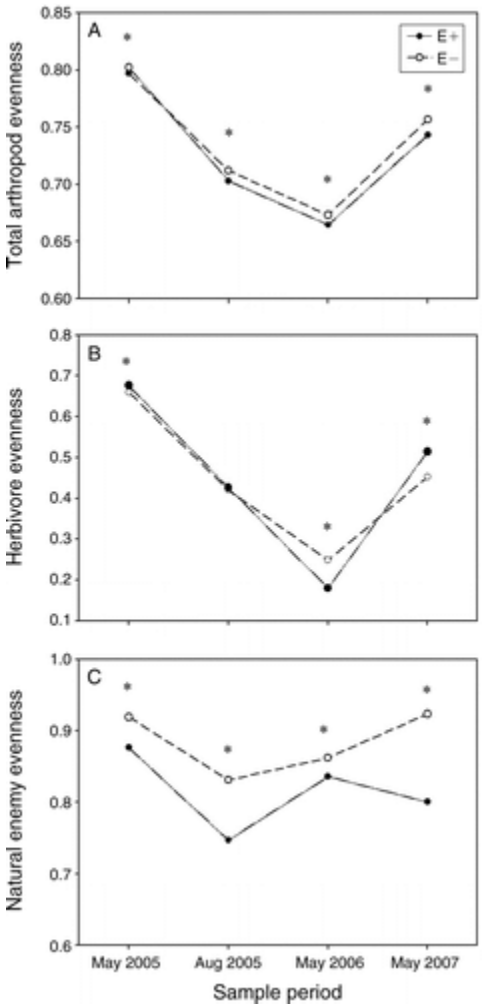

Fig. 3. Evenness of (A) total arthropods, (B) herbivorous insects, and (C) natural enemies (parasites plus predator) from May 2005 to May 2007 on endophyte-infected (E+) and endophyte-uninfected (E−) plants. Asterisks above symbols indicate significant differences (at $P < 0.05$) in each sampling period based upon 95% CIs from rarefaction.

Nonmetric multidimensional scaling and similarity

Fig. 4 illustrates the position of the 24 samples (by year, plant genotype, and infection status) along the first two ordination axes. Samples appear to aggregate by year but not by plant genotype or infection status. The ANOSIM analyses confirm this visual impression. When samples are grouped by genotype within year, year is significant ($P = 0.02$) but genotype is not ($P = 0.13$). When samples are grouped by infection status nested within genotype, neither infection status ($P = 0.97$) nor genotype ($P = 0.14$) are significant. When year, genotype, and infection status are analyzed separately as groups, year is significant ($P = 0.01$) but infection status ($P = 0.32$) and genotype ($P = 0.59$) are not.

