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Does a foliar endophyte improve plant fitness under flooding?

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Abstract Although endophytic fungi are ubiquitous in plants, their full range of ecological effects has yet to be characterized, particularly in non-agronomic systems. In this study, we compared the responses of two congeneric bluegrass species to flooding. Both plant species co-occur in subalpine zones of the Rocky Mountains. Marsh bluegrass (*Poa leptocoma*) commonly hosts a vertically transmitted fungal endophyte (*Epichloë* sp.) and naturally grows in wetter conditions than does nodding bluegrass (*Poa reflexa*), which lacks an epichloid endophyte. We investigated the novel hypothesis that endophyte symbiosis promotes host fitness under flooded conditions, contributing to niche differentiation between the two bluegrass species. We used a factorial greenhouse experiment to test whether endophyte presence improved survival, growth, or reproduction of *P. leptocoma* under flooded versus non-flooded edaphic conditions by experimentally removing the endophyte from half of the plants. We compared *P. leptocoma* responses to those of the endophyte-free congener. In contrast to expectations generated from the natural distributions of the two plant species, endophyte presence was more beneficial to *P. leptocoma* under ambient soil moisture than under flooding. Increased benefits of symbiosis in drier soils are consistent with studies of other grass endophytes. Flooded soils also unexpectedly improved the growth of *P. reflexa* more than that of the wet habitat specialist, *P. leptocoma.* While our results demonstrate an overall benefit of fungal symbiosis in this system, ecological factors other than flooding per se likely underlie the observed geographical distributions of these congeneric grasses in nature.

Keywords Mutualism · Symbiosis · Niche · Microsite · Distribution · Biogeography

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Introduction

Mutualistic interactions may change the ecological niches of host organisms by influencing host responses to both abiotic and biotic factors. For example, some mutualisms promote niche expansion or niche shifts, potentially facilitating species coexistence with close competitors (Elias et al. 2008; Forister et al. 2011; Joy 2013; Kazenel et al. 2015; Müller and Godfray 1999). Niche shifts due to mutualisms could affect species

range limits and species responses to future climates, especially when mutualisms influence partner resilience to abiotic stress (Afkhami et al. 2014; Compant et al. 2010; Hoeksema et al. 2010; Kivlin et al. 2013).

Endophytic fungi form mutualisms with plants that can ameliorate both abiotic and biotic stress (Rodriguez et al. 2009). For example, ~30% of grasses host fungal endophytes in the Clavicipitaceae, including genus *Epichloë* (Leuchtmann 1992). Although endophytic fungi are ubiquitous in plants, their full range of ecological effects on hosts have yet to be characterized, particularly in non-agronomic systems (Malinowski and Belesky 2000; Rodriguez et al. 2009). Prior studies have mainly focused on endophyte-mediated increases in herbivore resistance (Schardl et al. 2007, 2013; Tanaka et al. 2005) and drought tolerance (Davitt et al. 2011; Hesse et al. 2005; Worchel et al. 2013). Fungal endophytes have also been documented to confer resistance to pathogens (Arnold et al. 2003; Niones and Takemoto 2014) and tolerance of nutrient deficiency, heat, and salinity (Malinowski and Belesky 2000; Rodriguez et al. 2008). A broader understanding of fungal endophyte effects on hosts could create new opportunities for managing or introducing fungal symbioses in both non-agronomic and agronomic ecosystems.

We focused on flood tolerance as a potential plant response to symbiosis with *Epichloë* species. Among prior work on endophyte-mediated plant water relations, studies examined thirteen native grass-*Epichloë* symbiota, all but two of which focused on amelioration of drought stress (Davitt et al. 2011; Emery et al. 2015; Gibert and Hazard 2011; Kane 2011; Kannadan and Rudgers 2008; Morse et al. 2002; Oberhofer et al. 2014; Ren et al. 2014; Rudgers and Swafford 2009; Saona et al. 2010; Zhang et al. 2011; Zhang and Nan 2007) rather than flood stress (Song et al. 2015). One prior study compared endophyte-symbiotic and symbiont-free clones of a tall fescue cultivar and found no benefit of symbiosis under flooding (Arachevaleta et al. 1989). A more recent study suggested that an endophyte benefits a native grass under flooding but did not experimentally manipulate endophyte presence, precluding assignment of causality to the symbiosis (Song et al. 2015).

Observed distributions of two bluegrass species in the Rocky Mountains of Colorado, USA led us to hypothesize that endophytes may influence flood tolerance in hosts. *Poa leptocoma* (marsh bluegrass) hosts a fungal endophyte in the genus *Epichloë* and often grows in wet habitats (Barkworth et al. 2007; Kazenel et al. 2015). An endophyte-free congener, *Poa reflexa* (nodding bluegrass) commonly grows in the same geographic regions as *P. leptocoma* but is typically found in drier microsites (Barkworth et al. 2007; Kazenel et al. 2015). This natural distribution was unexpected because endophytes in the genus *Epichloë* are documented to promote drought (not flood) tolerance in hosts (Cheplick and Faeth 2009; Malinowski and Belesky 2000). Because *Epichloë* species associate with a variety of grass partners, their benefits to hosts may vary among host species and environments (Hamilton et al. 2012; Schardl 2010). In particular, the up-regulation of antioxidants in response to endophyte presence may benefit plants under multiple types of abiotic stress (Hamilton et al. 2012; Torres et al. 2012), including the oxidative stress associated with flooding roots (Song et al. 2015). Thus, it is possible that symbiosis with *Epichloë* sp. induces flood tolerance in host plants, thereby shifting host niche dimensions and contributing to ecological niche differentiation.

