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Understanding context-dependency in plant–microbe symbiosis: The influence of abiotic and biotic contexts on host fitness and the rate of symbiont transmission

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ABSTRACT

Understanding the dynamics of a hereditary symbiosis requires testing how ecological factors alter not only the fitness consequences of the symbiosis, but also the rate of symbiont transmission to the next generation. The relative importance of these two mechanisms remains unresolved because studies have not simultaneously examined how the ecological context of the symbiosis influences both costs/benefits and the rate of vertical transmission. Fungal endophytes in grasses have provided particularly tractable systems for investigating the ecological and evolutionary dynamics of hereditary symbiosis. Here we examine interactions between a fungal endophyte, *Epichloë amarillans*, and its grass host, *Agrostis hyemalis*, under altered abiotic and biotic contexts: a gradient of water availability and in the presence versus absence of soil microbes. We show that benefits of the symbiosis were strongest when water was limiting. Symbiotic plants at the lowest watering level produced ~40% more inflorescences and greater seed mass than non-symbiotic plants, while at the highest watering level, symbiotic and non-symbiotic plants did not significantly differ in reproductive fitness. Benefits appear to accrue by allowing hosts to escape from drought, a response that has not been previously reported to be endophyte-mediated. Symbiotic plants at the lowest watering level flowered 9 days earlier than non-symbiotic plants. Interestingly, our results suggest the symbiosis may be costly in the presence of soil microbes, as on live soil, the biomass of symbiotic plants was lower than the biomass of symbiont-free plants. We detected no effect of either the biotic or abiotic context on the rate of symbiont vertical transmission, suggesting that the context-dependent benefits of the symbiosis are the more important driver of variation in symbiont frequency in this system.

1. Introduction

Nearly all plants form symbiotic relationships with microbes (Petrini, 1986; Fitter and Moyersoen, 1996; Saikkonen et al., 1998). These symbionts can alter the ecology of their hosts, often by enhancing nutrient uptake, increasing tolerance to stress, or providing protection from host enemies (Smith and Read, 1997; Clay and Schardl, 2002; Hartley and Gange, 2009). They can also affect community composition (Clay and Holah, 1999; Hartnett and Wilson, 1999), succession (Janos, 1980; Rudgers et al., 2007), and nutrient cycling (Franzluebbers et al., 1999; van der Heijden et al., 2008). Given their potential for strong ecological impacts, not only on host plants but also on the surrounding community and ecosystem, there is great interest in understanding the factors that influence the persistence of these symbioses.

Theory suggests that the persistence of symbionts in host populations is dependent upon the net effect of the symbiont on host fitness and the mode and rate of symbiont transmission (Gundel et al., 2008). Thus, understanding the dynamics of the symbiosis at the population level requires testing how ecological factors alter not only the fitness consequences of the symbiosis, but also the rate of symbiont transmission. Positive, negative and neutral effects of symbionts on plant fitness are expected to arise from variation in the relative costs and benefits of the interaction under different ecological contexts. Variation in the mode and rate of symbiont transmission is also common, with some symbionts transferred horizontally through contagious spread and others transferred vertically from parent to offspring (Bright and Bulgheresi, 2010).

Fungal endophytes in grasses have provided particularly tractable systems for investigating the ecological and evolutionary dynamics of symbiosis. Fungal endophytes occur commonly, can be easily manipulated, and are not obligate for the plant; thus allowing for comparisons of symbiotic and symbiont-free hosts. Within the grass family, Poaceae, approximately 20–30% of species host systemic class 1 endophytic fungi in the fungal family Clavicipitaceae (Leuchtmann, 1992). These symbionts are often vertically transmit-

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ted through seeds of the host plant and can increase host growth and reproduction by enhancing host resistance to both biotic and abiotic stress (Cheplick and Faeth, 2009).

It remains unclear what factors contribute to the context-dependency and long-term persistence of grass–endophyte symbioses. Across host species, there exists substantial variation in the frequency of endophyte symbiosis, both within and among populations (Rudgers et al., 2009), suggesting that endophytes may not be universally beneficial and/or may have low rates of transmission across generations. Experimental evidence generally supports the hypothesis that the outcome of grass endophyte symbioses spans a continuum from parasitism to mutualism, with outcomes dependent upon the biotic and abiotic context (Cheplick et al., 1989; Schardl et al., 2004; Saikkonen et al., 2004; Muller and Krauss, 2005). For example, in tall fescue grass (*Lolium arundinaceum*) the endophyte symbiosis can enhance herbivore resistance (Rudgers and Clay, 2007), competitive ability (Clay et al., 1993) and drought tolerance (Elmi and West, 1995), but can also reduce host biomass under nutrient poor conditions (Cheplick et al., 1989). These context-dependent benefits may ultimately underlie the observed variation in endophyte frequency or persistence. For instance, one study has shown that increased herbivore pressure can drive increases in endophyte frequency (Clay et al., 2005). However, observational evidence suggests that imperfect vertical transmission of symbionts is also common (Afkhami and Rudgers, 2008), and could provide an alternative explanation for variation in symbiont frequency. The relative importance of these two mechanisms remains unresolved because studies have not simultaneously examined both the costs/benefits of the symbiosis and the rates of vertical transmission under altered abiotic or biotic contexts. This is an important step for understanding the degree to which intrinsic dynamics vs. factors extrinsic to the symbiosis influence symbiont frequencies in natural populations.

