

An Epichloë endophyte alters the ecology of Poa leptocoma

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Abstract

Determining mechanisms behind species ranges is vital to creating the most accurate models of species distributions under climate change. Mutualisms are an important factor to consider when examining mechanisms behind range limits of host plants, as they can have a large influence on community organization, competition, population distributions and landscape-scale community dynamics. Mutualistic relationships with endophytic fungi are ubiquitous in vascular plants, and can have strong effects on the individual fitness of host plants. The advantages gained from fungal endophytes can allow host species to have a broader realized niche than their fundamental niche would typically allow. In this experiment conducted in a subalpine meadow in the Rocky Mountains, USA, we examined the effects of a vertically transmitted fungal endophyte in the *Epichloë* genus on the distribution of *Poa leptocoma*. The observed range of *Poa leptocoma*, which always is found with the *Epichloë* endophyte, lies in wetter soils. We found that endophyte-symbiotic individuals of *Poa leptocoma* had a lower survival rate in wetter conditions, but produced more tillers and seeds in wetter conditions. These results demonstrate that the effects of the endophyte vary with host demography, that symbionts have the potential to affect host distribution, and that *Poa reflexa* may have a previously undocumented cryptic seed-borne pathogen.

Introduction

Making accurate ecological predictions of species responses to climate change will require understanding future shifts in species distributions. Relative to antagonisms, positive species interactions have received little attention as factors influencing a species range (Bruno et al. 2003). The presence of a mutualistic relationship can have a large influence on community organization, competition, population distributions and landscape-scale community dynamics (Bruno et al. 2003). Mutualisms are an important factor to consider when examining mechanisms behind range limits of host plants, and the effects of these interactions are important to investigate in order to create accurate models of how species respond to climate change (Afkhami et al. in prep).

Studies examining mutualisms have shown that these relationships can affect community composition and dynamics (Umbanhower and McCann 2005). In some cases, a mutualist may expand its host's range, and in others it may constrain it. An example of a mutualist expanding its host's niche by providing competitive dominance is seen in *Lycaeides melissa* caterpillars, whose mutualistic relationship with ants allowed them to expand their niche to a new host plant (Forister et al. 2011). In contrast, an example of a mutualist constraining the range of its host is *Erigone Atra*, which has an endosymbiont that negatively affects its long-range airborne dispersal (Goodacre et al. 2009).

Mutualistic relationships with endophytic fungi are ubiquitous in vascular plants, and can have strong effects on the individual fitness of host plants (Morse et al 2002, Clay and Schardl 2002). The advantages gained from fungal endophytes can allow host species to have a broader realized niche than their fundamental niche would typically allow (Bruno et al. 2003). Endophytic fungi provide a number of benefits for the host plant. These include enhanced

nutrient uptake, protection against plant pathogens via the production of antibacterial secondary metabolites and extra cell wall deposits, enhanced growth, protection against herbivory through alkaloid production, and tolerance against drought, heat, low light availability and low soil fertility (Clay and Schardl 2002, Gao et al. 2010). These effects can allow the host plant to garner more resources, escape constraints and gain a competitive advantage, with possible effects on plant distributions and densities (Bruno et al. 2003).

The effects of the presence of fungal endophytes in grasses under contrasting water availability have been examined in other study systems, mostly in species of fescue. In Arizona fescue, which has a mutualistic relationship with a *Neotyphodium* endophyte, it was found that endophyte-free individuals produced more biomass and had higher growth rates with high water availability, while endophyte-positive plants produced more biomass under low water availability. It was reported in this study that endophyte-positive plants conserved soil moisture more efficiently through lower water vapor conductance and transpirational losses. Subsequently, once water stress was imposed in the form of lower water availability, they had higher soil moisture availability than endophyte-negative plants (Morse et al. 2002). In *Poa alsodes*, a grass infected with a *Neotyphodium* endophyte, it was found that the presence of an endophyte had increased growth under drought stress as compared to endophyte-negative plants. Under high water availability there was no significant difference in performance between endophyte-positive and endophyte-negative plants (Kannadan and Rudgers 2008).