We used a factorial experimental design to test whether the presence of an *Epichloë* sp. improved survival, growth, or reproduction of *P. leptocoma* under flooded versus non-flooded edaphic conditions, and we compared plant responses to those of the endophyte-free congener, *P. reflexa*. Specifically, we addressed the question: does the fungal endophyte *Epichloë* sp. improve plant fitness under flooded conditions? We made two predictions: (1) Endophyte-bearing *P. leptocoma* will show no difference in fitness between flooded and non-flooded treatments, whereas endophyte-free *P. leptocoma* will have reduced fitness when flooded (Fig. 1), and (2) *Poa reflexa* will have lower fitness in flooded conditions relative to non-flooded conditions, based on its natural distribution in drier microsites and lack of an epichloid endophyte (Fig. 1).

Methods

Study organisms

Poa leptocoma and *P. reflexa* grow in subalpine to low alpine habitats in western North America, including the Colorado Rocky Mountains (Barkworth et al.

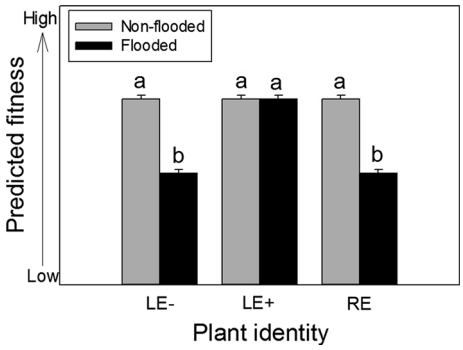

Fig. 1 Predicted fitness of endophyte-free *P. leptocoma* (LE−), endophyte-bearing *P. leptocoma* (LE+), and *P. reflexa* (RE) plants under non-flooded (*gray*) and flooded (*black*) treatments. We expected the endophyte to ameliorate flood stress, such that LE+ plants should achieve similarly high fitness in flooded and non-flooded conditions. The LE− and RE plants should have lower fitness when flooded because they lack the endophyte. Predictions were based on the plants' natural distributions in wetter (LE+) versus drier (RE) microsites

2007). The grasses commonly occur in wet meadows near streams, and while their distributions sometimes overlap, *P. leptocoma* tends to occupy wetter habitats than *P. reflexa* and can grow partially submerged in flowing water (Barkworth et al. 2007; Kazenel et al. 2015). *Poa leptocoma* has a larger geographic distribution (western North America to Alaska and west to Russia), whereas *P. reflexa* appears to be restricted to the central and southern Rocky Mountains in the United States. Our data on flowering phenology, collected in August–September 2010 at a site in the Gunnison National Forest (Colorado, USA; 38°58.314, −106°58.942, 3229 m elevation), where the species co-occur, showed that *P. reflexa* flowers earlier in the growing season than *P. leptocoma* and typically has produced seeds while *P. leptocoma* is still in the anther dehiscence phase of flowering.

Despite similarities in morphology, *P. leptocoma* and *P. reflexa* are estimated to be distant relatives within the *Poa* clade (Gillespie and Soreng 2005). *Poa* taxonomy is complex because many species are polyploid or apomictic, and they commonly hybridize (Barkworth et al. 2007). The genus, which likely originated in Eurasia, is currently estimated at 17.6–9.9 million years old, dating to the Middle Miocene (Giusanni et al. 2016), long after the formation of the Rocky Mountains, where the focal species reside. *Poa leptocoma* is a hexaploid species in section *Oreinos*, which is non-monophyletic (Soreng et al. 2010), and has not, to our knowledge, been subjected to molecular dating analysis. *Oreinos* is a circumboreal clade common to soggy areas and bogs in boreal, arctic, and alpine habitats (Anderton and Barkworth 2009). *Poa reflexa* is a tetraploid species placed in section *Homalopoa*, the biggest and most geographically widespread section of the genus (>170 species) (Anderton and Barkworth 2009). Although the origin of the *P. reflexa* lineage has not been dated, *Homalopoa* likely originated in Eurasia 8.4–4.2 million years ago (Giusanni et al. 2016). Subsequent diversification and radiation of the monophyletic, New World *Homalopoa* clade also occurred during the Late Miocene–Early Pliocene (Giusanni et al. 2016), a period of global cooling and drying, during which grassland ecosystems expanded (Herbert et al. 2016).

