Endophyte symbiosis benefits a rare grass under low water availability

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Summary

1. Symbiotic relationships with microbes may influence how plant species respond to environmental change. Here, we investigated how a fungal endophyte symbiosis affects the growth and survival of a rare, mid-western United States grass species under altered water availability. In a 12-week long greenhouse experiment, we compared the performance of endophyte-infected and endophyte-disinfected grove bluegrass (*Poa alsodes*, Poaceae) subjected to two levels of water availability. Gravimetric water measurements indicated that the low water treatment had 24% less soil moisture than the high water treatment.

2. In the high water treatment, the performance of endophyte-infected plants and disinfected plants was similar. However, under low water, endophyte-infected plants had 17% more total biomass than disinfected plants. Despite localization of the endophyte in above-ground plant tissues, effects of the endophyte were stronger below-ground: Under low water, endophyte-infected plants produced 24% more root biomass, but just 14% more shoot biomass than disinfected plants. When water-limited, disinfected plants had 29% greater leaf senescence than endophyte-infected plants.

3. We investigated several plant traits that may underlie benefits of symbiosis. Under low water, disinfected plants had significantly higher relative leaf water content than endophyte-infected plants, consistent with the hypothesis that endophytes cause plants to up-regulate water conservation mechanisms faster in response to drought. There were no strong differences between endophyte-infected and disinfected plants in root morphology, leaf area or water use efficiency, although plants with the endophyte had slightly shorter leaves.

4. Our results suggest that endophyte symbiosis may ameliorate the negative effects of drought stress for grove bluegrass. The heritable transmission of endophytes from plants to seeds provides opportunities for environmental change to influence selection on native grass-endophyte symbioses.

Key-words: *Neotyphodium*, mutualism, climate change, drought, *Poa alsodes*

Introduction

Symbiotic relationships with microbes may influence how plant species respond to environmental change. For example, endophytic fungi have been demonstrated to ameliorate drought stress for a few species of agronomically important forage and turf grasses, namely *Lolium* and *Festuca* spp. (reviewed by (Bacon 1993; Malinowski & Belesky 2000; Clay & Schardl 2002; Malinowski *et al*. 2005; Muller & Krauss 2005; Saikkonen *et al*. 2006). Under drought conditions, the systemic endophyte *Neotyphodium coenophialum* in tall fescue grass (*Lolium arundinaceum*) can enhance host growth and survival relative to uninfected plants (Arachevaleta *et al*. 1989; Bouton *et al*. 1993; West 1994; Malinowski & Belesky 2000). Since systemic fungal endophytes are estimated to occur in 20%–30% of all grass species (Leuchtmann 1992), these

symbionts may have strong influences on plant community composition by altering the performance of host plants relative to other species in the community (Clay *et al*. 2005; Rudgers *et al*. 2007). Although a primary function of endophytes appears to be increased host resistance to herbivores (Clay & Schardl 2002; Clay *et al*. 2005), endophytes may also provide other benefits that depend on the level of abiotic stress experienced by the host (Rudgers & Swafford, in press).

Prior surveys of natural grass populations have revealed higher frequencies of endophyte symbiosis in grass populations located in drier environments (Lewis *et al*. 1997; Leyronas & Raynal 2001), suggesting a widespread role for endophytes in enhancing host performance under water stress. To our knowledge, however, the question of whether endophytes improve drought tolerance in natural grass populations has been examined for just three native grass species. In a greenhouse study, Arizona fescue (*Festuca arizonica*) naturally infected with *Neotyphodium* had greater above-ground biomass than

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naturally endophyte-free plants under drought stress relative to regular watering, although total biomass was not affected (Morse *et al*. 2002). In this system, benefits and costs of the endophyte also varied among endophyte genotypes (Morse *et al*. 2007). In contrast, an endophyte-disinfection experiment found no benefits of endophyte symbiosis for *F. rubra* or *F. pratensis* under low water availability in the greenhouse (Ahlholm *et al*. 2002). In total, few studies (and fewer controlled experiments) exist that are useful in predicting how plant-endophyte symbioses will respond to altered water availability.