This study focused on the ability of endophytic fungi to shift the niche of their host plant. To do this, we investigated two grass species, one of which forms a mutualistic relationship with a systemic, vertically transmitted fungal endophyte from the genus *Epichloë*, family Clavicipitaceae. *Epichloë* endophytes grow in the intercellular spaces of their host, where they

subsidist on sugars and amino acids in the apoplast. Some *Epichloë* reproduce through the plant's seeds and ovules, thereby infecting host seeds (Kazenel et al. sin review; Clay & Schardl, 2002). We investigated how an *Epichloë* endophyte may alter the niche of *Poa leptocoma* and *Poa reflexa* in subalpine meadows of the Rocky Mountains, USA (see figure 1). *Poa letocoma* and *Poa reflexa* are two morphologically similar grass species that grow in western North America in subalpine meadows and forest understory near bodies of water. *Poa leptocoma* has a mutualistic relationship with an *Epichloë* endophyte, while *Poa reflexa* does not. *Poa leptocoma* grows most often in wetter habitats, although these species do co-occur (Barkworth et al. 2007). Recent results have focused on the early life history and seedling stage of these grasses, and here we compare adult performance over a period of four years, testing two questions through the manipulation of soil moisture: 1) Does the presence of an *Epichloë* endophyte affect the survival, growth, or reproductive fitness of *P. leptocoma*? and 2) Does the presence of an *Epichloë* endophyte alter the realized niche of *P. leptocoma* relative to its congener, *P. reflexa*?

Methods

Study System

We examined two ecologically and morphologically similar species, *Poa leptocoma* and *Poa reflexa*. These two species are difficult to distinguish with morphological characters, apart from *Poa leptocoma* having scabrous panicle branches, while *Poa reflexa* has smooth branches (Barkworth et al. 2007). *Poa leptocoma* has a mutualistic relationship with a vertically transmitted *Epichloë* endophyte, whereas *Poa reflexa* is endophyte-free (Kazenel et al. in review). *Poa leptocoma* (marsh bluegrass) and *Poa reflexa* (nodding bluegrass) are both perennial grasses native to most of the western United States (see figures 2 & 3).

Fungal endophyte removal

Prior to greenhouse propagation, a benomyl treatment was applied to the treatment plants representing both species on 13 October 2010. In order to control for any direct effects of benomyl, a fungicide used to experimentally remove the *Epichloë* endophyte from our *Poa leptocoma* plants, was also applied to *Poa reflexa* individuals that were part of the treatment population. This treatment entailed germinating seeds in 2% benomyl for two weeks. Control plants were germinated in water.

Common Garden

Plants from an earlier cohort were grown in 40 cm deep pots with root-trainers until 8 July 2011. They were then moved to a shaded location next to a lab building at the Rocky Mountain Biological Laboratory, CO, until 24 August. On 24 August 2011, they were planted .5 m apart along three transects that were spaced 1 m apart. These transects were located between the intermittent stream and permanent stream next to our experimental site. Roughly equal numbers of each treatment species were planted in each transect (approx. n=5). 15 control-treatment *Poa leptocoma*, 13 benomyl-treated *Poa leptocoma*, 21 control-treatment *Poa reflexa*, and 14 benomyl-treated *Poa reflexa* were planted in total. Minimal disturbance was caused during the planting process, and initial tillers were counted as they were planted.