The focal grass species differ in their associations with foliar endophytes. *Poa leptocoma* hosts a vertically transmitted fungal endophyte in the *Epichloë typhina* subspecies *poae* subclade (Clavicipitaceae) previously described in Kazenel et al. (2015). In contrast, *P. reflexa* lacked an *Epichloë* species in all individuals of all populations that we surveyed in Colorado (Kazenel et al. 2015; Ranelli et al. 2015).

Seed sources and endophyte treatment

In late August 2009, we harvested seeds from naturally occurring *P. leptocoma* and *P. reflexa* individuals chosen randomly along transects in Virginia Basin, Gunnison County, Colorado (38°58.314, −106°58.942, elevation 3229 m). We stored the seeds at 4 °C following collection. To obtain *P. leptocoma* plants that lacked the fungal endophyte *Epichloë* sp., a subset of seeds were extracted from the lemma and palea and then treated with the fungicide benomyl (2 g/L) for five weeks on wet filter paper in standard plastic Petri plates. Control seeds were placed on Petri plates that contained only DI water. Following germination, plants were grown in 10-cm square pots for ∼6 weeks in the Rice University greenhouse (∼22 to 25 °C, no supplemental lighting) and were then transplanted into 6.4 cm diameter × 36 cm deep root-trainer pots (Stuewe and Sons, Tangent, OR). All pots contained a 50:50 mix of play sand and potting mix (MetroMix 220, Sun-Gro, Inc., Agawam, MA). We moved plants from the greenhouse to the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado (38°95.848, −106°98.909, elevation

2891 m) on July 8, 2011. At this time, we divided each plant into ramets of 1–2 tillers, which were then planted into 3.8 cm diameter × 14 cm root-trainer pots filled with a 1:1 mixture of sterile sand and potting mix. During July 2011, a first generation of 24 fungicide-treated plants and 25 control plants, each representing 10 genotypes, was transplanted into a common garden at the Virginia Basin site. Individuals were planted at 1-m intervals along three transects, spaced 2 m apart.

To obtain individuals that were one-generation distant from the original fungicide treatment, we harvested seeds from 21 individuals each of the fungicide and control plants that reproduced in the common garden field plot (August 2012). Seeds were stored at 4 °C following collection and were then planted into cone-tainers (3.8 cm diameter × 14 cm deep, Stuewe and Sons, Tangent, OR) filled with field soil collected from a nearby site at the RMBL on July 11–12, 2013. Due to low germination rates under field conditions, we moved the cone-tainers to a rooftop greenhouse at the University of New Mexico (Albuquerque, NM) on August 19, 2013. Sprayers misted the seeds daily, and 81% of seeds germinated within a month. We did not provide supplemental light, and we maintained a temperature range of ~15 to 24 °C. We confirmed endophyte status by staining thin sections of the inner leaf sheath with aniline blue lactic acid and examining at 200X (Bacon and White 1994). Once the seedlings had two to five tillers, we split each into two ramets with approximately equal root mass and 1–3 tillers each. When re-potting the ramets, we mixed soil from the RMBL field site in a 1:1 ratio with 360 MetroMix (Sun-Gro, Inc., Agawam, MA).

Flood treatment

Ramets were allowed to recover for ~2 weeks following re-potting and were then subjected to one of two flood treatments: 'flooded' or 'non-flooded.' One ramet from each original plant was assigned to each flood treatment, resulting in 24 *P. reflexa*, 25 endophyte-bearing (E+) *P. leptocoma*, and 22 endophyte-free (E−) *P. leptocoma* individuals ($N = 142$ plants) in each flood level. The plants were distributed across six trays and spaced with ~5 cm between plants to prevent shading and light competition.

The flood treatments began on December 3, 2013. For the flooded treatment, we added 33.4 mL of tap water to each plant to create and maintain flooding conditions. In addition, we placed flooded treatment pots into plastic bags (3.8 cm diameter × 14 cm deep) that retained water; thus roots were submerged in standing water for the duration of the flood treatment. No aboveground mass was submerged during treatment. We changed plastic bags on a monthly basis because they tended to leak after one month. Based on field measures of soil volumetric water content (Kazenel et al. 2015), we estimated that non-flooded conditions were 18% of flooded conditions, so non-flooded treatment plants received 6 mL of water. To impose these treatments, we hand-watered individual pots every Monday, Tuesday, Thursday, and Saturday. We did not place plants in the non-flooded treatment in plastic bags.

As the grasses grew, we increased the non-flooded watering level on January 4, 2014 from 6 to 25 mL to better match field soil volumetric water measurements. We did not change flooded conditions because soils were completely water-saturated. Due to rapid plant growth, plants were transplanted into larger cone-tainers (6.4 cm diameter × 25.4 cm deep, Stuewe and Sons, Tangent, OR) in early March 2014, and watering amounts were re-adjusted following transplanting. Based on the water holding capacities of the new cone-tainers, flooded plants received 232 mL of water into plastic bags and non-flooded plants received 162 mL per watering event.