The mechanisms through which fungal endophytes enable host grasses to mitigate water stress remain unclear (Malinowski *et al*. 2005; Assuero *et al*. 2006). Prior studies suggest a combination of drought stress avoidance and drought tolerance mechanisms. In drought stress avoidance, water deficits may be reduced in endophyte-infected tall fescue (*L. arundinaceum*) and meadow fescue (*F. pratensis*) by earlier and faster stomatal closure in response to water stress. However, the short-term benefits of rapid stomatal closure could be offset by eventual reductions in photosynthetic rates (Belesky *et al*. 1987; Elmi & West 1995; Buck *et al*. 1997; Malinowski *et al*. 1997; Elmi *et al*. 2000), and endophyte presence does not appear to alter stomatal conductance in perennial ryegrass (*L. perenne*) (reviewed by West 1994; Malinowski & Belesky 2000; Malinowski *et al*. 2005). In addition, endophyte-mediated reductions in root diameter, increases in root hair length, and reductions in root production near the soil surface may increase plant water absorption and decrease susceptibility to drought (Richardson *et al*. 1990; Malinowski *et al*. 1999; Crush *et al*. 2004). Drought tolerance mechanisms may include osmotic adjustment (the accumulation of solutes to retain cell turgor under water stress), with lower osmotic potentials reported in endophyte-infected plants compared to endophyte-free plants (West 1994; Malinowski & Belesky 2000; Malinowski *et al*. 2005). Osmotic adjustment appears to occur, in part, through increased accumulation of solutes in the presence of the endophyte (Richardson *et al*. 1992). Additionally, increased turgor pressure in the basal regions of stems may promote survival of the apical meristem, and thereby account for the increased drought tolerance of infected plants (West 1994; Elbersen & West 1996; Buck *et al*. 1997). Finally, endophytes may promote plant water use efficiency under low water availability, if the reduced transpiration rates of infected plants promote conservation of soil moisture (West 1994; Morse *et al*. 2002).

To expand understanding of the ecological consequences of endophyte symbiosis and examine potential functional traits involved in these interactions, we investigated the symbiosis between a rare native plant, grove bluegrass (*Poa alsodes*, Poaceae), and a systemic endophytic fungus (*Neotyphodium* sp., Clavicipitaceae) under two levels of water availability in the greenhouse. Specifically we asked, are the benefits of endophyte symbiosis greater under low water availability? We sought to identify possible mechanisms through which the endophyte affects grove bluegrass by examining several above- and below-ground plant traits. Based on prior literature for agronomic grasses, we predicted that, above-ground, endophyte-infected plants would exhibit greater water conservation mechanisms than disinfected plants, including reductions in the relative water content of leaves and in leaf area, as well as increases in water use efficiency. Below-ground, we predicted increased water uptake mechanisms, such as increased root : shoot ratios, longer root hairs, greater root hair densities and reduced root diameters.

Methods

STUDY ORGANISMS

Grove bluegrass (*P. alsodes*, Poaceae) is a perennial C3 grass native to moist woodland habitats from the northeastern to central United States of America (Gleason & Cronquist 1991). Grove bluegrass is classified as rare in Indiana and endangered in Illinois (USDA & NRCS 2007). We collected from a natural population in the Indiana University Research and Teaching Preserve at Lilly Dickey Woods, near Bloomington, IN (39°14′29″N, 086°13′7″W). We collected seeds from 20 plants on 12 June 2005 and 30 plants on 25 May 2006, and combined seeds in our experiment.

The endophyte in grove bluegrass has not been identified using molecular methods, but is likely in the genus *Neotyphodium* (rather than *Epichloë*) because it is vertically transmitted to seeds (M.E. Afkhami & J.A. Rudgers, unpublished data), and stromata formation has not been observed (J.A. Rudgers, personal observations). This symbiosis is known to produce the anti-oxidant and anti-fungal compound, resveratrol (Powell *et al*. 1994), but no other ecological effects have been reported. It has been hypothesized that resveratrol production may precondition plants to respond more rapidly to stress, including drought stress (Malinowski & Belesky 2000).

The frequency of endophyte symbiosis for grove bluegrass has not previously been reported in the literature. For the Indiana population at Lilly Dickey Woods, the percentage of endophyte-infected seeds was 74% (*n* = 286 seeds), the percentage of endophyte-infected greenhouse grown seedlings was 87% (*n* = 223 seedlings), and the percentage of endophyte-infected adult plants in the field was 100% (*n* = 37 plants). While data on endophyte frequencies are not currently available for other populations of grove bluegrass (in Indiana or elsewhere), among-population variation in endophyte frequency is common in other plant species (e.g. Afkhami & Rudgers in review; Clay & Schardl 2002).