Historically, endophyte symbioses have primarily been recognized for benefitting host plants through increased resistance to herbivores (Clay, 1996; Bush et al., 1997; Clay and Schardl, 2002). However, research has also shown that endophytic fungi can increase host competitive ability (Clay et al., 1993), drought tolerance (Elmi and West, 1995; Malinowski and Belesky, 2000; Kannadan and Rudgers, 2008), pathogen resistance (Gwinn and Gavin, 1992; Mahmood et al., 1993), and the accumulation of nutrients (Malinowski et al., 2000; Rahman and Saiga, 2005), suggesting these symbionts may play important roles in ameliorating a wide variety of environmental stressors. These alternative pathways of benefits have received less attention than herbivory in the current literature.

Endophyte-mediated benefits to hosts under water stress have been well documented in several agronomically important forage and turf grass species from the genera *Festuca* and *Lolium* (reviewed by Bacon, 1993; Malinowski and Belesky, 2000; Clay and Schardl, 2002; Muller and Krauss, 2005; Saikkonen et al., 2006), and more recently in two native grass species (Morse et al., 2002; Kannadan and Rudgers, 2008). Surveys of native grasses have also documented higher frequencies of symbiosis in drier habitats (Lewis et al., 1997; Leyronas and Raynal, 2001; Novas et al., 2007; Saona et al., 2010), suggesting the potential for a widespread function of endophytes in mediating plant responses to water stress.

Local and seasonal availability of soil moisture is a critical factor determining the distribution and abundance of plant species (Cornwell and Grubb, 2003). Limited water availability can have strong, negative impacts on plant productivity, and plants have evolved adaptations in numerous physiological, developmental, and life history traits to cope with this stress (Geber and Dawson, 1990; Ackerly et al., 2000). These traits have historically been grouped into strategies that enable plants to avoid, tolerate, or escape drought, although it has been recognized that these strategies are not mutually exclusive (Levitt, 1980; Ludlow, 1989).

When subjected to slowly developing water shortages (days to months) some plants can optimize their long-term resource gain through acclimation responses that allow them to avoid or tolerate drought. Avoidance mechanisms allow plants to maintain tissue water potential as high as possible by minimizing water loss or maximizing water uptake. Endophyte-mediated drought avoidance mechanisms have been documented in several species, including changes in the timing and rate of stomatal closure (Elmi and West, 1995; Malinowski et al., 1997), increases in root dry matter (Latch et al., 1985; De Battista et al., 1990; Malinowski et al., 1997), and greater storage of water in tillers (Elbersen and West, 1996; Buck et al., 1997) of symbiotic plants relative to symbiont-free. Endophyte-mediated changes in root traits associated with enhanced water uptake, such as increased root hair length, have also been reported (Malinowski et al., 1999). Tolerance mechanisms allow plant tissues to withstand negative water deficits through changes in physiological and biochemical properties. Endophyte-mediated changes in several drought tolerance mechanisms have also been documented, including the translocation of assimilates to leaves (Richardson et al., 1992), osmotic adjustment (reviewed in Malinowski and Belesky, 2000), and changes in cell wall elasticity (White et al., 1992). As an alternative to acclimation responses, plants can escape drought stress. Escape strategies rely on successful reproduction before the most intense period of drought, allowing plants to maximize fitness. This can be accomplished by increasing growth rates, flowering early, and allocating more resources to reproduction to maximize resource use while water is available. Historically, most attention has been placed on avoidance and tolerance, and, to our knowledge, no investigations have examined the potential for endophyte-mediated escape.

In addition to changes in the abiotic context, the costs and benefits of grass–endophyte interactions may also vary with biotic factors other than herbivory. In this study, we additionally examined the potential role of soil microbes in altering the costs and benefits of the symbiosis. Some prior studies suggested antagonism between foliar endophytes and soil communities, including decreased soil microbial biomass (Jenkins et al., 2006), suppressed plant pathogenic nematodes (Kimmons et al., 1990; Elmi et al., 2000), and reduced mycorrhizal fungi colonization and spore abundance in the soil (Chu-chou et al., 1992; Guo et al., 1992; Mueller, 2003; Omacini et al., 2006; Mack and Rudgers, 2008). Endophyte density has also been negatively correlated with rates of mycorrhizal colonization of roots, and it has been hypothesized that variation in endophyte density could influence rates of vertical transmission of the endophyte (Mack and Rudgers, 2008). While endophytes can clearly have strong impacts on belowground communities and processes, little is known about how microbes belowground influence the costs, benefits, and transmission of these aboveground symbioses.

Here we examine interactions between a fungal endophyte, *Epichloë amarillans*, and its grass host, *Agrostis hyemalis*, under altered abiotic and biotic contexts: a gradient of water availability and in the presence versus absence of soil microbes. Specifically, we address the following questions:

1. Do the costs or benefits of endophyte symbiosis vary with changes in water availability and/or the presence of the soil microbial community?
2. Do these ecological factors influence the rate of vertical transmission of the endophyte?

To our knowledge, this is the first study to evaluate the context-dependency of these two, alternative pathways (costs/benefits, symbiont transmission) that can influence symbiont frequency and persistence.