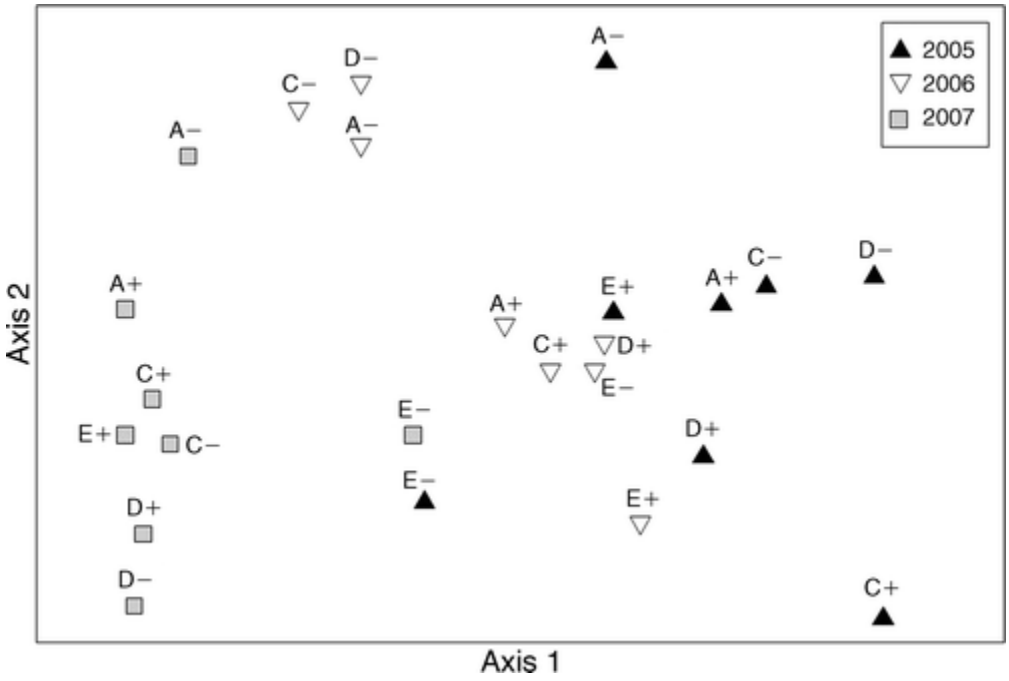

Fig. 4. Ordination diagram of the first two axes of the nonmetric multidimensional scaling analysis for arthropod composition samples by plant maternal genotype, infection status, and year of sampling. Each year is designated by a different symbol. The symbols are labeled by the four maternal plant genotypes (A, C, D, and E) and by infection status (+, infected; −, uninfected).

Discussion

Asexual endophytes, as maternally inherited fungal symbionts of grasses, comprise a tiny fraction of the biomass in the community (Clay and Holah 1999). Yet these inherited symbionts

may have profound and often complex effects on abundances, species richness, and evenness of the associated arthropod community via changes in plant phenotypes. Our results show that the phenotypic variation contributed by the presence of infection appears to greatly affect arthropod abundances but often in ways unexpected by the conventional concept of endophytes as defensive mutualists. In addition, plant maternal genotype and a key environmental factor, soil moisture, also contribute to and interact with endophyte infection to alter abundances of some arthropod groups. Furthermore, endophyte effects on arthropod abundances and diversity are not static but change with the ontogeny of the host plant.

Endophyte-mediated changes in abundances

In terms of abundances, the E+ plants supported higher abundances of arthropods than did the E− plants in early ontogeny, and most of these arthropods were herbivorous insects in terms of number and biomass (Figs. 1 and 2A). Generally, the effects of endophyte infection appear to cascade upward from the host to herbivores to other trophic levels in terms of abundance and biomass. Predator (and natural enemies collectively) and detritivore abundances on the E+ plants were generally higher than on the E− plants during sampling periods when herbivore abundances were also greater. However, there is not a uniform resource-based or bottom-up effect (e.g., Powers 1992) derived from endophyte-mediated changes in the host plant. For example, parasite and omnivore abundances were unaffected by the endophyte, and the effect of infection varied over time for abundances of predators and detritivores.

The general increase in arthropod abundances may be due to more rapid growth and larger size of E+ relative to E− plants during the course of the experiment (Faeth 2009). However, size alone cannot account for the increase in arthropods for two reasons. First, arthropods were sampled on a per unit volume basis rather than entire plants. Second, plant biomass was used as a covariate in all analyses, and thus E+ plants had statistically more arthropod and herbivores per unit volume despite differences in size. Thus, arthropods, and herbivorous insects in particular, were not just more numerous per plant but also denser per unit volume on E+ plants. *Neotyphodium* infection is also known to alter physiological (e.g., Morse et al. 2007), nutritional (e.g., Malinowski et al. 2000), and biochemical (e.g., Rasmussen et al. 2008) aspects of their hosts that affect herbivores. Thus, increases in herbivores may be due to higher nutritional quality of E+ plants relative to E− plants. Infected Arizona fescue also has increased water use efficiency under drought conditions than E− plants (Morse et al. 2007), and thus herbivores may be attracted to E+ plants because of increased water content, especially since the study area is a semiarid grassland that has experienced a prolonged drought. However, herbivore abundances were unaffected by water treatments, so this reason seems less tenable than other host plant changes due to infection.