ENDOPHYTE TREATMENT

To remove the endophyte, we heat-treated a subset of randomly chosen seeds in a convection drying oven (VMR International 1390FM, Sheldon Manufacturing Inc., Cornelius, OR). Because disinfection procedures have not been established for this species, we initially treated seeds for 0, 2, 3, 4, 5, 6, 7, 8, 9 or 10 days at 60 °C to determine optimal treatment time. Endophyte-infected and disinfected seeds were planted in container pots (Stuewe & Sons, Inc., Corvalis, OR) filled with Metromix 200 (Sun Gro Horticulture Inc., Bellevue, WA) on 6 July 2006. To assess treatment effectiveness, we made several leaf peels from each plant, stained them with lacto-phenol cotton blue or rose bengal, and examined tissue under a microscope at 200× (Clark *et al*. 1983; Belanger 1996). We scored a total of 89 seedlings with an average of nine seedlings per number of days in the oven (range = 7–29 seedlings). In addition, we assessed potential effects of the heat treatment on seed germination, for a total of 258 grove bluegrass seeds, with *c.* 20 seeds per number of days in the oven.

GREENHOUSE EXPERIMENT

For the experiment, we cloned material from plants grown from seeds that were heat treated for either 0 day (control) or 7–10 days (disinfection). For the disinfection treatment, we only cloned plants for which endophyte removal was successful. We grew plants in the greenhouse for *c.* 5 months prior to cloning. This procedure allows the assessment of plant performance (e.g. growth, survival) to be separated from the initial heat treatment (or control) by a round of vegetative reproduction, and is commonly used in endophyte studies (e.g. Faeth & Sullivan 2003; Morse *et al.* 2007). On 19 January 2007, we removed two tillers from each initial plant and transplanted each tiller into a 10-cm square pot (8·7-cm deep) containing Miracle Grow Potting Mix (Miracle-Grow Lawn Products Inc., Marysville, OH, 0·21% nitrogen, 0·07% phosphorous and 0·14% potassium). We randomly assigned each clone to either a high or low water treatment, and arranged the pots in a randomized order in the greenhouse. Final sample sizes were E+ low water = 19; E+ high water = 20; E– low water = 19; E– high water = 19. Greenhouse temperature was maintained at *c.* 24°C with no supplemental light.

WATER TREATMENT

We imposed a water manipulation from 20 February 2007 to 14 May 2007 in the greenhouse. We watered the plants with tap water according to the following regimen: high water – twice daily with *c.* 50 mL each time using automatic emitters (Rain Bird, San Diego, CA); low water – once every other day with *c.* 50 mL. Due to increases in light intensity and heat during the spring, we altered the low water treatment on 4 March 2007 to 50 mL of water daily to avoid death of the plants. We measured gravimetric water content on 18 April 2007 by taking a soil core from each pot (2·35 cm^3 plastic tube) immediately after applying the high water treatment; this captured the maximum difference between treatments. The low water treatment represented a maximum 24% reduction in water per gram of soil relative to the high water treatment (mean percentage soil moisture ± SE, high water = 50·2% ± 1·5%; low water = 38·2% ± 1·9%). There was no effect of the endophyte or endophyte × water interaction for soil water content (all $P > 0.15$).

RESPONSE VARIABLES: PLANT GROWTH

To assess plant growth non-destructively, we counted the total number of leaves on each plant on 4 February 2007 (prior to the water treatment), on 15 March 2007 and on 11 May 2007 (before harvest). On the latter two dates, leaf senescence was also determined as the percentage of brown leaves. The plants were harvested during 14–17 May 2007, before plants became pot bound. We washed roots through a 1-mm sieve, and separated root and shoot biomass with scissors. Biomass was dried for 3 days in a convection oven to constant weight. After harvest, we examined the correlation between leaf number and above-ground biomass ($r = 0.77$, $P < 0.0001$, $n = 78$ plants).