Experimental Design

To observe the effect of endophyte presence on the range of host plants, we used a 4X2X2 experimental design applied to two species, *P. leptocoma* and *P. reflexa*. We planted

benomyl-treated and control plants of both species near an intermittent stream at distances of 0m, 1m, 4m, and 8m. A permanent stream was near to this, with distances from the transects measuring 7.5m, 8.5m, 11.5m and 15.5m. Plants were grown in 10cm square pots for 2-8 weeks before transplanting them into 6.4 cm diameter x 36 cm deep root-trainer pots for approximately six weeks (Stuewe and Sons, Tangent, OR). Plants were then shipped from the Rice University Greenhouse to the Rocky Mountain Biological Laboratory in Gothic, Colorado on 8 July 2011. Five genotypes were used from each treatment: *P. leptocoma* Fungicide E-, *P. leptocoma* Control E+, *P. reflexa* Fungicide E-, and *P. reflexa* Control E-. On 8 July 2011, we divided each plant into eight clones, which were then planted in 3.8 cm x 14 cm root-trainer pots filled with a 1:1 mixture of sterile sand and organic potting mix. This approach allowed us to have each genotype represented in all transects and both water treatments. With 5 genotypes per treatment, we had a total of 20 genotypes and 160 plants. At each distance of 0m, 1m, 4m and 8m from the intermittent stream, we planted individuals 0.5m apart on a linear transect, with genotypes and treatments assigned in random order along each transect. Planting occurred on 23 July 2011, during which we did not clear the area around the plants and caused as little disturbance in the natural vegetation as possible.

Precipitation treatment

Over a period of four summers (July to August from 2011 to 2014), we supplemented our water treatment plants with .5323 liters of water from the nearby streams twice each week. The volumetric water content (%) of the soil directly adjacent to our control and treatment plants was measured once weekly using a TDR probe (FieldScout, Spectrum Technologies, Aurora, IL).

Response variables

To measure plant success, we scored tillers at the beginning and end of each growing season (June and August), along with survival, gopher presence, number of inflorescences, and number of seeds. The number of initial tillers before planting occurred in 2011 was also recorded.

Environmental variables

To describe environmental variability within the watering experiment, we measure light level (PAR) on 8 August, 2014 using a LI-250A Light Meter (LI-COR Biosciences, Lincoln, NE, USA). In addition, we used a 17 cm diameter circle placed around each individual location in the experiment to estimate total vegetative cover (percentage) visually as a way to assess effects of distance from the stream on the potential for plant competition.

Data analysis

Data were analyzed using repeated measures ANVOA in the statistical program SAS (SAS Institute, Cary, NC). First, we used models that examined plants in the precipitation experiment (ambient precipitation only) as well as plants receiving ambient precipitation that were planted in the common garden established at the same time as the precipitation experiment. These analyses excluded the watered plants, and allowed us to describe survival, growth, and reproduction responses along a broader spatial gradient of water availability than was captured by the four distances in our precipitation manipulation. We tested for effects of survival assuming a binomial distribution of the response variable (Proc GLIMMIX). We log-transformed some variables to achieve normality of residuals and homogeneity of variances.

Second, we constructed models that included only the plants in the watering experiment. We tested for effects of survival using Proc GLMMIX with a binary response variable; fixed factors were distance from stream (continuous, fungal treatment, year, and month (June or August) including interactions among the treatments, and the random effect of plant identity (nested within distance and fungal treatments). Second, we examined only the responses in the precipitation experiment, including the fixed factors of distance from stream (4 levels), fungal treatment, water treatment, year, and month (June or August) including interactions among the treatments, and the random effect of plant identity (nested within distance, fungal, and water treatments).

Expected results

Based on the observed distribution of *Poa leptocoma* in wetter soils, we would expect to see that the survival, growth and reproductive fitness of control-treatment *Poa leptocoma* would be significantly better than that of *Poa reflexa* and endophyte-free *Poa leptocoma* in wetter microsites. This expected result is also supported by a previous germination experiment conducted in which endophyte-positive *Poa leptocoma* showed preferential germination rates for similar microsites (Kazanel et al. sin review). However, in a previous flooding experiment conducted by Rudger's lab, endophyte-positive *Poa leptocoma* did worse than endophyte-free *Poa leptocoma* and *Poa reflexa* in flooded conditions on an individual basis where survival was the response variable. Therefore, based on this previous study, we might expect to see lower survival rates of endophyte-positive *Poa leptocoma* when precipitation was augmented and when nearer to the stream.