2. Methods

2.1. Study organisms

A. hyemalis is a short-lived perennial or facultatively annual C_3 grass distributed throughout eastern North America and frequently occurring in pastures, along roadbanks and ditches, and in open woods. It is widely distributed throughout the state of Texas, flowering in late-April to early May. *A. hyemalis* hosts the endophyte *E. amarillans* (White, 1994; Craven et al., 2001). In our Texas field sites, mean endophyte frequency (% symbiotic plants/population) was 84% (range 50–96%; 8 populations), and all populations surveyed were symbiotic (Rudgers et al., 2009). During summer 2006, we collected seeds from approximately 100 individuals in a natural population at the Stephen F. Austin Experimental Forest (31°29′52″N, 94°46′46″W). Endophyte frequency in the source population was 96%. In this population, the endophyte appears to be primarily vertically transmitted to seeds, as stromata formation has rarely been observed in the field (<1% of plants across all of our sampled populations). However, other populations of *A. hyemalis* can show higher rates of stromata formation (White and Chambless, 1991; White et al., 1993), which has the potential to reduce plant fitness. In addition, there is some suggestion in the literature that the endophyte in *A. hyemalis* could spread contagiously via epiphyllous conidia (White et al., 1996).

2.2. Endophyte-disinfection treatment

By using experimental removal of the endophyte rather than comparing naturally symbiotic and symbiont-free plants, we can quantify the costs and benefits of endophyte symbiosis without the confounding effects of plant genotype. To remove the endophyte, we heat treated a subset of randomly chosen seeds in a water bath at 62 °C for 6 min. To minimize the potential side effects of the endophyte removal treatment, the treated seeds were grown in the greenhouse until they flowered and set seed; this second generation of seeds was then used to plant the experimental treatments. Our disinfection treatment was designed to mimic the natural process of endophyte loss from symbiotic lineages, which can occur through imperfect vertical transmission (Afkhami and Rudgers, 2008).

Prior to establishment of the experiment, we made several leaf peels from 30 plants per E+ and E− treatment, stained the leaves with rose bengal, and examined tissue under a microscope at 200× (Leica Microsystems, Wetzlar, Germany) to check the effectiveness of the disinfection process (Belanger, 1996). In addition, to assess if the treatment had any effects on seed germination for the seeds used in our experiment, 20 seeds from each treatment were planted in 10 replicate petri dishes in a growth chamber (12 h day length, 15–24 °C), and we recorded the proportion of seeds that germinated.

2.3. Soil community treatment

Our experiment included two soil community treatments: sterilized and control. We manipulated the biotic component of the soil community via sterilization of live field-collected soil. We collected soil from Stephen F. Austin Experimental Forest on 16 Jun 2008 at a site near the source *A. hyemalis* population. Soil was taken from the top *c.* 15 cm of the soil horizon to match the rooting zone of *A. hyemalis*, sieved to 4 mm (U.S. Standard Sieve No. 5, Soil Test Inc., Lake Bluff, IL) and stored at 4 °C until establishment of the experiment. Soil was sterilized twice in an autoclave at 121 °C for 1.5 h. Control soil remained untreated. At the end of the experiment, root tissue samples (~1 g) were stained to assess colonization by arbuscular mycorrhizal fungi, following the procedure described by INVAM (http://invam.caf.wvu.edu/methods/mycorrhizae/staining.htm) using 0.05% trypan blue. After staining, we mounted roots onto slides and examined them under a microscope at 400× (Leica Microsystems, Wetzlar, Germany).

2.4. Greenhouse experiment

On 15 July 2008, symbiotic and symbiont-free seeds that were one generation removed from the endophyte-disinfection treatment were planted in plastic seedling trays (4.1 cm × 4.1 cm cells) filled with an autoclave-sterilized 50:50 mixture of screened and washed sand (QUIKRETE® International Inc., Atlanta, GA) and Metromix 200 (SunGro Horticulture Inc., Corvalis, OR). Each of 96 E+ and 96 E− seedlings were randomly assigned to a soil (live or sterile) and watering treatment (20 ml, 40 ml, 60 ml, 80 ml per day), for a total of 16 treatment combinations (12 replicates per endophyte × water × soil combination, 192 individual plants). On 25 August 2008, seedlings were transplanted into 10 cm × 10 cm × 10 cm deep plastic pots filled with 600 ml of the sterile 50:50 sand and Metromix, and 20 ml of live or sterile field soil. Thus, our soil inoculum constituted of ~3% of the total soil volume in experimental pots; therefore, any differences between the live and sterile treatments were not likely driven by nutrient differences caused by sterilization. To reduce splash contamination, the 20 ml of soil used as inoculum was sandwiched in the middle of the pot between layers of the sterile soil mixture. We arranged pots in a randomized order in the greenhouse, and watered them with 40 ml tap water twice per day for 1 week prior to establishment of the watering treatment. Throughout the experiment, greenhouse temperature was maintained at *c.* 24 °C with no supplemental light.

2.5. Watering treatment

We imposed the water manipulation on 1 September 2008. Using automatic emitters (Rain Bird, San Diego, CA), we watered plants with tap water twice per day with 10 ml, 20 ml, 30 ml, or 40 ml. Using a soil moisture probe (TDR 100 Soil Moisture Probe, 7.5 cm probes, Spectrum Technologies, Inc., Plainfield, IL), we measured soil moisture 1 week after the establishment of the water treatment and again just prior to harvesting to check the effectiveness of our treatment. TDR probes were calibrated with measurements of gravimetric water content from a subset of trial pots ($r^2 = 0.81$, $F_{1,19} = 75.9$, $P < 0.0001$, $n = 20$ pots).