RESPONSE VARIABLES: ABOVE-GROUND PLANT TRAITS

We measured four above-ground traits to examine potential mechanisms of drought tolerance or avoidance. For the relative water content of leaves [RWC = (fresh weight – dry weight)/(turgid weight – dry weight)], we collected a randomly selected leaf from each plant on 5 April 2007, cut a 4-cm section from the blade, and determined fresh weight. Turgid weight was determined after soaking each leaf section separately in DI water for 4 h, and dry weight was obtained after 3 days in the convection oven at 60 °C following methods in Barrs (1968). Additionally, we used Scion Image (ScionCorp, Frederick, MD) to measure leaf surface area and leaf length (as estimated by the major axis function) for one randomly chosen leaf from each plant on 10 May 2007. We measured leaf length by hand to test that the major axis provided a good estimate of leaf length ($r = 0.85$, $P < 0.0001$, $n = 78$ leaves). To determine water use efficiency from the carbon isotope ratio (δ^{13}C), we collected a second randomly chosen leaf, air-dried it, and sent a 2·5-mg subsample to the UC Davis Stable Isotope Facility (Europa Hydra 20/20 continuous flow isotope ratio mass spectrometer; Europa Scientific, Cambridge, UK). A more positive stable isotope signature indicates higher water use efficiency (Farquhar *et al.* 1989; Lajtha & Michener 1994).

RESPONSE VARIABLES: BELOW-GROUND PLANT TRAITS

We measured five important root traits that may influence water uptake. We used plant dry biomass to calculate the root : shoot ratio. In addition, we extracted a randomly selected subsample of fresh roots from each plant, which was stored in distilled water at 4 °C until morphology could be measured. A randomly chosen subsection of the fresh root sample was photographed with a Leica DFC-480 digital camera attached to a Leica MZ 12·5 stereoscope (Leica Microsystems, Wetzlar, Germany). Using Scion Image, we measured root diameter, and root hair length following methods in Malinowski *et al.* (1999). We calculated root hair density by counting the number of root hairs along a 3·2-mm transect following the main root, and we determined mean root hair length and maximum root hair length from the lengths of five randomly chosen root hairs per sample.

STATISTICAL ANALYSIS

We constructed three MANOVA models (SAS Institute 2004). The first model tested for effects on plant growth by combining responses of above and below-ground biomass, percentage of senescent leaves (final census, i.e. census 2), and leaf number (final census). The second model included above-ground plant traits: leaf relative water content, leaf area, leaf length and water use efficiency (as measured by δ^{13}C). The third model included below-ground plant traits: root : shoot ratio, root diameter, root hair density and root hair length. All statistical models included the fixed factors of endophyte treatment (E+ or E–), water treatment (high or low) and their interaction, as well as the random factor of clone (nested within the endophyte treatment). In each model, we additionally tested two *a priori* contrasts: (i) Do E+ and E– differ under low water? and (ii) Do E+ and E– differ under high water? If MANOVA detected significant treatment effects, we decomposed effects using individual ANOVA. For leaf number and leaf senescence, we applied repeated measures ANOVA, and included initial leaf number (prior to the water treatment) as a covariate for the percentage of senescent leaves. Analyses met assumptions of normality of residuals and homogeneity of variances following arcsin square-root transformation of the percentage of senescent leaves and the root : shoot ratio.

Results

ENDOPHYTE TREATMENT EFFECTIVENESS

The heat treatment was effective at endophyte removal after 6 days in the convection oven (Fig. 1). The heat treatment had no significant effect on seed germination according to logistic

Table 1. Statistical results for *Poa alsodes* from M/ANOVA examining the effects of the endophyte treatment, water treatment, time, and clone on plant biomass and two repeatedly measured variables: leaf number and leaf senescence. *P*-values < 0·05 are shown in bold. Initial leaf count was included as a covariate in the repeated measures analysis of leaf senescence ($F_{1,36} = 2·79$, $P = 0·10$)