Pest control

Two outbreaks of pests occurred during the greenhouse experiment. First, spider mites (*Tetranychus urticae*) appeared during the second month of treatments and were eliminated within a week. Second, bird cherry oat aphids (*Rhopalosiphum padi*) appeared towards the beginning of the final month and were never completely eliminated, though we regularly patrolled and manually killed them. We used Avid Miticide (Syngenta Crop Protection, LLC, Greensboro, NC) and Fertilome Triple Action (Harwell's Green Thumb Nursery Inc., Montgomery, AL) on the spider mites and Safer Brand Insect Killing Soap (Safer Brand, Lititz, PA) and Fertilome Triple Action on the aphids, following manufacturer's instructions.

Response variables

We counted initial tillers and leaves on each plant on December 5, 2013. We subsequently counted tillers and leaves once per month to measure plant growth. To estimate reproduction, we counted and harvested flowering tillers and seeds as they were produced.

We harvested all plants on June 3–4, 2014, six months after the flood treatments began. For each plant, we separated aboveground and belowground biomass and rinsed roots with tap water over a metal sieve (1 mm mesh). We weighed the biomass after drying for 24 h in a convection oven at 60 °C. We determined final aboveground, belowground, and total biomass, as well as the root-to-shoot ratio.

Statistical analyses

If endophyte presence altered host plant response to flooding, then we would expect a statistically significant interaction between plant identity and flood treatment, with the observation that endophyte-bearing *P. leptocoma* were more buffered against flood stress than either endophyte-free *P. leptocoma* or the congener, *P. reflexa*. We used multivariate analysis (MANOVA) including the independent variables of watering treatment and plant identity (3 levels: *P. leptocoma* E+, *P. leptocoma* E−, and *P. reflexa*). We included plant genotype (nested within plant identity) as a random factor to account for the non-independence of ramets that were subjected to each watering treatment. Model comparisons (via *AICc*) showed better fits for models that included plant genotype than models without. MANOVA included the dependent variables of average tiller count (over the four census dates), average leaf count, number of seeds produced, and above/belowground biomass (Proc MIXED using restricted maximum likelihood and an unstructured variance–covariance matrix, SAS v.9.3, SAS Institute Inc., Cary, NC). In order to meet assumptions of normally distributed residuals and homogeneity of variances, we log-transformed the number of seeds and belowground biomass. When treatments significantly affected overall plant fitness estimates in MANOVA, we conducted individual ANOVA on each response variable to isolate the treatment effects on different components of plant fitness. For the repeatedly measured variables of tiller count and leaf count, we used repeated measures, mixed model ANOVA. When treatment interactions were statistically significant, we used a Tukey–Kramer adjustment for multiple comparisons to decompose differences among the means. In addition, we used mixed model ANOVA to examine how plants allocated to roots versus shoots; the root-to-shoot ratio was log-transformed. All statistical analyses were conducted in SAS v. 9.3 (SAS Institute Inc., Cary, NC).

Comparison against root endophyte-mediated flood tolerance

In order to further investigate the hypothesis that endophyte symbiosis ameliorates flood stress in plants, in November 2016, we conducted a comprehensive literature search via Web of Science to determine if foliar and root symbionts have similar effects on host plants under flooding. We applied the following search terms: (endophyt* or mycorrhiza*) and (waterlog* or flood*). We filtered to include only studies with fungi and freshwater to improve comparison against our work. The search yielded 1232 publications. We then applied the following criteria to filter our literature search: (1) the study must include experimental, factorial manipulation of symbionts and flooding and (2) the study must measure at least one plant fitness or function metric. There were 17 total studies that met these criteria (Supplementary Material). We then recorded the one fitness and/or one function metric from each study that showed the strongest interaction between symbiont and flood treatments. We applied vote counting to this subset of records to indicate cases in which the symbiont increased, reduced, or had no significant effect on measures of plant flood tolerance. The vote counting method is a liberal estimate of symbiont mediation of flood response because we picked a maximum of two responses measured in each study: one related to plant fitness and one related to function. We used this analysis to contextualize our results on the influence of foliar endophytes on flood tolerance within the current literature on root endophytes.

Results

Endophyte presence did not promote fitness in flooded *Poa leptocoma*

We expected *P. leptocoma* fitness to be highest when the endophyte was present and soils were flooded, but our data did not support this prediction. Endophyte presence increased *P. leptocoma* aboveground biomass by ~40% under non-flooded conditions, but conferred no significant benefit under flooding (Fig. 2a; Table 1). Other growth responses, including belowground and total biomass (Figs. 2b, 3a; Table 1) and tiller and leaf production (Fig. 2c, d; Table 2) did not significantly differ between non-flooded and flooded *P. leptocoma* plants. Endophyte presence did however confer some benefits regardless of flooding. Endophyte-bearing *P. leptocoma* had ~40% more belowground biomass and ~25% more total biomass than endophyte-free *P. leptocoma* (Fig. 2b, 3a).