2.6. Response variables

To assess treatment effects on plant growth, we counted tillers five times throughout the experiment. Here, we report final tiller numbers, as results were consistent through time. At the end of 10 weeks (beginning 15 November 2008), we harvested all plants and measured above- and belowground biomass. Roots were washed through a 1.0 mm U.S. Standard Sieve (No. 18, Soil Test Inc., Lake Bluff, IL). Above- and belowground mass was obtained following drying at 60 °C to a constant mass and used to calculate the root:shoot ratio (root mass (g) × shoot mass^{-1} (g)). To track changes in plant phenology, we recorded the number of days to flowering (i.e., the date that flowers opened on the first inflorescence produced). To quantify plant reproduction, we collected all inflorescences individually. *A. hyemalis* exhibits dual dispersal modes, such that some seeds are dispersed locally, falling off the mature inflorescence while it is still attached to the plant, while others are dispersed when the inflorescence breaks loose from the parent and rolls in the wind (Rabinowitz and Rapp, 1979). In order to obtain accurate measures of seed production, we removed individual inflorescences from plants just prior to the release of seeds. In addition, for the highest and lowest

watering treatments, we manually removed seeds from all inflorescences per plant and weighed them to obtain total seed mass; we limited this assessment to the treatment extremes due to the labor intensiveness of seed removal. To assess treatment effects on reproductive allocation, we calculated inflorescences per tiller (inflorescence number × total tiller number^{-1}) and reproductive effort (seed weight × total biomass^{-1}).

2.7. Seed viability and endophyte transmission

To assess treatment effects on the viability of seeds produced, a randomly selected subset of 20 seeds from each symbiotic and symbiont-free plant from the highest and lowest watering treatments were planted in sealed petri dishes filled with wet sterile sand then placed in a growth chamber (12 h day length, 15–24 °C). After 4 weeks, representing the typical germination window, we recorded the number of seeds that germinated. For the subset of symbiotic plants, we assessed the rate of vertical transmission by scoring endophyte presence/absence for a minimum of 10 seedlings per parent plant using rose bengal stain (Belanger, 1996). We then calculated the proportion of seeds that germinated as an additional measure of reproductive fitness of the plant, and the proportion of symbiotic seedlings as a measure of the rate of vertical transmission and therefore, the fitness of the endophyte.

2.8. Statistical analysis

We constructed two MANOVA models (SAS Institute, 2004). The first model tested for treatment effects on plant growth by combining the responses of total biomass, aboveground biomass, belowground biomass, final tiller number, and root:shoot ratio. The second model tested for treatment effects on plant reproduction by combining the responses of days until flowering, inflorescence number, total seed mass, inflorescences per tiller, and reproductive effort. For this model, our MANOVA was restricted to the highest and lowest watering levels, because seed mass was not measured for the intermediate watering levels. All statistical models included the fixed factors of endophyte treatment (E+ or E−), water treatment (two or four levels), and soil type (live or sterile). If MANOVA detected significant treatment effects, we decomposed the effects using individual ANOVA. For the plant reproductive traits, for which we had data at all four water levels, we present results from the full model, as results did not qualitatively differ from the model restricted to the highest and lowest water level. Post-hoc Tukey HSD tests were used to compare treatment means. At the lowest watering level, we also calculated Pearson correlation coefficients to examine the relationships between days to flowering, the number of inflorescences produced, and seed mass. For examining treatment effects on the rate of vertical transmission, we performed ANOVA with the fixed factors of water availability and soil type. All analyses met assumptions of normality of residuals and homogeneity of variances following log transformation of aboveground biomass, total biomass and seed mass, and square-root transformation of inflorescence number.

3. Results

3.1. Treatment effectiveness

3.1.1. Endophyte treatment

The heat treatment to remove the endophyte had no effect on the proportion of seeds that germinated in the second generation ($F_{1,19} = 0.66$, $P = 0.4287$; means ± SE: E+ = 0.785 ± 0.039, E− = 0.822 ± 0.033). Symbiont frequency in the seedlings used to establish the experiment was 0% in the E− treatment and 100% in the E+ treatment ($n = 35$ plants per treatment).

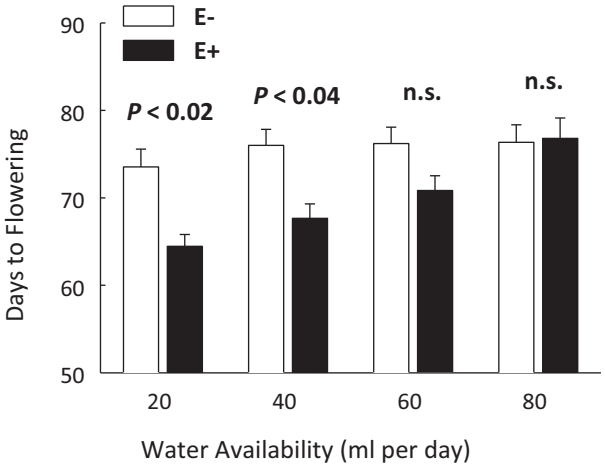

Fig. 1. Effects of the fungal endophyte and water availability on days to flowering in *Agrostis hyemalis*. Bars show means ± SE. Dark bars (E+) are plants grown from symbiotic seeds collected from a population in the field. Light bars (E−) are plants grown from experimentally disinfected seeds from the same population. Significant differences between the endophyte treatments within each water level are noted on top of bars.