| Effect	MANOVA F	P	Shoot biomass F	P	Root biomass F	P	Total biomass F	P	Leaf number F	P	Leaf senescence F	P
Endophyte	3·2	**0·0254**	1·8	0·1913	3·9	0·0551	2·4	0·1327	9·6	**0·0038**	1·3	0·2582
Water	3·7	**0·0143**	1·6	0·2122	6·0	**0·0193**	2·9	0·0993	0·4	0·5163	10·5	**0·0026**
Endophyte × Water	1·7	0·1770	2·6	0·1126	1·0	0·3138	3·2	0·0815	5·2	**0·0288**	5·7	**0·0227**
Clone (Endophyte)	1·2	0·1226	2·6	**0·0024**	1·2	0·2642	2·4	**0·0057**	2·3	**0·0056**	0·6	0·9512
A priori contrast												
High water: E+ vs. E−	0·5	0·7379	0·0	0·8383	0·5	0·5019	0·0	0·8602	0·3	0·5665	0·6	0·4388
A priori contrast												
Low water: E+ vs. E−	4·5	**0·0053**	4·5	**0·0411**	4·5	**0·0406**	5·7	**0·0224**	14·4	**0·0005**	4·9	**0·0340**
Time									707·7	**0·0001**	0·8	0·3938
Time × Endophyte									5·4	**0·0261**	0·4	0·5308
Time × Water									0·8	0·3792	0·7	0·4137
Time × Endophyte × Water									0·1	0·7898	0·5	0·4938
Time × Clone (Endophyte)									1·6	0·0724	1·2	0·3009
A priori contrast												
Time × High water: E+ vs. E−									2·1	0·1559	1·1	0·3005
A priori contrast												
Time × Low water: E+ vs. E−									3·3	0·0756	0·0	0·9825
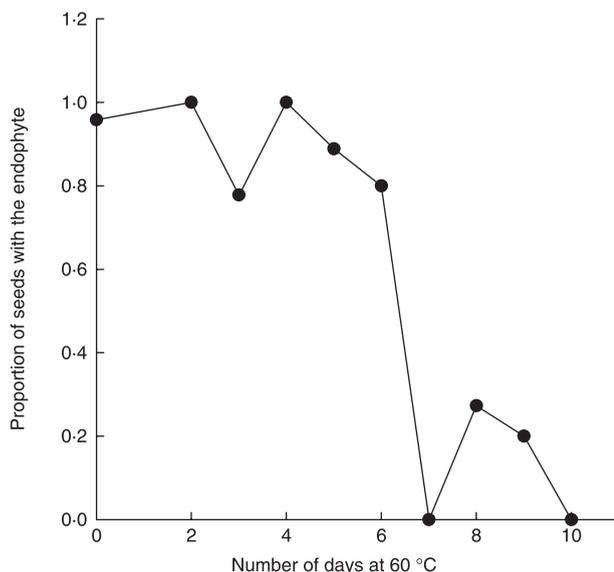
Fig. 1. Effect of the number of days in the heat treatment on the proportion of *Poa alsodes* seedlings infected with a fungal endophyte (likelihood ratio $\chi^2 = 40·0$, $P < 0·0001$). Each seed was treated as a categorical response (E+ or E−).

regression, which tested whether the number of days in the oven affected the probability that a seed germinated (likelihood ratio $\chi^2 = 0·005$, $P = 0·9$, $n = 7–29$ seeds per heat treatment level).

PLANT GROWTH

When combined in MANOVA, estimates of plant growth were significantly greater for endophyte-infected plants, but only under low water availability (Table 1, MANOVA *a priori* contrast low water: E+ vs. E−, $P = 0·0053$). At the final census (census 2), E+ plants produced, on average, 24 more leaves than E− under low water, but only nine more leaves (a non-significant difference) under high water (Fig. 2; Table 1). Furthermore, under low water, E− plants had significantly greater leaf senescence (29% greater on census 1) than E+ plants (Fig. 3; Table 1). Finally, shoot, root and total biomass of E+ plants was significantly greater than E− plants, but only in the low water treatment (Fig. 4a–c; Table 1, low water: E+ vs. E−, all $P < 0·05$). Across both endophyte treatments, low water significantly increased leaf senescence (Table 1, water $P = 0·0026$) and reduced root biomass (Table 1, water $P = 0·0193$).