Results

We found that the *Epichloë* endophyte has different effects on *Poa leptocoma* depending on its demographic stage. *Poa leptocoma*'s survival rate was significantly worse at all distances from the stream when watered and where the endophyte was present ($p = .0427$)(see figure 4). In ambient precipitation there was less of an effect of distance on survival, suggesting that there is another mechanism affecting the range of *Poa leptocoma*, such as competition or reduced light. *Poa reflexa* had no significant differences in survival rate across distances to the stream, although it did better closer to the stream.

Poa leptocoma's growth as measured by the mean number of tillers produced per year was significantly better at closer distances to the stream, where the soil is wetter ($p = .0011$). Endophyte-positive *Poa leptocoma* had a higher growth rate closer to the stream as opposed to endophyte-free *Poa leptocoma* ($p = .0284$)(see figure 5). We found that *Poa reflexa*'s growth as measured by tillers and when it had been germinated in benomyl fungicide was better with ambient precipitation and worse when precipitation was augmented, with increased growth farther from the stream under ambient conditions ($p = .0012$)(see figure 6). This suggests that there is a cryptic seed-borne pathogen present that decreases the performance of *Poa reflexa* in drier areas. This is interesting considering that their observed range has typically been farther away from bodies of water.

Lifetime seed production in *Poa leptocoma*, as measured by the total number of seeds produced over four years, was significantly higher in plants living closer to the stream ($p = .0063$)(see figure 7). This relationship fit better with a quadratic equation ($p = .0437$). *Poa reflexa*'s lifetime seed count was significantly affected by distance from stream, treatment and watering treatment, with control plants doing better at farther distances under augmented

precipitation ($p = .0076$)(see figure 8). Under ambient conditions, plants that had been germinated in fungicide had the highest performance.

Discussion

We found that endophyte-positive *Poa leptocoma* had lower survival rates in wetter conditions, but had increased lifetime seed production and growth (as measured by tillers) in those same microsites. This shows us that the *Epichloë* endophyte has different effects on *Poa leptocoma* depending on its demographic stage. Although the results we found for survival indicate that the *Epichloë* endophyte hinders *Poa leptocoma* in wetter environments, higher seed production and tiller growth in these areas could explain the natural tendency of *Poa leptocoma* to inhabit wetter soils. Although the presence of a fungal endophyte in other systems has been reported provide a higher tolerance to low light conditions, this is a possible reason for the lower survival rates at farther distances from the stream of endophyte-positive *Poa leptocoma*, which we are currently investigating (Clay & Schardl 2002).

Our study correlates with other studies carried out in other similar study systems. One study that investigated the effect of a fungal endophyte on *Poa alsodes* found that the endophyte aided the host in times of drought stress, but had no effect in flooded conditions (Kannadan and Rudgers 2008). Another experiment conducted on a species of fescue found similar results for drought stress, but found that endophyte-free hosts had increased growth under high water availability as compared to endophyte-positive hosts (Morse et al. 2002). In a future experiment, we will investigate the role that the *Epichloë* endophyte in the competition between *Poa leptocoma* and *Poa reflexa*, as well as the entirety of the plant community under contrasting

water availability regimes. This will help us to further understand the mechanisms behind how the presence of a fungal endophyte might affect species ranges.

In a flooding experiment previously conducted by the Rudger's lab, we saw that endophyte-positive *Poa leptocoma* had lower survival rates in flooded conditions than both endophyte-free *Poa leptocoma* and *Poa reflexa*. That outcome is further supported by this study, which found that endophyte-positive had the lowest survival rate in wetter conditions as compared to endophyte-free *Poa leptocoma* and *Poa reflexa*. These results are surprising considering that the natural range of *Poa leptocoma* has been reported to be in these microsites (Barkworth et al. 2007). Many studies have been done to show that the presence of fungal endophytes are beneficial in terms of providing drought tolerance for their host, but future work should strive to understand why a fungal endophyte might prove deleterious to its host in situations of flood-induced stress (Clay and Schardl 2002).

