

**CAN FUNGAL SYMBIONTS SHIFT HOST NICHE DIMENSIONS TO PROMOTE SPECIES  
COEXISTENCE?**

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## Abstract

A central question in the study of biodiversity is, what mechanisms allow species to coexist? While most attention to this question has focused on antagonistic species interactions, positive species interactions have recently gained attention and may rival antagonisms in their importance. Grasses with vertically transmitted fungal endophytes provide useful systems for studying symbiont-mediated species coexistence because the endophyte does not spread contagiously and the infection status of a plant can be experimentally controlled. Here, we examined whether the vertically transmitted fungal endophyte *Neotyphodium* sp. can shift the niche of the subalpine bluegrass *Poa leptocoma* so that it overlaps less with the niche of the ecologically similar grass *Poa reflexa*. *P. leptocoma* often grows in wetter environments closer to streams than *P. reflexa*, although the two species can grow intermixed in some environments. Our results show that the endophyte shifts the niche of *P. leptocoma* so that it overlaps less with the niche of *P. reflexa*, potentially decreasing interspecific competition. Symbiont-mediated niche shifts were strong at the early seedling life history stage, but not at the adult plant stage. Our study provides support for the role of mutualisms in affecting the composition of ecological communities.

## Introduction

A central question in the study of biodiversity is, what mechanisms allow species to coexist? Most attention to this question has focused on antagonistic species interactions, namely competition and predation, as controls on species coexistence (Begon, Towsend, and Harper 2006). However, positive species interactions have recently been gaining more attention and may rival antagonistic species interaction in their importance, although they have been much less studied (Bruno et al 2003, Agrawal et al. 2007, Bertness and Callaway 1994). Mutualisms may give species larger ranges by expanding or shifting the niche of individual partners (Bruno et al. 2003).

Studies on mutualisms, especially mycorrhizal fungi, have shown that symbionts can affect species coexistence and the composition of communities (Umbanhower and McCann 2005). In some cases, symbionts increase the dominance of their host species, thereby decreasing species coexistence and the species richness of competitors in the community (Harnett and Wilson 1999, Rudgers et al. 2007). This is often the case for mutualistic symbionts that have an exclusive relationship with their hosts such that benefits are only received by one host species. For example, tall fescue grass *Lolium arundinaceum* is host to a fungal endophyte that prevents herbivores from eating it. This causes the herbivores to eat more of the competing plants, decreasing the species richness of other plants in the surrounding community (Rudgers et al. 2007). In other cases, mutualisms appear to increase species diversity and coexistence (Müller and Godfrey 1999, Hart et al. 2003), often when benefits are provided to multiple species in the community. For example, more aphid species were able to persist in communities where mutualist ants tended them and protected against aphid enemies than in communities where no ants were tending (Müller and Godfrey 1999).

Few studies have directly tested whether association with a mutualist can promote species coexistence by altering the niche of the host organism. For example, ants that distribute seeds of *Hexastylis arifolia* were not found to have any effect on the niche of the plants (Warren et al. 2010). In contrast, *Lycaeides melissa* caterpillars were able to expand to a new host, alfalfa, with the help of mutualistic ants (Forister et al. 2010). Similarly, arbuscular mycorrhizal fungi can increase species coexistence by reducing the ability of one species to repress the other via competition for shared resources (Wagg et al. 2011).

Many plants have endophytic bacteria or fungi, which live inside plant tissues (Bacon and White, 2000). These symbionts can affect the plant in many ways, and function along a continuum, from mutualists to parasites. In some cases, endophytes are responsible for the secondary chemicals previously thought to be produced by plants (Strobel 2002). In some plants, fungal endophytes are well documented to enhance plant resistance to stress, including resistance to drought, herbivory, and pathogens (Clay and Schardl 2002). These effects on host plants can amplify to affect the community (Rudgers et al. 2007) and ecosystem as well (Lemons et al. 2005). However, it remains unknown whether endophytes can shift the niche of their hosts in ways that could promote coexistence with closely related competitor plants.