Survival and reproduction responses also did not support our prediction that *Epichloë* sp. promotes host fitness under flooding. Survival was not a major fitness factor in this experiment, as only three endophyte-bearing and three endophyte-free *P. leptocoma* plants died. Seed production did not differ significantly between non-flooded and flooded plants (Fig. 3b; Table 1). However, 73% (32 of 44) of the endophyte-free *P. leptocoma* produced seeds during the experiment, while only 2% (1 of 50) of the endophyte-bearing *P. leptocoma* reproduced, demonstrating a possible effect of symbiosis on the timing of plant reproduction (Fig. 3b; Table 1). Because these perennial grasses are long-lived, it remains unclear whether the early reproduction of endophyte-free plants has net fitness benefits or net fitness costs.

Flooding increased the fitness of *Poa reflexa*

Contrary to our prediction that *P. reflexa* would be less fit in flooded conditions, flooding *P. reflexa* increased its fitness estimates. Flooded *P. reflexa* grew ~30% more aboveground biomass and tillers than non-flooded *P. reflexa* (Fig. 2a, c). In addition, *P. reflexa* leaf production was 15% higher when flooded (Fig. 2d). There was no significant difference in total or belowground biomass between flooded and non-flooded *P. reflexa* (Fig. 2b, 3a; Table 1). Only three *P. reflexa* plants died, and no *P. reflexa* reproduced during the experiment (Fig. 3b), indicative of a difference in life history strategy relative to *P.*

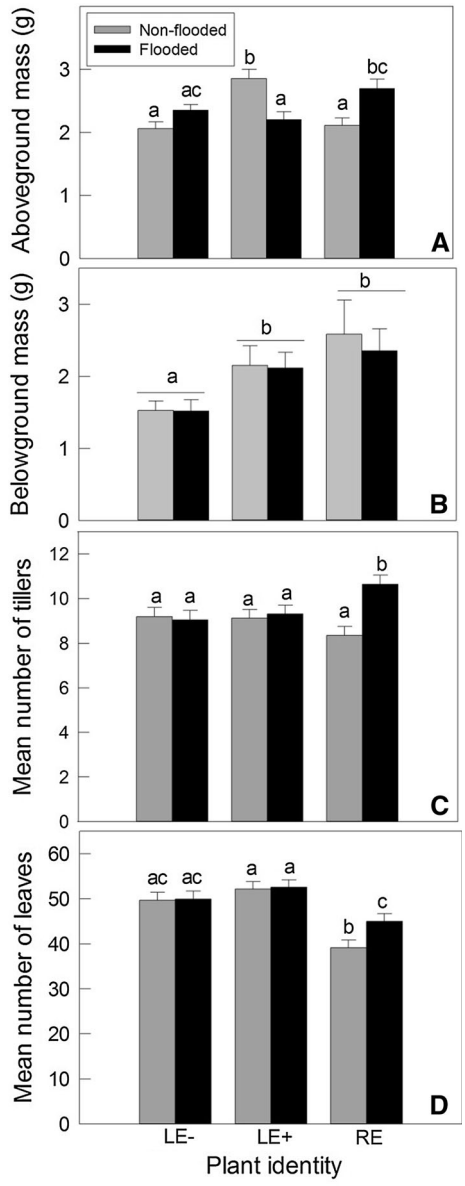

Fig. 2 Mean (±1 SE) aboveground, belowground, and foliar growth responses of endophyte-free *P. leptocoma* (LE−), endophyte-bearing *P. leptocoma* (LE+), and *P. reflexa* (RE) plants under non-flooded (*gray*) and flooded (*black*) treatments. **a** Aboveground biomass (plant identity × flood treatment $p < 0.0001$). **b** Belowground biomass (plant identity $p = 0.007$). **c** Mean number of tillers through time (plant identity × flood treatment $p = 0.0015$). **d** Mean number of leaves through time (plant identity × flood treatment $p = 0.0241$). Means or pairs of means annotated with *different letters* significantly differed

Table 1 Results of statistical models testing the effects of plant identity (endophyte-free *P. leptocoma*, endophyte-bearing *P. leptocoma*, or *P. reflexa*), flood treatment (non-flooded or flooded), and their interaction on (a) MANOVA: aboveground biomass, belowground biomass, average tiller production, average leaf production, and number of seeds produced, (b) aboveground biomass, (c) belowground biomass, (d) total biomass, (e) number of seeds produced, and (f) root-to-shoot ratio