Whereas it is uncertain whether nutritional, water content, or other factors associated with E+ plants underlie the increase in herbivore abundances in early ontogeny, our results indicate that alkaloids associated with E+ Arizona fescue plants do not deter herbivores, reduce herbivore

abundances, or reduce herbivore species richness. Asexual, systemic endophytes in grasses have been long assumed to act primarily as defensive mutualists by producing alkaloids that deter herbivores (e.g., Clay 1988, 1990, Clay and Schardl 2002). Much of the evidence for this hypothesis is derived from studies of two introduced, agronomic grasses (tall fescue and perennial ryegrass) using short-term bioassays and experiments involving generalist insect pest species (Faeth 2002, Saikkonen et al. 2006, Faeth and Saikkonen 2007). Here, endophyte infection increases herbivore abundances, at least in the first two growing seasons in this native grass.

Our results are in stark contrast to a recent three-year study of the effects of *Neotyphodium* infection in agronomic tall fescue on arthropod abundances and diversity in old-field plots. Rudgers and Clay (2008) found that herbivore abundances were reduced up to 70% and species richness reduced 20% in E+ vs. E− plots. In Rudgers and Clay's (2008) study, plant genotype was not controlled but rather homogenized among the E+ and E− plots, arthropods were sampled per plot (rather than by per plant as we did here), arthropod biomass was not determined, and plant resources were not controlled or manipulated. Here, we show that plant genotype also affected arthropod, herbivore, and omnivore biomass, as well as omnivore abundances and that plant genotype may interact with endophyte infection to alter arthropod biomass. Because we used only four plant genotypes and natural populations are typically comprised of many different plant genotypes, we cannot say how plant genotypic diversity may affect arthropod abundances in large populations. Previous studies have shown that plant genotype alone can dictate the structure and abundances of arthropod communities (e.g., Crutsinger et al. 2006). Nonetheless, our results suggest that endophyte infection plays an important role in determining arthropod abundances and likely interacts with plant genotype to influence plant-associated arthropod communities.

Our results also indicate that the local environmental factors also interact with plant genotype and endophyte infection to affect arthropod abundances. Greater soil moisture availability increased the abundances of predators and abundances and biomass of detritivores. Also, soil moisture interacted with plant genotype to affect predator abundances and biomass and with infection status to affect omnivore abundances. Our study occurred in semiarid grasslands where precipitation is relatively low and temperatures are relatively high during the growing season (e.g., Sullivan and Faeth 2004). Predators may be more sensitive to stressful environmental conditions than their herbivore prey, which derive metabolic water and temperature amelioration from their host plants (e.g., Menge and Sutherland 1987). Thus, predators may increase on plants in the supplemented water treatment because of the increased availability of free water from irrigation, increased local humidity, or reduced temperatures. Likewise, detritivores likely increased on plants with supplemented water because they feed on dead plant material and live in or near the soil and typically respond positively to soil moisture (e.g., Staley et al. 2007). It is interesting to note that detritivores, as consumers of dead plant material, which is critical to decomposition and nutrient recycling in communities, also increased in abundance and biomass

on the E+ plants. Previous studies with agronomic grasses indicate that endophyte infection often reduces decomposition rates and alters diversity and abundances of detritivores, presumably because of alkaloids present in grass litter (e.g., Omacini et al. 2004, Lemons et al. 2005). We controlled only one of many environmental factors that may affect plant phenotype variation. Other environmental factors, such as soil nitrogen, may also affect key host plants traits, such as alkaloid levels (e.g., Cheplick and Faeth 2009). Therefore, because of these limits in our experiment, caution is necessary in extrapolating our results.