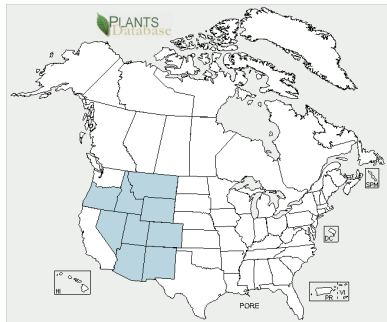
Our results also build on previous research looking into how having a relationship with an *Epichloë* endophyte affects the germination and seedling stages of *Poa leptocoma*. By looking into how the endophyte impacts the survival, growth and reproductive fitness of *Poa leptocoma* we can start to form a cohesive model of how positive species interactions influence species ranges, which is vital to creating accurate predictions of how plant distributions will change with the climate. A previous experiment conducted in the same study system found that endophyte positive *Poa leptocoma* seeds germinated with success rates that showed a preference for their observed niche, while endophyte negative *Poa leptocoma* seeds and *Poa reflexa* seeds showed no differential proclivity to germinate in a given niche (Kazanel et al. in prep). Through these studies, we see that endophytes play a more complex role in shaping community dynamics and structure than may have been previously realized.

Figures

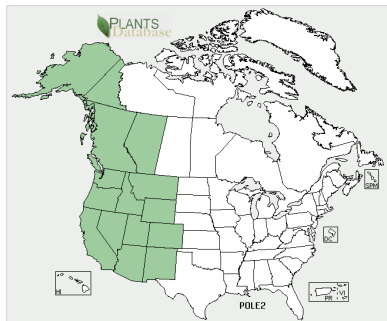
1). GIS map of experimental watering site and common garden.



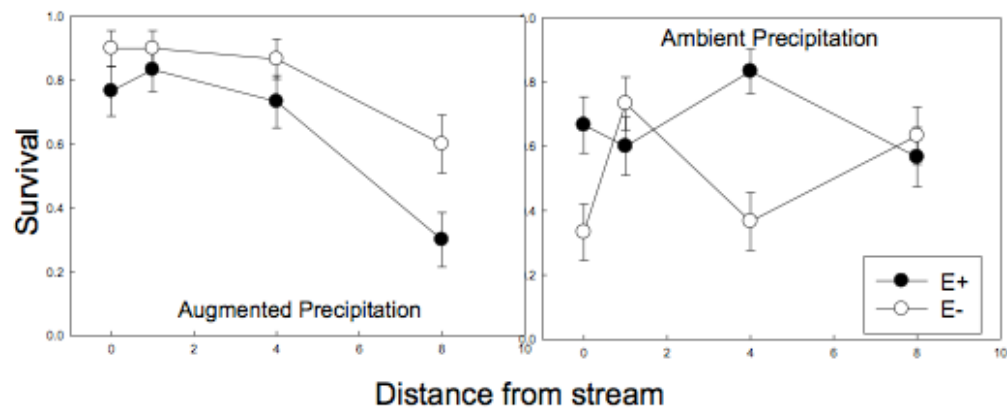
2). Distribution map of *Poa leptocoma* (USDA Plants Database).



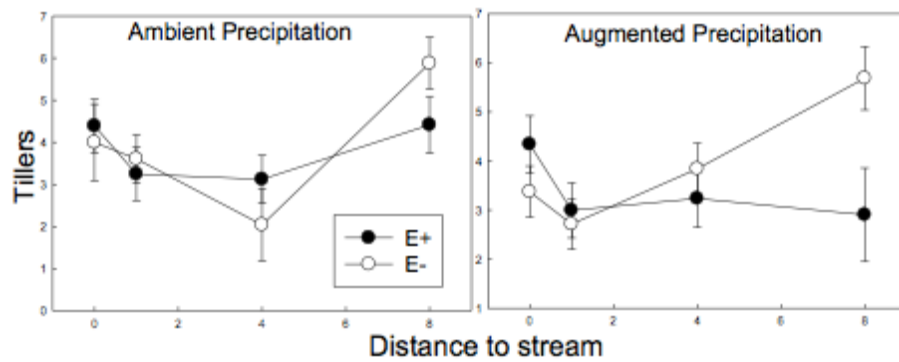
3). Distribution map of *Poa reflexa* (USDA Plants Database).