3.1.2. Water treatment

One week after the establishment of the water treatment, the lowest water level represented a 53% reduction in soil volumetric water content relative to the highest water level, and all four levels of the treatment differed significantly from each other ($F_{3,188} = 47.35$, $P < 0.0001$; means ± SE: 20 ml = 12.4 ± 0.55; 40 ml = 15.64 ± 0.68; 60 ml = 19.41 ± 0.99; 80 ml = 26.08 ± 1.22). After 10 weeks, the percentage reduction in soil volumetric water content between the highest and lowest watering level increased to 85%. All treatment levels remained significantly different from each other ($F_{3,188} = 118.17$, $P < 0.0001$; means ± SE: 20 ml = 2.29 ± 0.13; 40 ml = 4.61 ± 0.24; 60 ml = 9.06 ± 0.47; 80 ml = 15.72 ± 0.99). Unexpectedly, the endophyte treatment also affected volumetric water content, with on average 11% higher soil water content in pots with endophyte-symbiotic plants relative to the endophyte-free treatment ($F_{1,191} = 5.23$, $P < 0.0233$; means ± SE: E+ = 13.82 ± 0.75; E− = 12.48 ± 0.60).

3.1.3. Soil treatment

Examination of 30 slides from the live soil treatment revealed no colonization by arbuscular mycorrhizal fungi, suggesting our live soil treatment was not effective in manipulating this component of the biotic soil community. Other soil microorganisms were likely present in the live soil, but were not directly assayed.

3.2. Plant reproduction responses

The endophyte symbiosis increased several plant reproduction responses at low water availability, as indicated by a significant interaction between the endophyte and water treatments (Table 1). When water was limiting, the symbiosis altered plant phenology. Endophyte-symbiotic plants began flowering 9 days earlier than non-symbiotic plants at low water availability, but did not differ from non-symbiotic plants at high water availability (Fig. 1 and Table 1). The pattern was similar for the number of inflorescences produced. At the lowest water level, days to flowering was negatively correlated with the number of inflorescences produced ($r = -0.33$, $P = 0.0224$, $n = 48$). Symbiotic plants at the lowest watering level produced 42% more inflorescences than non-symbiotic plants, but the endophyte treatments did not significantly differ in the number of inflorescences produced at high water availability (Fig. 2a and Table 1). These differences in inflorescence number also

Table 1
Statistical results from M/ANOVA examining the effects of the endophyte treatment, water treatment, and soil treatment on plant reproductive responses: days to flowering, the number of inflorescences, inflorescences per tiller, seed weight, and reproductive effort. *P*-values <0.05 are shown in bold face. MANOVA was restricted to the highest and lowest watering levels, because seed mass was not measured for the intermediate watering levels. ANOVA (full) results presented for days to flowering, inflorescences, and inflorescences per tiller are from the full dataset ($N = 192$ plants). ANOVA (subset) results for seed mass and reproductive effort include only the highest and lowest watering treatment ($N = 96$ plants).

Effect	MANOVA			ANOVA (full)	Days to flowering		Inflorescences		Inflorescences per tiller		ANOVA (subset)	Seed mass		Reproductive effort	
	d.f.	*F*	*P*	*d.f.*	*F*	*P*	*F*	*P*	*F*	*P*	*d.f.*	*F*	*P*	*F*	*P*
Endophyte	5,84	3.24	**0.010**	1	17.8	**<0.001**	27.1	**<0.001**	15.6	**<0.001**	1	2.41	0.124	4.97	**0.028**
Water	5,84	154	**<0.001**	3	5.70	**0.001**	55.8	**<0.001**	1.94	0.125	1	384	**<0.001**	3.72	0.057
Soil type	5,84	0.96	0.447	1	0.01	0.922	0.25	0.620	1.08	0.301	1	0.06	0.814	2.78	0.099
Endo × water	5,84	4.55	**0.001**	3	2.68	**0.049**	2.82	**0.041**	3.00	**0.032**	1	5.68	**0.019**	14.8	**<0.001**
Endo × soil type	5,84	1.02	0.412	1	1.57	0.213	0.18	0.676	0.17	0.681	1	0.00	0.946	0.77	0.381
Water × soil type	5,84	0.54	0.748	3	0.94	0.421	0.14	0.935	0.30	0.828	1	0.00	0.959	1.02	0.316
Endo × water × soil	5,84	0.39	0.852	3	0.57	0.636	0.32	0.821	0.15	0.933	1	0.03	0.859	0.64	0.427

corresponded with differences in the total seed mass produced by the plants ($r = 0.62$, $P < 0.0001$, $n = 48$). Symbiotic plants produced 43% more seed mass than non-symbiotic plants at low water availability, but did not differ from non-symbiotic plants at high water availability (Fig. 2b and Table 1).

In addition to modulating total reproductive output, the endophyte symbiosis and water availability also interacted to alter plant allocation towards reproduction. Symbiont presence increased the number of inflorescences per tiller by 23% at low water availability, but at high water availability, symbiotic and symbiont-free plants did not significantly differ (Fig. 2c and Table 1). Reproductive effort showed a similar pattern. Symbiotic plants invested 35% more in seed mass/vegetative mass than non-symbiotic plants at low water availability, but did not differ from symbiont-free plants at high water availability (Fig. 2d and Table 1).