ABOVE-GROUND PLANT TRAITS

MANOVA revealed that above-ground plant traits responded to the endophyte treatment under low water availability (Table 2, *a priori* contrast low water: E+ vs. E−, $P = 0·0456$). Individual traits had treatment-specific responses. Leaf length was, on average, 8% shorter for endophyte-infected plants regardless of the water treatment (Fig. 5a; Table 2, endophyte $P = 0·0218$). Total leaf area, however, was not significantly affected by either treatment (Table 2). Under low water, leaf relative water content was significantly greater for E− than E+ plants, consistent with the hypothesis that E+ plants up-regulate water conservation mechanisms sooner than E− in response to water stress (Fig. 5b; Table 2). Only the water treatment affected water use efficiency, which was significantly greater (i.e. more positive δ^{13}C) under low water than high water, although the magnitude of difference was small (mean δ^{13}C ± SE, low water: E+ = −28·03 ± 0·14, E− = −28·06 ± 0·20; high water: E+ = −28·48 ± 0·22, E− = −28·57 ± 0·17; Table 2, $P = 0·0157$).
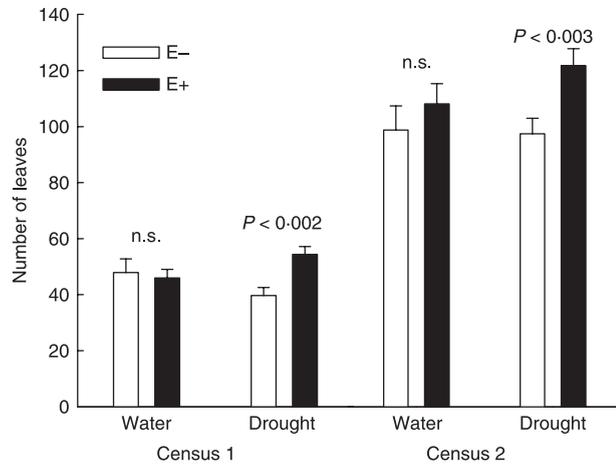
Fig. 2. Effects of the fungal endophyte and water availability treatments on leaf number in *Poa alsodes*. Bars indicate means ± SE. Dark bars are E+ = plants cloned from plants naturally infected with endophyte. Open bars are E– = plants cloned from experimentally disinfected plants. Significant differences between treatments are noted on top of the bars. Sample sizes were E+ low water = 19; E+ high water = 20; E– low water = 19; E– high water = 19.