In this study, we used plants that have symbioses with systemic, vertically transmitted fungal endophytes as a model for understanding how symbionts affect the ecology of their hosts. These Class 1 endophytes (family Clavicipitaceae) occur in 20-30% of all grass species and have also been reported in sedges and morning glories (Leuchtman 1992, Rodriguez et al. 2009). These fungi are typically found in all aboveground tissues of the plant and are often passed from mother plant to daughter seed (Clay and Schardl 2002). Cases of exclusive vertical transmission of the symbiosis make grass-endophyte interactions particularly good models for investigating the mechanisms affecting the ecology of host plants because the presence of the fungus can be experimentally manipulated and there is no contagious spread. Furthermore, the vertical mode of transmission ties the endophyte and its host's evolutionary history in a close association via a process called partner fidelity feedback (Sachs et al. 2004), which creates positive interactions between the endophyte and its host (Rudgers et al. 2009). If an exclusively vertically transmitted endophyte were detrimental to its host plant, that plant, and therefore endophyte, would be selected against.

Here, we focus on whether endophytic fungi could alter the niche of host grasses by affecting aspects of host ecology. Endophytes are known to improve host tolerance to drought, increase nitrogen and phosphorus content, and enhance resistance to herbivory in some host grasses (reviewed by Clay and Schardl 2002, Schardl 2011, Malinowski and Belesky 2000, Cheplick and Faeth 2009). Endophytes could make grasses more able to live in stressful environments (Davitt et al. 2010). Fungal endophytes in grasses produce alkaloids that decrease herbivory, possibly allowing host plants to live in areas where they might otherwise be eaten (Schardl 2011).

In this study we tested whether the presence of a fungal endophyte altered the niche of marsh bluegrass, *Poa leptocoma*, and its coexistence with the similar but endophyte-free species, nodding bluegrass, *Poa reflexa*, in subalpine meadows of the

Rocky Mountains, USA. These two species commonly co-occur, and although it has been reported that *Poa leptocoma* prefers wetter habitats (Barkworth et al. 2007), no data exist that document niche differences. It is surprising that the endophytic species is associated with wetter areas given that endophytes are known to promote drought tolerance in other species (Cheplick and Faeth 2009), and it may be that endophytes can confer tolerance to stress caused by too much water -- although this hypothesis has never been tested. Specifically, we tested three questions to explore the effect of endophyte symbiosis on multiple stages of host life history. 1) Do *P. leptocoma* and *P. reflexa* occupy different ecological niches? 2) Does endophyte presence affect seed germination and seedling growth in different putative niches?, and 3) What are the effects of water availability and endophyte presence on the growth of adult *Poa reflexa* and *Poa leptocoma*?

## Methods

To study effects of endophyte symbiosis on niche and plant species coexistence, I examined two ecologically similar species of bluegrass: *Poa leptocoma* and *Poa reflexa*. Both species grow in western North America in subalpine environments near streams (Barkworth et al. 2007). *P. leptocoma* has scabrous panicle branches, whereas *P. reflexa* has smooth branches, but it is otherwise difficult to differentiate between the two species using morphological characters.

To characterize endophyte symbiosis in the bluegrass hosts, we made thin sections of the inner leaf sheath and stained them with aniline blue lactic acid dye following methods in Bacon and White (1994). We collected from several populations in the Rocky Mountains, from Rocky Mountain National Park south to Gunnison County (Table 1). In our surveys, *P. leptocoma* was infected with a systemic, asexual, vertically transmitted fungal endophyte *Neotyphodium* sp. (we never observed stromata) approaching 100% of plants with the endophyte (Table 1), while no systemic endophyte was associated with *P. reflexa* (Table 1). *Neotyphodium* sp. is a Class 1 endophyte that grows between the cells of its host plant. It appears to reproduce exclusively asexually, growing into the host seeds. In the greenhouse, the endophyte was passed on to the daughter seedlings ~95% of the time, but it remains at very high frequencies in the natural populations surveyed (Table 1), suggesting that the fungus confers some benefit to *P. leptocoma*.