Root-to-shoot ratio, belowground biomass, and seed number data were log-transformed prior to analysis

p-values < 0.05 are shown in bold

	Num *df*	Den *df*	*F* ratio	*p*
(a) MANOVA				
Plant identity	2	69	16.36	**<0.0001**
Flood treatment	1	69	0.29	0.5919
Plant identity × flood treatment	2	69	6.14	**0.0035**
(b) Aboveground biomass				
Plant identity	2	68	2.98	0.0576
Flood treatment	1	65	0.51	0.4767
Plant identity × flood treatment	2	65	14.43	**<0.0001**
(c) Belowground biomass				
Plant identity	2	68	5.40	**0.0067**
Flood treatment	1	65	0.04	0.8416
Plant identity × flood treatment	2	65	0.00	0.9969
(d) Total biomass				
Plant identity	2	68	5.02	**0.0092**
Flood treatment	1	65	0.17	0.6772
Plant identity × flood treatment	2	65	2.45	0.0939
(e) Number of seeds				
Plant identity	2	68	76.91	**<0.0001**
Flood treatment	1	67	1.66	0.2025
Plant identity × flood treatment	2	67	0.71	0.4950
(f) Root-to-shoot ratio				
Plant identity	2	68	3.80	**0.0272**
Flood treatment	1	65	0.60	0.4429
Plant identity × flood treatment	2	65	3.74	**0.0289**

leptocoma, for which endophyte-free individuals did flower during the experiment.

Plant allocation patterns

Root-to-shoot ratios compared how plants allocated resources in response to our treatments. The two bluegrass species invested differently (Fig. 4; Table 1). For *P. reflexa*, flooding decreased the root-to-shoot ratio, while for endophyte-bearing *P. leptocoma* flooding increased the root-to-shoot ratio, though these differences were non-significant (Fig. 4). The only significant difference occurred between flooded endophyte-free *P. leptocoma* (lowest ratio) and non-flooded *P. reflexa* (highest ratio). However, when flooded, endophyte-bearing *P. leptocoma* had a 58% higher root-to-shoot ratio than endophyte-free *P. leptocoma*, a difference that was only marginally non-significant (Fig. 4).

Flood tolerance mediated by root endophytes

In the 17 published studies uncovered in our filtered literature search, we found 27 total cases of experimental work on root endophytes and plant flood tolerance (Table 3). Half of the studies featured agronomic systems, and the majority of the studies (85%) featured arbuscular mycorrhizal fungi. Root endophytes generally had a neutral effect on host plant response to flood stress, although they ameliorated flood stress in 30% of cases (Table 3).

Discussion

Based on the natural history and microsite distributions of our focal grasses, we originally made two predictions: (1) Flooding will have minimal effect on *Epichloë*-symbiotic *P. leptocoma*, but flooding will

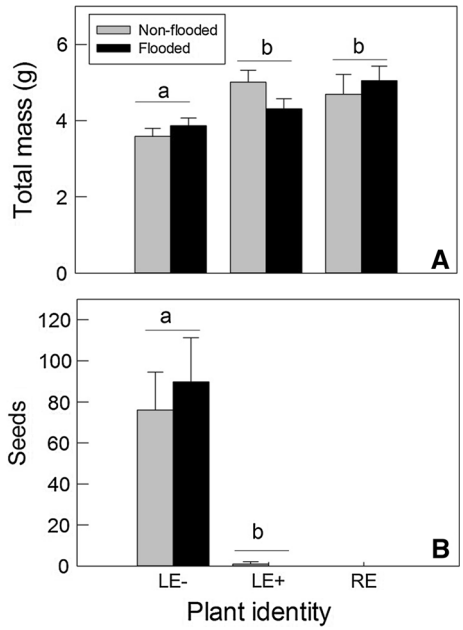

Fig. 3 Mean (±1 SE) total biomass and seed production of endophyte-free *P. leptocoma* (LE−), endophyte-bearing *P. leptocoma* (LE+), and *P. reflexa* (RE) plants under non-flooded (*gray*) and flooded (*black*) treatments. **a** Total biomass (plant identity $p = 0.0092$). **b** Number of seeds produced per plant, including zeros for non-reproductive plants (plant identity $p < 0.0001$). Pairs of means annotated with *different letters* significantly differed

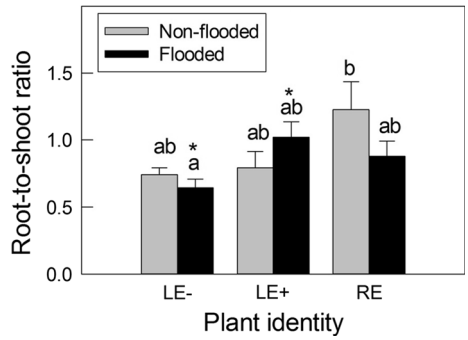

Fig. 4 Mean (±1 SE) root-to-shoot ratio of endophyte-free *P. leptocoma* (LE−), endophyte-bearing *P. leptocoma* (LE+), and *P. reflexa* (RE) plants under non-flooded (*gray*) and flooded (*black*) treatments. Means annotated with *different letters* significantly differed (plant identity × flood treatment $p = 0.0289$). *Asterisks* indicate a pairwise difference that was marginally non-significant ($p = 0.09$)

reduce fitness when the symbiont is removed and (2) flooded conditions will reduce the fitness of *P. reflexa* relative to non-flooded conditions (Fig. 1). Our results did not support the prediction that the epichloid endophyte contributes to ecological niche differentiation between *P. leptocoma* and *P. reflexa* by promoting host fitness under flooded conditions. Instead, we found increased benefits of symbiosis in non-flooded soils, consistent with prior studies on