BELOW-GROUND PLANT TRAITS

Below-ground traits responded less to the endophyte or water treatments than above-ground traits (endophyte effect, MANOVA $F = 2.6$, $P = 0.0473$). The only treatment that significantly altered root morphology was water availability. Low water increased maximum root hair length by 18% regardless of the endophyte (means (mm) ± SE, low water = 0.77 ± 0.04, high water = 0.65 ± 0.04; $F = 6.4$, $P = 0.0162$). There were no significant treatment effects on the root : shoot ratio (Fig. 4d), root diameter, mean root hair length or root hair density (all $P > 0.07$).
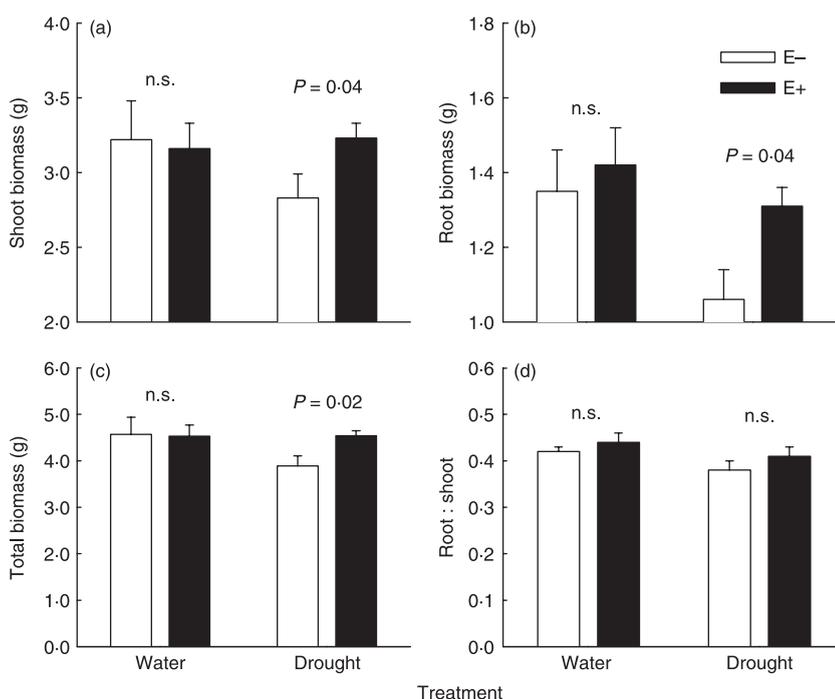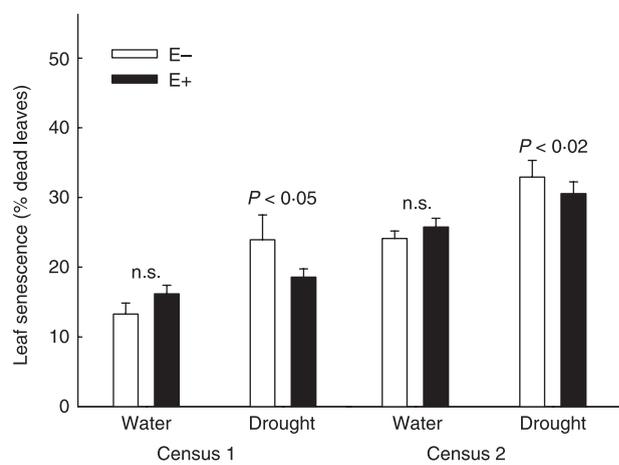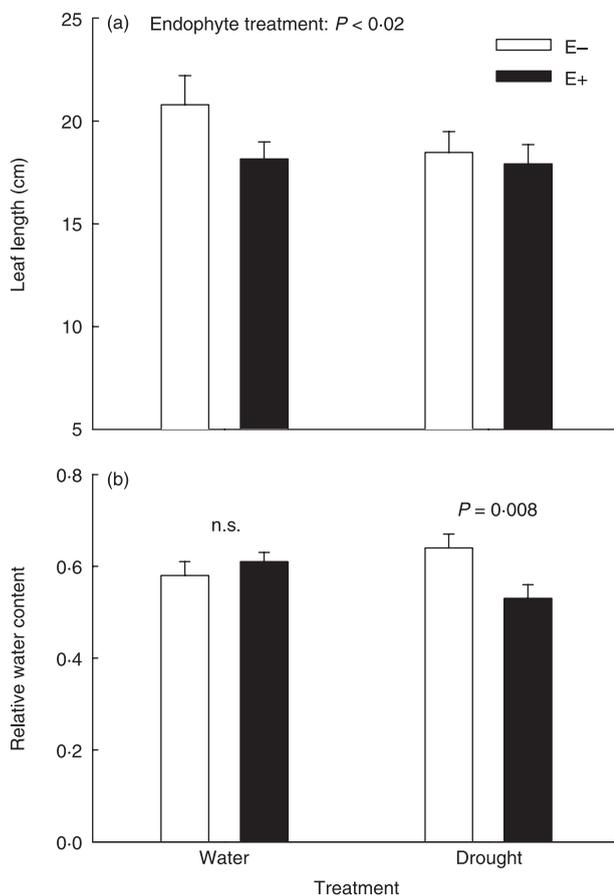

Fig. 3. Effects of the fungal endophyte and water availability treatments on leaf senescence (% of total leaves that were dead) in *Poa alsodes*. Bars indicate means ± SE. Sample sizes and labelling conventions as in Fig. 2.

Discussion

Here we show that symbiosis between the rare plant, grove bluegrass and a fungal endophyte (*Neotyphodium* sp.) can become more beneficial to the plant when water becomes more limiting. Our work expands understanding of the ecological consequences of grass-endophyte symbioses in natural ecosystems and suggests that endophyte benefits may be more common in non-agronomic systems than previously predicted (Faeth 2002; Saikkonen *et al.* 2006). The benefits of plant symbionts may be especially important in a changing climate. Under current climate models, it remains unclear how increases in surface temperature will interact with predicted changes in precipitation to affect soil moisture levels

Fig. 4. Effects of the fungal endophyte and water availability treatments on (a) shoot biomass, (b) root biomass, (c) total biomass and (d) root : shoot ratio of *Poa alsodes*. Bars indicate means ± SE. Sample sizes and labelling conventions as in Fig. 2.