During August 2008, we marked 77 *Poa leptocoma* plants and 40 *Poa reflexa* plants near a stream system in Virginia Basin, Gunnison County, CO (38°58.314N, 106°58.942W, elevation 3229 m). Plants were marked approximately every 2 m along transects in three regions of the watershed: directly in and along a shady streambed (only *P. leptocoma* was present), next to a sunny streambed, and in a wet meadow between the two streams.

### 1) Do *P. leptocoma* and *P. reflexa* occupy different ecological niches?

We characterized the niches of the marked *Poa leptocoma* and *Poa reflexa* plants at the Virginia Basin site, as well as at two sites near Schofield Pass (sites 1 and 2) and at one site on Snodgrass Mountain (Table 1). Since anecdotal data suggested that *P. leptocoma* prefers wetter environments than *P. reflexa* (Barkworth et al. 2007), we focused on water availability at each site. We recorded volumetric

water content (VWC) of the soil at each plant using a soil moisture meter (HydroSense meter, Campbell Scientific, Logan, UT) with probes 12cm-20cm long. We also measured the distance of the plant (to the nearest 0.01 m) from the nearest likely source of water (a stream or snow bank, depending on which was closer). It was important to measure both soil moisture and distance to the stream because while the two are often correlated, distance from the stream can include more factors that could contribute to the niche than soil moisture alone.

At the Virginia Basin site, all previously marked plants were measured, excepting a few for which it was too rocky to obtain accurate soil moisture data. At the Schofield and Snodgrass sites, we used the stream as our transect line and recorded data on the individual plant of each species that was found closest to the stream. At these sites, each plant measured was located at least 3m away from the next plant of the same species. Data were analyzed with ANOVA including the fixed factors of plant species identity, site and the siteXspecies interaction (SAS version 9.2, SAS Institute, Inc., Cary NC). The responses of distance and VWC were both log-transformed to improve the normality of residuals and homogeneity of variances.

## **2) Does endophyte presence affect seed germination and seedling growth in different putative niches?**

*Experimental approach.* We used the marked plants at the Virginia Basin site to 'black box' the niche of each species. These plants provided replicates of the specific microhabitat of each species allowing us to compare plant performance without having to replicate the precise abiotic and biotic conditions characteristic of each species' niche. We compared the germination and growth of seeds planted into natural *P. leptocoma* and *P. reflexa* niches to test whether niche requirements differed between species and/or with endophyte symbiosis.

*Seed sources.* Surrounding each marked, naturally occurring plant, we planted 1 *P. leptocoma* E+ seed, 3 *P. leptocoma* E- disinfected seeds, 2 *P. leptocoma* naturally collected seeds, and 3 *P. reflexa* naturally collected and naturally E- seeds (Figure 1). All seeds were originally collected from the Virginia Basin site. *P. reflexa* and *P. leptocoma* 'natural' seeds were collected in the field during late August 2009 and stored at 4°C. *P. leptocoma* E+ and E- seeds came from plants grown in a common greenhouse environment. First, the endophyte was experimentally removed by germinating seeds for 5 weeks on wet filter paper in standard plastic Petri plates containing the fungicide benomyl (2g/L). Control Petri plates had water only. Following germination, seedlings were transplanted into 2.5 cm X 4 cm plastic pots and grown for 2 - 6 weeks on a light bench. Once seedlings had established a root system, they were transplanted into 10 cm square pots with a 1:1 mixture of Pro-mix BX BX (Premier Horticulture, Quakertown, PA) to sterile sand and grown until reproductive. Seeds for the experiment were collected in the greenhouse during July 2010. This approach provided a control on maternal effects by decoupling the effect of endophyte presence from any maternal effects associated with plant genotype or with growth in different microhabitats.

*Experimental methods.* To track seeds we lightly glued them to colored plastic toothpicks with water-soluble glue (Elmer's Washable School Glue, Elmer's Products, Inc. Columbus, OH). We used a different color of toothpick for each type of

seed, and stuck the toothpicks in the ground to position the seeds just at the soil surface, providing exposure to light for germination (Figure 1). The seeds were planted on 9 July 2011 in randomized order in a circle with a radius of 5cm around the original plant. We scored germination of the seeds weekly for 5 weeks.