Table 2 Results of statistical models testing the effects of plant identity (endophyte-free *P. leptocoma*, endophyte-bearing *P. leptocoma*, or *P. reflexa*), flood treatment (non-flooded or flooded), time since initial counting of tillers and leaves (days), and all possible interactions on (a) number of tillers per plant and (b) number of leaves per plant

	df	χ^2	*p*
(a) Number of tillers			
Plant identity	2	34.66	**<0.0001**
Flood treatment	1	3.45	0.0631
Plant identity × flood treatment	2	7.67	**0.0216**
Time	3	422.94	**<0.0001**
Time × plant identity	6	25.48	**0.0003**
Time × flood treatment	3	14.34	**0.0025**
Time × plant identity × flood treatment	6	10.15	0.1183
(b) Number of leaves			
Plant identity	2	0.35	0.8392
Flood treatment	1	5.86	**0.0155**
Plant identity × flood treatment	2	13.66	**0.0011**
Time	3	756.78	**<0.0001**
Time × plant identity	6	35.18	**<0.0001**
Time × flood treatment	3	1.68	0.6411
Time × plant identity × flood treatment	6	4.29	0.6372

p-values < 0.05 are shown in bold

Table 3 Vote counting table derived from a review of the root endophyte literature. Amelioration indicates that having a fungal symbiont either decreased the negative effect of flooding, or in cases where flooding was beneficial, enhanced the positive effect. Worsening indicates that a fungal symbiont magnified the negative effect of flooding, or in cases where flooding was beneficial, reduced the positive effect. Neutral indicates cases where the interaction between fungal symbiont presence and experimental flooding was non-significant. The table also indicates the percentage of studies conducted in agronomic versus non-agronomic systems and featuring arbuscular mycorrhizal fungi, rhizosphere soil microbes, or ectomycorrhizal fungi

Total cases	$N = 27$
Effect on plant response to flooding	
Amelioration	30%
Worsening	26%
Neutral	44%
Study system	
Agronomic	50%
Non-agronomic	50%
Fungal symbiont type	
Arbuscular mycorrhizal fungi	85.2%
Rhizosphere soil microbes	11.1%
Ectomycorrhizal fungi	3.7%

other native grass-endophyte associations in which endophytes were beneficial in drier soils (reviewed by Cheplick and Faeth 2009; Malinowski and Belesky 2000). Alternative ecological factors may therefore underlie the observed distributions of *P. leptocoma* and *P. reflexa* in nature.

To our knowledge, just two previous studies have subjected grass-*Epichloë* symbiota to flooding. Arachevaleta et al. (1989) compared symbiotic and symbiont-free tillers of one clone (genotype) of tall fescue grass, but the endophyte did not affect plant response to flooding. Song et al. (2015) compared naturally symbiotic and symbiont-free individuals of *Hordeum brevisubulatum*; however, because the endophyte was not experimentally manipulated, it was not possible to separate the endophyte effect from differences due to plant genotype. In flooded *H. brevisubulatum*, endophyte presence was associated with greater root vitality, root biomass, tiller production, and chlorophyll content, along with lower leaf wilt rates, than occurred in endophyte-free plants (Song et al. 2015). Differences in plant physiology included wider leaves, a larger initial proline burst, and stronger increases in malondialdehyde content and electrolyte leakage, all of which may influence plant osmotic potential (Song et al. 2015). Compared to Song et al. (2015), we observed approximately twice the effect size in root biomass ($\sim$40% higher) for endophyte-bearing compared to endophyte-free *P. leptocoma*, but we lack data on associated physiological responses. Recent studies have suggested antioxidant up-regulation as a potent mechanism underlying the benefits of symbionts to plants experiencing stress (Hamilton et al. 2012; Torres et al. 2012). If such effects are common in hosts of *Epichloë* species, then they could explain benefits to plants under both drought and anaerobic stress. However, in our system, endophyte benefits were clearly stronger in drier soils than in standing water, suggesting there is not a single mechanism underlying benefits to host plants under abiotic stress.

Our results align with prior evidence of opposing effects of endophyte symbiosis at different host life history stages (e.g., Chung et al. 2015; Gundel et al. 2012; Rudgers et al. 2012). First, despite their overall higher biomass, endophyte-symbiotic *P. leptocoma* plants produced fewer seeds than endophyte-free individuals, suggesting a negative effect of symbiosis on early host reproductive effort. Because these plants are long-lived (some of our field-marked adults are at least eight years old), the net fitness effect of early reproduction cannot be assessed from greenhouse experiments. Second, in our prior 3-year field experiment, endophyte presence constrained the germination and seedling survival of *P. leptocoma* to wet microsites (Kazenel et al. 2015). However, endophyte symbiosis ultimately provided a net benefit to early plant growth in the field, regardless of microsite moisture conditions (Kazenel et al. 2015). Similarly, in the present study, the endophyte conferred growth benefits in both watering treatments. Only aboveground biomass showed strong context dependency in endophyte benefits, with higher benefits in drier soils. Combined across these two studies, our results support the interpretation that the endophyte contributes to niche differentiation between the two bluegrasses by initially constraining *P. leptocoma* germination and seedling survival to wetter microsites, rather than by providing a fitness benefit in such sites. However, results thus far do not explain the enigma of *P.*

reflexa's distribution, leaving unresolved the question of why this species occurs in drier microsites when it clearly benefits from flooded conditions.