One explanation for the differences in our results from those of Rudgers and Clay (2008) and others (e.g., Omacini et al. 2001) showing increased herbivore resistance in agronomic grasses is that alkaloids in Arizona fescue are not of the type or level that deter or reduce performance of herbivores and thus reduced their abundances. Although Rudgers and Clay (2008) did not measure alkaloids, E+ tall fescue (Kentucky 31 variety) is known for having high levels of multiple alkaloids that deter and reduce performance of agronomic, generalist insect pests (e.g., Leuchtmann et al. 2000). Generally, previous studies with agronomic tall fescue (e.g., Clay 2001, Finkes et al. 2006), perennial ryegrass (e.g., Meister et al. 2006, Härri 2007), and Italian ryegrass (Omacini et al. 2001) show reduced herbivore abundances on E+ grasses relative to E− grasses, and all three infected agronomic grasses produce relatively high levels of alkaloids (e.g., Leuchtmann et al. 2000).

The E+ Arizona fescue plants produce the alkaloid peramine, a known chemical deterrent to herbivorous insects (e.g., Siegel and Bush 1996). Indeed, peramine levels far exceeded 3 ppm, the minimal concentration known to deter or reduce survival of generalist sucking or chewing insects in bioassays with infected agronomic grasses (Siegel and Bush 1996), and levels in Arizona fescue exceed those typically found in E+ tall fescue (e.g., Leuchtmann et al. 2000). Tall fescue does produce high levels of loline alkaloids that Arizona fescue does not, and this may account for reduction in herbivore abundances in tall fescue in the Rudgers and Clay (2008) study. Our results corroborate previous bioassay studies showing that native insect herbivores prefer, consume more tissue of, and survive better on E+ relative to E− Arizona fescue plants (e.g., Saikkonen et al. 1999). Furthermore, in a recent study examining herbivore abundances on a native grass, *Achnatherum robustum*, Jani et al. (2009) found that not only were herbivore abundances greater on E+ plants relative to E− plants, but herbivore abundances were positively correlated with ergot alkaloid content of infected plants. Infected *A. robustum* produces extraordinarily high levels of ergot alkaloids, far higher than those found in any of the aforementioned introduced, agronomic grasses (Faeth et al. 2006).

Whereas there are relatively few community-level studies with infected agronomic grasses and still fewer with native grasses, these dramatic differences in results suggest that explanations other than levels or types of alkaloids dictate differences between the two. One explanation is that insect herbivores in natural communities are more specialized and can detoxify alkaloids and perhaps even require alkaloids as oviposition cues and nutritional components or sequester them in their own defenses against natural enemies (e.g., Faeth 2002, Cheplick and Faeth

2009, Hartley and Gange 2009). Generalist insect herbivores are usually more susceptible to plant and endophytic-associated allelochemicals than specialists (e.g., Faeth 2002, Hartley and Gange 2009) and many previous tests of the defensive mutualism hypothesis have involved generalized, agricultural pest species and introduced, agronomic grass hosts (e.g., Faeth 2002). Determining whether the many herbivores species collected in our study (see Appendix A) were generalists or specialists was beyond the scope of this study. However, if the insect herbivores collected in our study are representative of other insect communities associated with native plant communities (e.g., Fritz and Simms 1992) and particularly native grassland insect communities (e.g., Hollier et al. 2005), we would expect a large fraction to be comprised of specialist species. It would be enlightening for future studies of the effects of endophytes at the community level to examine relative effects of endophyte-associated alkaloids on generalist and specialist herbivorous insects.