*Expectations and data analysis.* We expected that endophyte presence would shift the niche of *P. leptocoma*, so we expected *P. leptocoma* E+ seeds to germinate best in *P. leptocoma* habitats, *P. reflexa* seeds to germinate best in *P. reflexa* habitats, and the *P. leptocoma* seeds without endophyte to germinate best in *P. reflexa* habitats. Data were analyzed with a log-linear model, including the binomial response of germination (0/1) with a logit link function (SAS version 9.2, SAS Institute, Inc., Cary, NC). The model included the fixed effects of seed identity, site identity (*P. leptocoma* or *P. reflexa* niche) and their interaction. Following a significant interaction, we performed *a priori* contrasts for each seed type to compare the likelihood of germination between the *P. leptocoma* and *P. reflexa* niches.

### **3) What are the effects of water availability and endophyte presence on the growth of adult *Poa reflexa* and *Poa leptocoma*?**

*Experimental design.* To observe the effect of endophyte presence in *Poa leptocoma* in the adult stage and to directly observe the effect of distance from the stream on growth of *Poa reflexa* and *Poa leptocoma*, we planted benomyl-treated and control plants of both species at distances of 0m, 1m, 4m, and 8m from a stream (Fig. 2). On 13 October 2010, the benomyl treatment was applied to both *P. leptocoma* and *P. reflexa* seeds as described for question 2; this allowed us to test for any direct effects of benomyl, as treatment of naturally endophyte-free *P. reflexa* would not affect endophyte symbiosis. We grew plants in 10 cm square pots for 2-8 weeks, then transplanted them into 6.4 cm diameter X 36 cm deep root-trainer pots for approximately 6 weeks (Stuewe and Sons, Tangent, OR). Plants were shipped from the Rice University greenhouse to the Rocky Mountain Biological Lab on 8 July 2011. We used 5 genotypes of each treatment: *P. leptocoma* Fungicide E-, *P. leptocoma* Control E+, *P. reflexa* Fungicide E-, *P. reflexa* Control E-. On 8 July 2011, we divided each plant into eight clones, which were planted into 3.8 cm X 14 cm root-trainer pots filled with a 1:1 mixture of sterile sand and organic potting mix so that we could have the same genotype represented at each distance and water treatment. With 5 genotypes per treatment, we had a total of 20 genotypes and 160 plants. Along each distance from the stream, we planted individuals 0.5m apart along a linear transect, with genotypes and treatments assigned in a random order along each transect. Planting occurred on 23 July 2011. When planting, we did not clear an area around the plants and disturbed the natural vegetation as little as possible.

*Water treatment.* We added 0.5L of water to half of the plants twice a week. Water was collected directly from the nearby stream and applied with a cup.

*Response variables and analysis.* To score plant success, we counted number of tillers of each plant before planting (21 July 2011) and near the end of the growing season (7 August 2011). The response variable was the change in tiller number (final - initial). A few plants ( $n=9$ ) died during the season, and these were

excluded from the analysis. Data from each plant species were analyzed with a general linear model including the fixed effects of fungicide (benomyl or control) and water (control or addition), and the continuous factor of distance (0, 1, 4, or 8 m from the stream), as well as all interactions and the random effect of plant genotype (nested within the fungicide treatment) (SAS version 9.2., SAS Institute, Inc., Cary, NC).

## Results

### 1) Do *P. leptocoma* and *P. reflexa* occupy different ecological niches?

At the Virginia Basin site, *Poa leptocoma* grew in environments an average of 8.3 m from streams, while *Poa reflexa* grew in environments an average of 22.0 m from streams (Figure 3). This means that *P. reflexa* typically occupies environments 165% further from streams than *P. leptocoma*. *P. leptocoma* sites were 77% wetter than *P. reflexa* sites, with an average volumetric water content (VWC) of 32%, compared to an average VWC of 18% for *P. reflexa* sites (Figure 4).