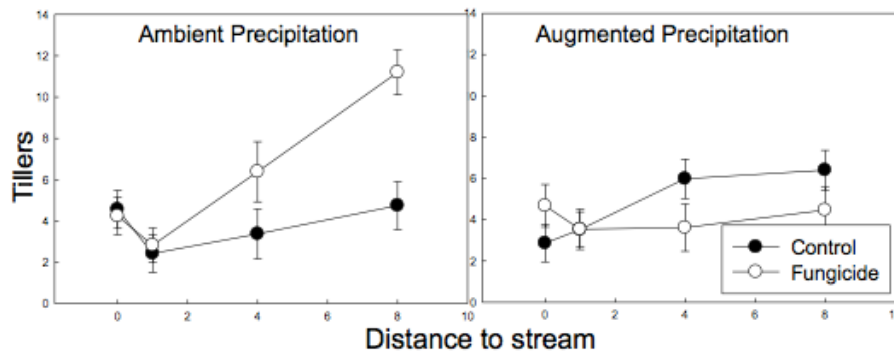
4). The survival rate of *Poa leptocoma* at each distance from the stream, depending on treatment and water treatment ($p = .0427$).



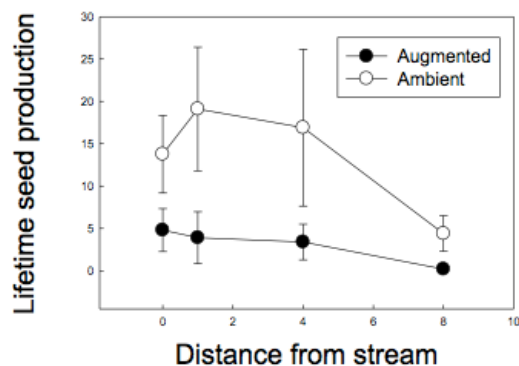
5). Growth of *Poa leptocoma* as measured by tillers at each distance from stream depending on treatment and water treatment ($p = .0284$).



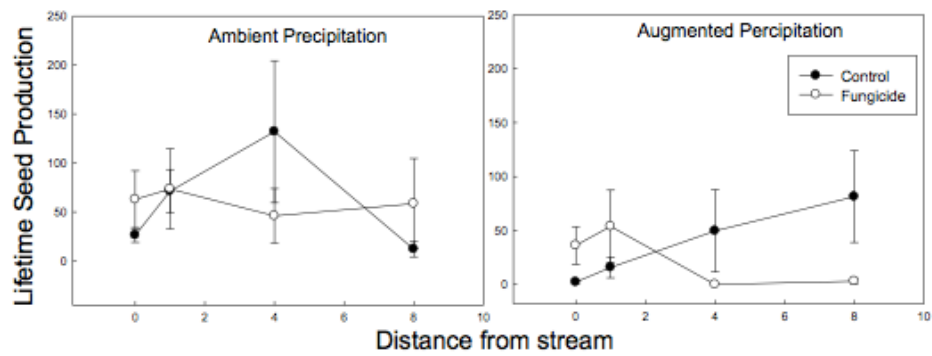
6). *Poa reflexa*'s growth as measured by tillers at each distance from stream, depending on treatment and water treatment ($p = .0012$).



7). Lifetime seed production in *Poa leptocoma*, as measured by the total number of seeds produced over four years, at each distance from stream and depending on water treatment ($p = .0063$).



8). *Poa reflexa*'s lifetime seed count at each distance from stream and depending on treatment and watering treatment ($p = .0076$).



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