There is another explanation for the lack of increased host resistance to herbivores that is contrary to the defensive mutualism hypothesis. Faeth and Sullivan (2003) proposed that asexual endophytes such as *Neotyphodium* are reproductive parasites and control host reproduction to increase their own fitness at the expense of their host. In a companion paper in which growth and reproductive effort of Arizona fescue was measured over several years, Faeth (2009) showed that infection promoted early flowering and increased allocation to reproduction in early ontogeny of the host. Furthermore, infected plants with higher herbivore loads allocated more to seed production than uninfected hosts in early ontogeny. Because asexual endophytes can be lost from perennial hosts as they mature (e.g., Afkhami and Rudgers 2008), increased early production of seeds enhances endophyte transmission and hence fitness of the endophyte, but likely reduces long-term fitness of the host (Faeth 2009). Because herbivory in earlier stages promotes enhanced seed production, endophytes in perennial native grasses may have evolved to promote, rather than deter herbivores, if herbivory increases seed production and transmission success and if asexual endophytes control reproductive allocation in hosts (Faeth 2009).

Certainly there are other differences between agronomic and native grass systems and grass species and habitats within each system that could affect the outcome of endophyte effects on arthropod abundances (e.g., Faeth and Saikkonen 2007), such as differences in climate, plant diversity, soil nutrients and moisture, and plant and endophyte genotypic diversity. Regardless of which explanation holds for the lack of endophyte-mediated herbivore resistance in Arizona fescue, it is evident that the general effects of infection on arthropod abundances appear different from previous studies and are influenced by interactions with varying plant genotypes and environmental factors.

Endophyte-mediated changes in diversity

Previous studies with agronomic grasses in simplified agro-ecosystems and more complex old fields have shown that endophytes and their alkaloids may alter species richness. Finkes et al. (2006) showed herbivore abundances and richness and predator richness declined in old-field

plots originally seeded with agronomic E+ tall fescue relative to those seeded with E− tall fescue. Likewise, Härri (2007) and Meister et al. (2006) showed decreased herbivore abundances but increased coexistence of two herbivore species on E+ agronomic perennial ryegrass. Omacini et al. (2001) again showed decreased abundances but unchanged richness of herbivores on E+ Italian ryegrass. To our knowledge, our study is the first long-term experimental study of endophyte-mediated effects on arthropod abundances, richness, and evenness in a native grass in a complex natural community. Our results are much different from those conducted on infected agronomic grasses and also show endophyte-mediated effects on diversity over several years of host plant ontogeny.

Generally, total richness and that of the herbivore feeding guild did not differ on the E+ and the E− plants (Table 3), while herbivore abundances increased on the E+ plants. In the one sampling period during which total arthropod richness differed (May 2006), species richness on the E+ plants was less than that on the E− plants, despite higher abundances. Detritivore species richness, like detritivore abundances, was generally higher on E+ plants.

Infection, however, appeared to have more profound effects on diversity of the natural enemy guild. The E+ plants were often lower in species richness (Table 3) and always lower in evenness (Fig. 3A) than the E− plants, even though parasite abundance and biomass were unaffected by infection while predator abundance (Fig. 2B) and biomass increased on E+ plants (Tables 1 and 2). This suggests that infection reduces parasite species richness and shifts dominance hierarchies among parasitic species of herbivores and thus may inhibit upward trophic cascades by decreasing the quality of herbivore hosts and prey for some species of natural enemies (e.g., Omacini et al. 2001, de Sassi et al. 2006).

This change in natural enemy species richness and evenness in E+ plants may also provide another explanation for increased herbivore and possibly detritivore abundances on E+ plants. Recent studies of endophytes (e.g., Omacini et al. 2001, de Sassi et al. 2006, Härri et al. 2008) in more complex arthropod communities indicate that endophytes and their alkaloids may have positive effects on herbivores by negatively affecting their natural enemies (e.g., Hartley and Gange 2009). Alkaloids may be sequestered by herbivores in their own defense or natural enemies may be less tolerant to alkaloids than their prey or hosts (Bultman et al. 1997, Faeth 2002, Müller and Krauss 2005, Cheplick and Faeth 2009). Thus, endophyte infections and their alkaloids may alter top-down effects, and these may flow downward to herbivores by providing "enemy-reduced space" against some natural enemy species (Jani et al. 2009). This hypothesis is further supported by the herbivore:natural enemy ratios, which were greater on the E+ plants relative to the E− plants, at least in May 2006 (see Appendix D). Not only were herbivore abundances increased on the E+ plants (Fig. 2A), but the relative number of natural enemies was also reduced (Appendix D).