3.3. Plant growth responses

In contrast to its effects on plant reproduction, endophyte symbiosis did not modulate plant growth responses to water availability. However, the endophyte did interact with the soil treatment to alter total plant biomass. Specifically, non-symbiotic plants on live soil had 17% greater biomass than symbiotic plants on live soil, whereas symbiotic and symbiont-free plants performed equally well on sterile soil (Fig. 3 and Table 2). In general, decreased water availability caused strong reductions in plant growth, reducing biomass by 68%, reducing tiller number by 56%, and increasing root:shoot ratio by 30% (Table 2), demonstrating that our treatments were effective in generating abiotic stress.

3.4. Endophyte transmission

On average across all treatments, seed germination was 75% ± 1.3 SE. Neither the endophyte treatment, water availability, nor the soil treatment influenced the proportion of progeny seeds that germinated (endophyte $F_{1,95} = 0.43$ $P = 0.5154$; water availability $F_{1,95} = 0.41$ $P = 0.5227$; soil treatment $F_{1,95} = 0.10$ $P = 0.7497$). In addition, neither the soil nor water treatment influenced the rate of endophyte transmission to seedlings (water availability $F_{1,47} = 0.61$ $P = 0.4388$; soil treatment $F_{1,47} = 0.11$ $P = 0.7393$). However, transmission to seedlings was imperfect, as the mean symbiont frequency in seedlings was 90% (mean ± SE: 0.90 ± 0.02) whereas the parental endophyte frequency was 100%.

4. Discussion

To our knowledge, this is the first study to simultaneously investigate how changes to the ecological context of a symbiosis alter both the costs and benefits of the interaction and the rate of symbiont vertical transmission. Specifically, we showed that fitness benefits of the symbiosis between the native grass *A. hyemalis* and the fungal endophyte *E. amarillans* were strongest when water was limiting. Symbiotic plants at the lowest watering level produced ~40% more inflorescences and greater seed mass than non-symbiotic plants, while at the highest watering level, symbiotic and non-symbiotic plants did not significantly differ in reproductive fitness. In addition, we found no differences in germination rates of the seeds produced by symbiotic and symbiont-free plants, suggesting that the increase in reproductive output did not decrease seed viability. Because symbiont transmission was not influenced by water availability in our study, variation in endophyte frequency in natural populations more likely reflects the relative performance of symbiotic and symbiont-free hosts in different environmental contexts.

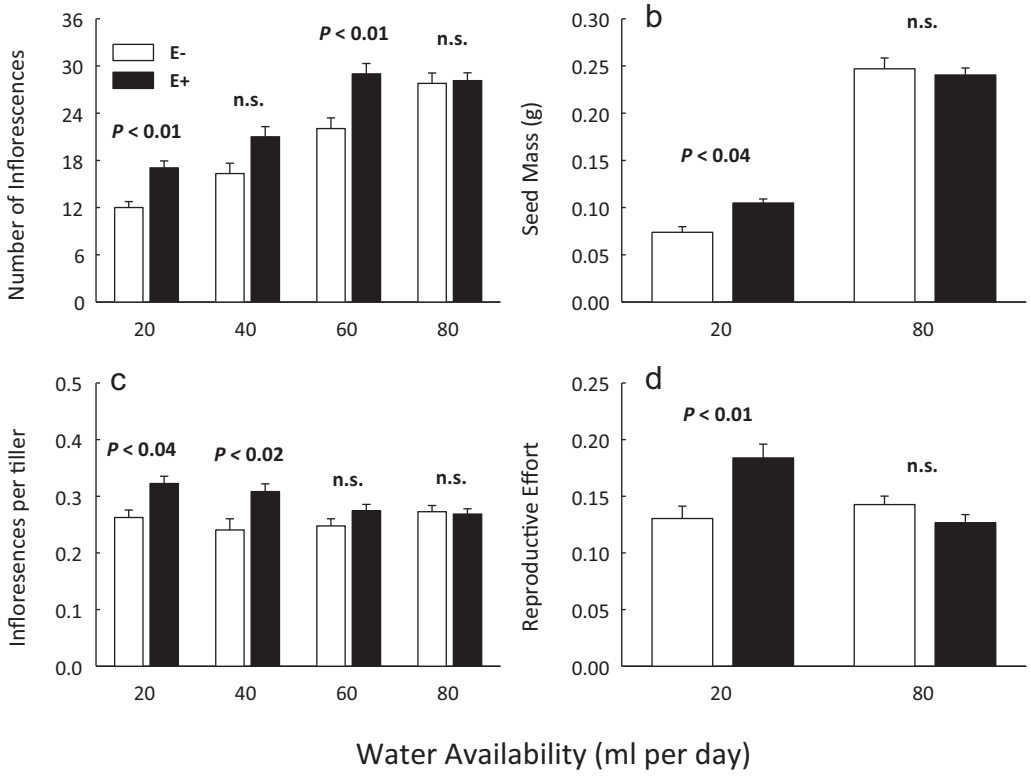

Fig. 2. Effects of the fungal endophyte and water availability on (a) the number of inflorescences produced, (b) seed mass, (c) inflorescences per tiller, and (d) reproductive effort measured as the ratio of seed mass to total plant biomass. Bars show means ± SE. Significant differences between the endophyte treatments within each water level are noted on top of bars.