Alternative hypotheses to explain niche differentiation between *P. leptocoma* and *P. reflexa* may include variation in the nutrient content of flooded and non-flooded soils. Some grasses symbiotic with *Epichloë* species exhibit improved uptake of soil nutrients, including phosphorus and calcium, in nutrient poor soils (Lewis 2004; Malinowski and Belesky 1999; Rahman and Saiga 2005). In our greenhouse experiment, plastic bags would have retained nutrients as well as water in the flooded pots, an effect that would not occur in nature. Flooding can flush nutrients from soils, with particularly strong effects on phosphorus, calcium, and nitrate (Kozlowski 2012; Reddy et al. 1984). Future experiments could assess plant performance under manipulated soil nutrient concentrations to evaluate this alternative hypothesis.

Endophyte-mediated alteration of plant traits appears to be common in grass hosts (reviewed by Malinowski and Belesky 2000). Our results suggest that endophyte symbiosis alters how host plants allocate resources under flood stress. Specifically, under flooding, endophyte presence increased the root-to-shoot ratio of *P. leptocoma* by 58% relative to endophyte-free plants (Fig. 4), whereas under non-flooded soil moisture, the root-to-shoot ratio was only 9% higher in symbiotic plants. In endophyte-free *P. reflexa*, the root-to-shoot ratio was highest under non-flooded soil moisture, similar to the response of endophyte-free *P. leptocoma*. Prior work on flooded *H. brevisubulatum* also showed increases in root biomass of endophyte-symbiotic plants subjected to waterlogging, along with other physiological changes (Song et al. 2015). Under drought stress, a number of endophyte-mediated trait changes can occur, including earlier closure of stomata, increased water storage in tiller bases, production of phenolic compounds, and increased concentrations of non-structural carbohydrates and loline alkaloids in tillers (Malinowski and Belesky 2000; Nagabhyru et al. 2013; Torres et al. 2012). Detailed characterization of the precise physiological mechanisms that underlie the responses reported here would improve understanding of how endophytes affect plant responses to soil moisture.

Plant nutritional status needs to be examined in more depth in flooded plant-endophyte systems. In our study, plant fitness was mainly assessed through measures of biomass accumulation, but prior findings suggest that effects on plant nutritional status are also important, and could offer clues about long-term benefits, particularly when plant biomass responses are non-significant. This is especially true regarding the content of specific nutrients such as phosphorus and nitrogen. Endophytes have been shown to influence plant nutritional status under controlled conditions. For example, dark septate endophytes in plant roots tend to increase nitrogen and phosphorus content in plant shoots, especially when the plants are <5 months old (reviewed by Newsham 2011). Endophytes may also improve or maintain plant nutrient status under flood stress. When we reviewed the literature for cases of root endophytes ameliorating flooding stress, we found amelioration in 30% of cases (Table 3). Half of those cases ($\sim$15% of the total) occurred when symbionts improved plant functional traits under flooding, including components of nutritional status. For example, root phosphorus uptake (Hajiboland et al. 2009), chlorophyll *a* and *b* contents (Tuo et al. 2015) and proline content (Tuo et al. 2015; Wu et al. 2013) all increased under symbiosis. In the remaining cases in which symbiosis ameliorated flood stress, symbionts mainly altered biomass accumulation, namely aboveground mass in studies on grasses or trees (Orchard et al. 2016; Tikvic et al. 2007; Zou et al. 2014). Symbionts may also reduce plant nutritional status under flood stress; for example, root phosphorus uptake actually decreased in one case investigating the effects of the arbuscular mycorrhizal fungus *Rhizophagus irregularis* and in cases investigating the effects of rhizosphere microbial communities on plants under flooding (Deepika and Kothamasi 2015). In our study, and in the literature we reviewed, plant nutritional status was not typically addressed together with biomass accumulation in the same publication or study system. Thus, in the future, combined assessments of plant fitness estimates and functional traits could provide further insight into the physiology and functional roles of fungal endophytes.

Anthropogenic change may cause species range shifts, potentially through altered aboveground–belowground interactions (Van der Putten 2012). Understanding the effects of mutualisms on partner species and their degree of context dependency is important for predicting constraints on species range limits and responses to future climates (Afkhami et al. 2014; Compant et al. 2010; Kivlin et al. 2013). Our work

suggests that endophyte effects on adult plant responses to soil moisture alone are not sufficient to explain differences in the niche dimensions and distributions of congeneric bluegrass species. Our study adds to growing evidence of differential effects of symbionts among host life history stages, and shows that benefits of foliar fungal symbiosis depend on the abiotic context.

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