Table 2. Statistical results for Poa alsodes from M/ANOVA examining the effects of the endophyte treatment, water treatment and clone on above-ground plant traits: leaf length, leaf surface area, relative water content (RWC) and water use efficiency. *P*-values < 0·05 are shown in bold face

Effect	MANOVA		Leaf length		Leaf surface area		RWC		Water use efficiency	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Endophyte	1·3	0·29	5·5	**0·02**	2·1	0·16	2·1	0·15	0·1	0·75
Water	2·1	0·09	1·5	0·22	0·8	0·37	0·1	0·74	6·4	**0·01**
Endophyte × Water	2·7	**0·05**	1·0	0·33	0·0	0·96	5·7	**0·02**	0·0	0·85
Clone (Endophyte)	0·9	0·76	2·6	0·08	1·0	0·50	0·6	0·91	0·9	0·66
A priori contrast										
High water: E+ vs. E–	1·2	0·32	6·3	**0·01**	1·0	0·33	0·4	0·52	0·1	0·72
A priori contrast										
Low water: E+ vs. E–	2·7	**0·05**	4·1	**0·05**	1·1	0·30	7·6	**0·01**	0·0	0·92

Fig. 5. Effects of the fungal endophyte and water availability treatments on leaf length and relative water content (RWC) of leaves for *Poa alsodes*. Bars indicate means ± SE. Sample sizes and labelling conventions as in Fig. 2.

(Christensen *et al.* 2007). However, during the past century, the Indiana region experienced on average 1%–2% increases in the Palmer Drought Severity Index (the most commonly used index of drought, based on observed precipitation and temperature and local available data on soil moisture) (Trenberth *et al.* 2007). Given natural variation among grass populations in endophyte frequencies (e.g. Clay & Leuchtmann 1989) and maternal inheritance of endophytes by seeds, we predict that climate changes have the potential to affect endophyte frequencies, with increased selection for symbiosis under drier conditions.

To our knowledge, this is one of only two published studies to show that endophyte symbiosis can confer drought tolerance or avoidance for a native species in a non-agronomic context (the other is Morse *et al.* 2007). Specifically under low water conditions, endophyte-infected grove bluegrass had greater overall shoot, root and total biomass as well as more leaves and less leaf senescence than disinfected plants. Despite localization of the endophyte in above-ground tissues, the effects of endophytes appear to be stronger below-ground, increasing root biomass by 24% and shoot biomass by just 14%. Root biomass may be critical for the survival and persistence of perennial C3 grasses, particularly under water stress and in competition with other plant species (e.g. Kemp & Culvenor 1994; Nippert & Knapp 2007). In prior work, endophyte infection of the agronomic grasses, perennial ryegrass (Latch *et al.* 1985) and tall fescue (De Battista *et al.* 1990), has been linked to increased root biomass, but water was not manipulated in these earlier studies. Our experiment on this perennial grass lasted 12 weeks and was conducted under controlled greenhouse conditions. Longer term experiments in the field will be necessary to confirm the observed patterns.

We tracked nine plant traits to identify potential mechanisms through which the endophyte may confer benefits. Only one trait varied with both the endophyte and water treatments: The relative water content of leaves was significantly higher in disinfected plants than in endophyte-infected plants only under the low water treatment. This result is consistent with the hypothesis that the endophyte promotes water conservation strategies in the plant (Malinowski & Belesky 2000). This result contrasts with prior work on both tall fescue and perennial ryegrass, which found no significant influence of endophyte symbiosis on relative water content, although trends for tall fescue were in the same direction as reported here (Eerens *et al.* 1998; Assuero *et al.* 2006). In our study, endophyte-infected plants also had shorter leaf lengths, which could improve drought tolerance by reducing surface area for water loss, excepting that leaf area did not significantly differ between

endophyte treatments. Previous work revealed that endophyte infection of tall fescue led to decreased root diameters and increased root hair lengths, potentially increasing root surface absorption (Richardson *et al.* 1990; Malinowski *et al.* 1999). While we found increases in both maximum root hair length and water use efficiency in the low water relative to high water treatment, there was no significant effect of the endophyte on these traits, a result that is inconsistent with enhanced drought avoidance. It is possible that, under low water availability, disinfected plants produce longer root hairs to help offset their lower total root biomass. Since we did not measure water in the base of the tillers or monitor stomatal conductance or photosynthetic rate, these assays would be useful next steps for understanding mechanisms of drought tolerance (e.g. Belesky *et al.* 1987). Future studies could also assess how other factors, such as herbivory and nutrient uptake, are influenced by the endophyte in grove bluegrass.

Microbial mutualisms could play a significant role in how plant species cope with environmental stress. By understanding how complex interactions between plants and their symbionts vary with stress, we can make better predictions about how climate change will influence species distributions and abundances in the future. For grove bluegrass, we predict that reduced water availability will result in greater benefits of the symbiosis for plant growth.

Acknowledgments

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