Altogether, our results show that changes in the abiotic context influence the costs and benefits of the symbiosis, enhancing understanding of the role of endophytes in ameliorating stress in native grasses and providing insight into the future implications of long-term changes to the environment. In other systems, changes in the costs and benefits of symbiosis in response to abiotic stress have been linked to symbiont loss in local populations. Perhaps most notably, in the symbiosis between reef-building corals and zooxanthellae, increased light and temperature can lead to coral bleaching, in which the algal symbionts are expelled from the host (Abrego et al., 2008). This process can have strong negative consequences for biodiversity and ecosystem functioning (Hughes et al., 2003; Baker et al., 2008), and highlights the importance of understanding how environmental changes influence the dynamics of symbioses. We hypothesize that changes in soil moisture levels, particularly those accompanying climate change, could ultimately impact endophyte persistence in native populations, and we predict increased frequencies of the endophyte in *A. hyemalis* in drier environments.

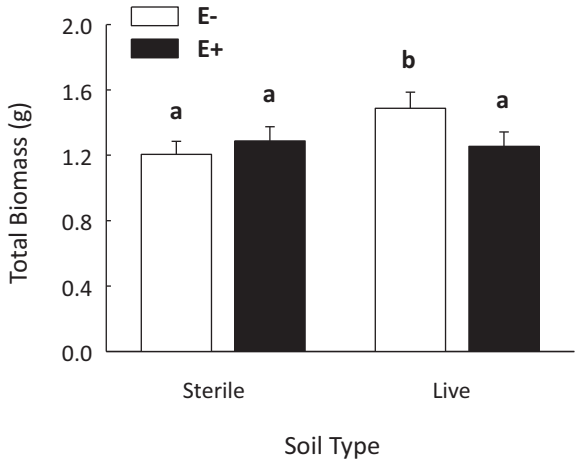

Fig. 3. Effects of the fungal endophyte and soil type (live or sterile) on total plant biomass. Bars show means ± SE. Different letters on top of bars denote means that significantly differ.

Table 2
Statistical results from M/ANOVA examining the effects of the endophyte treatment, water treatment, and soil treatment on plant growth responses: total biomass, belowground biomass, aboveground biomass, root:shoot ratio, and final tiller number. *P*-values <0.05 are shown in bold face.

Effect	MANOVA			ANOVA	Total biomass		Belowground biomass		Aboveground biomass		Root:shoot ratio		Tiller number	
	d.f.	*F*	*P*	*d.f.*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Endophyte	5,170	1.67	0.145	1	2.10	0.149	1.64	0.202	0.66	0.417	2.82	0.095	1.36	0.245
Water	15,516	17.5	**<0.001**	3	164.8	**<0.001**	40.1	**<0.001**	159.2	**<0.001**	5.94	**<0.001**	74.3	**<0.001**
Soil type	5,170	1.21	0.305	1	3.98	**0.048**	3.27	0.072	3.57	0.061	0.55	0.460	1.57	0.213
Endo × water	15,516	1.28	0.213	3	1.75	0.158	0.83	0.478	1.37	0.252	0.75	0.523	1.15	0.331
Endo × soil type	5,170	2.10	0.068	1	7.99	**0.005**	1.62	0.205	10.42	**0.002**	0.92	0.338	0.72	0.397
Water × soil type	15,516	1.21	0.265	3	0.80	0.498	1.24	0.296	0.66	0.577	0.86	0.464	1.62	0.186
Endo × water × soil	15,516	0.58	0.893	3	0.88	0.452	0.51	0.677	0.65	0.585	0.16	0.921	0.32	0.811

One caveat to this conclusion is that endophyte frequency will also depend on the degree of stromata formation and horizontal transmission, which did not occur in our experiment. Previous studies in this system have suggested that stromata formation may be costly when water is limited, due to changes in epidermal cell structure and increases in the rates of evapotranspiration in stromata-bearing individuals relative to symbiotic non-stromatal individuals (White and Chambless, 1991; White et al., 1993, 1997; White and Camp, 1995). Such a cost could produce the opposite effect on symbiont frequency in stromata-forming populations, with lower frequencies in drier habitats. Additionally, it has been suggested that rates of stromata formation may be related to genotypic differences in endophyte growth rates, because endophyte isolates from stromata bearing individuals grew more rapidly than endophyte isolates from non-stromata bearing individuals on various sugars (White and Chambless, 1991). Combined with results from our study demonstrating that fitness benefits of the symbiosis between *A. hyemalis* and the fungal endophyte *E. amarillans* were strongest when water was limiting, it appears that low water availability could simultaneously select against horizontal transmission and increase the frequency of vertically transmitted endophytes. In the future, studies examining how water availability influences both changes in endophyte frequency and rates of horizontal vs. vertical symbiont transmission in populations that vary in frequency of stromata formation could lead to a greater understanding of the factors that lead to the fixation of vertically transmitted symbioses.