Ontogenetic effects of endophytes

Asexual endophytes occur predominantly in long-lived, perennial grasses (e.g., Faeth and Hamilton 2006, Cheplick and Faeth 2009). However, most studies of endophytes and their effects on host grasses and the associated arthropod communities are of short duration, typically one growing season or less (e.g., Faeth and Saikkonen 2007). Although our study encompassed four growing seasons and three years of arthropod sampling, we caution that it does not provide a complete picture given that the life spans of perennial grasses are decadal (Faeth and Hamilton 2006). Furthermore, we caution that background environmental variation, such as changes in precipitation over the course of the study, also likely influenced absolute abundances of arthropods. Nonetheless, our results do indicate that the nature of endophyte–host grass interactions and the consequences for arthropod abundances, diversity, and community structure are dynamic through time. For example, if one examined herbivore abundances on mature plants in 2007, then one would conclude that endophytes in Arizona fescue have no effect on herbivore loads. However, during early ontogeny and rapid growth (2005–2006; see Faeth 2009), endophyte-mediated changes increased herbivore loads or decreased resistance, a very different conclusion. Likewise, there were ontogenic changes in species richness and evenness that vary by respective feeding guild, and NMDS shows that the composite arthropod community varied mainly by sampling year (Fig. 4). Whereas there is growing acknowledgement that endophyte–host interactions are not always mutualistic as once envisioned and depend on host and plant genotype and environmental factors (e.g., Faeth and Sullivan 2003, Hartley and Gange 2009), our results demonstrate that these interactions and their effects on the arthropod community are also highly labile in another dimension: time.

Hughes et al. (2008) emphasized the importance of separating the magnitude of genetic diversity from other factors, such as environmental variation, on community-level attributes in natural populations. In most studies to date, genetic diversity usually refers to genetic variation in plants and its repercussions on community structure and diversity and ecosystem processes (Hughes et al. 2008). Inherited plant symbionts also contribute to the genetic diversity of their hosts and effect marked changes in the phenotype of their hosts (e.g., Clay et al. 2005). We show that the phenotypic variation associated with asexual, inherited endophytes also have dramatic effects on arthropod abundances and diversity and interact with variation from plant genotypic diversity and a limiting environmental factor. Maternally inherited endophytes spur community-wide changes that parallel how certain plant genotypes and their extended phenotypes alter community structure and diversity (Whitham et al. 2003, Crutsinger et al. 2006, Hughes et al. 2008). In future studies, it is imperative to include these microbial plant symbionts as part of the genetic variation of the plant that influences community diversity and ecosystem processes.

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APPENDIX A

Arthropod taxa collected from Arizona fescue during the course of the study (*Ecological Archives* E091-092-A1).

APPENDIX B

The interaction of endophyte infection status and the four maternal plant genotypes on total arthropod abundances (*Ecological Archives* E091-092-A2).

APPENDIX C

The effect of soil moisture treatments on the number of predator and detritivore individuals (*Ecological Archives* E091-092-A3).

APPENDIX D

The effect of endophyte infection on the ratio of herbivore to natural enemy individuals (*Ecological Archives* E091-092-A4).

APPENDIX E

Significant rarefaction curves for differences in species richness of total arthropods, parasites, natural enemies (predators and parasites), omnivores, and detritivores on plants infected or

uninfected with the *Neotyphodium* endophyte over the four sampling periods of the study (*Ecological Archives* E091-092-A5).

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