Relatively few controlled experiments have examined the benefits or costs of endophyte symbioses for native grass species (Saikkonen et al., 2006; Cheplick and Faeth, 2009). To date, our work represents one of three published studies to show that endophyte symbiosis can confer benefits to native grasses under water stress (Morse et al., 2002; Kannadan and Rudgers, 2008). Prior studies in grass–endophyte systems have provided support for endophyte-mediated benefits to plants under water stress through enhancements of both drought avoidance and drought tolerance mechanisms (see references Section 1). However, our study demonstrates that benefits may also accrue by allowing hosts to escape from drought. Symbiotic plants at the lowest watering level flowered 9 days earlier that non-symbiotic plants, and this plastic change in flowering time was correlated with an increase in the number of inflorescences plants produced. Given that the severity of our watering treatment increased through time, this symbiont-mediated shift in phenology could have benefited hosts by allowing them to maximize resource utilization prior to the most severe level of stress, and is consistent with other studies that have demonstrated the adaptive significance of early flowering for escaping drought (Sherrard and Maherali, 2006; Franks et al., 2007). It is also in agreement with results from previous studies in the related species *Agrostis tenuis*. Bradshaw (1959a) reported evidence for population differentiation in flowering time, with lowland populations from warmer habitats flowering earlier than highland populations, when plants were grown in a common environment. Mcneilly and Antonovi (1968) found a similar result, with plants from a contaminated mine site flowering earlier than plants from a nearby pasture. In their study, early flowering was also correlated with warmer, drier soils. Both studies attributed these changes to genetic differentiation between populations, although it seems plausible that some changes could have been mediated by the presence of an endophyte, particularly because Bradshaw (1959b) reported variation in the frequency of choke disease (caused by sexual reproduction of an endophyte) in the populations of *A. tenuis* he surveyed.

In our study, in addition to flowering early, symbiotic plants at the lowest water level invested more resources in reproduction than vegetative growth than did non-symbiotic plants. Because *A. hyemalis* is a perennial, increased allocation to reproduction in one year could potentially decrease reproductive output in future growing seasons. However, if severe reductions in water availability reduce plant survival, a greater investment in current reproduction could maximize lifetime plant fitness. Our field data also suggest that *A. hyemalis* is likely not a long-lived perennial in the habitat where we collected seeds, as 12.5% of plants (out of 200) in field plots established near the source population were annuals and 71% of plants were biennials. Future work extending experiments through multiple years would help to elucidate longer-term effects of endophytes on plant survival.

In addition to potentially allowing plants to escape drought, symbiont-mediated changes to plant phenotypes under water stress could have interesting evolutionary implications. Specifically, a shift in the flowering phenology of symbiotic plants could increase assortative mating among symbiotic plants, ultimately leading to genetic divergence between symbiotic and symbiont-free subpopulations of hosts. Additionally, symbiont-mediated benefits to grass hosts at low soil moisture levels could lead to habitat specialization of symbiotic lineages, and further promote the reproductive isolation of symbiotic and symbiont-free hosts through spatial habitat segregation. Combined, these processes could ultimately lead to symbiont-induced speciation (Thompson, 1987).

Manipulation of the soil community suggested a potential cost of the symbiosis. In the presence of soil microbes, symbiont-free hosts accumulated more biomass than symbiotic hosts. These increases in biomass did not result in increases in reproductive fitness, but could alter reproductive output over the lifetime of a perennial host, or have consequences for long-term plant survival. Prior research has similarly demonstrated that endophytes can be costly under extreme resource limitation, such as the absence of soil nutrients (Cheplick et al., 1989; Cheplick, 2007). Although our soil treatment had no effect on mycorrhizal fungi (we found no root colonization in any treatment), other components of the soil microbial community could underlie the positive effects of live soil for endophyte-free plants. For example, plant-growth promoting bacteria or rhizospheric nitrogen-fixers (Bergmann et al., 2009; Lugtenberg and Kamilova, 2009) could benefit endophyte-free plants, and like other soil bacteria, may show no effect on endophyte-symbiotic plants if the endophyte generally suppresses bacteria populations (Franzluebbers et al., 1999; Jenkins et al., 2006).

Finally, our work also has potential implications for improvements to turfgrass production. The genus *Agrostis* includes 150–200 species, a few of which are widely planted for turf, particularly creeping bentgrass (*Agrostis stolonifera*). Bentgrass turfs are sensitive to summer heat and drought, and crop improvement efforts have aimed to increase drought tolerance for enhanced performance (e.g., Xu and Huang, 2000). To our knowledge, endophytes have not yet been investigated as a potential mechanism to improve climate tolerance in this group. However, artificial inoculations of endophytes into novel plant lineages can be achieved (Latch and Christensen, 1985; Tintjer and Rudgers, 2006), and thereby may benefit turf improvement efforts. Although benefits in our study appeared to primarily occur through changes in phenology that allowed symbiotic plants to escape drought and may not be relevant to mowed, non-reproducing turf systems, the symbiosis also influenced soil moisture levels, with symbiotic plants retaining higher soil moisture than symbiont-free plants across all water levels. This suggests that endophyte symbiosis may also improve drought avoidance by altering rates of transpiration or water use efficiency and reduce the need for intensive water additions, both of which could improve turf performance during summer heat and drought.

Understanding how changes in abiotic and biotic factors alter the costs and benefits of symbiosis and the rate of symbiont transmission is important for gaining insight into the mechanisms

of symbiont persistence. Our results highlight the complexity of plant–microbe symbiosis. Benefits to hosts were stronger when water was limiting, but the symbiosis may be costly in the presence of soil microbes. Given their ecological, evolutionary, and economic importance, elucidating the breadth of factors that influence symbiont persistence will be critical for understanding the impacts of anthropogenic changes to the environment.

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