Similar differences were observed for two of the three additional locations surveyed (Figures 3 & 4). At Site 1 and Site 2, both at Schofield, *Poa leptocoma* grew in environments closer to streams and with higher soil moisture than *Poa reflexa*. At Site 1, *P. leptocoma* grew 605% closer to streams and with VWC 153% higher than *P. reflexa*. At Site 2, *P. leptocoma* grew 1331% closer to streams and with VWC 62% higher than *P. reflexa*. However, at Site 3, Snodgrass, *P. reflexa* grew in environments 26% closer to a stream with 38% higher soil moisture than *P. leptocoma*. Soil moisture was lower overall at Site 3 than at the other three sites.

### 2) Does endophyte presence affect seed germination and seedling growth in different putative niches?

*Poa leptocoma* seeds with the endophyte were more likely to germinate at sites occupied by *Poa leptocoma* plants than at *Poa reflexa* sites. Likewise, *Poa reflexa* germinated more at sites occupied by *Poa reflexa* plants than at *Poa leptocoma* sites (Figure 5). For *P. leptocoma* plants in which endophyte had been removed, the seeds were equally likely to germinate at *P. reflexa* sites as *P. leptocoma* sites. Unfortunately, germination of the naturally collected *P. leptocoma* seeds was exceptionally low (< 3%); therefore, these seeds were excluded from the statistical analysis. In contrast, the *Poa leptocoma* seeds from plants that had been grown in the greenhouse (*P. leptocoma* E- and *P. leptocoma* E+ control seeds) germinated much more readily than the seeds collected from natural populations (Figure 5).

### 3) What are the effects of water availability and endophyte presence on the growth of adult *Poa reflexa* and *Poa leptocoma*?

We expected the results of our experiment on adult plants to be similar to the results of the germination experiment, with *Poa leptocoma* growing best closest to the stream and with water added, *Poa reflexa* growing best far from the stream and without water, and *P. leptocoma* with endophyte removed to grow best far from the stream and without water. We did not expect fungicide treatment to have any effect on *P. reflexa* plants, which are naturally endophyte-free.

*P. leptocoma* grew better closer to the stream than far from it (Table 2 & Figure 6). In contrast, distance did not appear to have any effect on *P. reflexa* growth (Table 2). The addition of water did not have an effect on either *P. reflexa* or *P. leptocoma*. Fungicide reduced the growth of *P. reflexa* but had no effect on *P. leptocoma*.

Since the effect of distance from the stream was significant for *P. leptocoma*, we conducted a regression analysis to examine the slope of this relationship, which was  $\beta = -0.11$ ,  $P = 0.0246$ ; thus, plants placed closer to stream had greater positive growth than those planted farther from the stream, which was our initial expectation based on reports that *P. leptocoma* prefers wetter habitats than *P. reflexa*. Surprisingly, neither the endophyte treatment nor the addition of water influenced the strength or direction of the effect of distance on *P. leptocoma*, as evidenced by non-significant interactions between treatments and distance.

## Discussion

*Poa leptocoma* grew closer to the stream and in wetter soils than *Poa reflexa* (Figures 3 & 4). This slight difference in niche in the two similar plant species is likely a facet of what allows them to coexist in the same area. Because the two species are so similar, if they inhabited the same niche, one would likely outcompete the other (Gause 1934). It is possible that these niche differences seemingly based on water availability are an artifact of some other, more important niche difference. Shade effects, soil nutrient effects, or other factors could all be correlated with the soil moisture or distance from water source, and be the true source of niche divergence. Since an organism's niche consists of many factors, the two we measured are not necessarily the only differences in the two species' niches. However, the fact that the differences in soil water content were generally stronger than differences in distance from the nearest water source (e.g., 165% difference vs. 77% for Virginia Basin) suggests that water availability may be an important niche difference between *P. leptocoma* and *P. reflexa*.

*Poa leptocoma* and *Poa reflexa* can inhabit similar habitats, as shown by the plants at Snodgrass (site 3). At that site, *P. reflexa* was in fact in slightly wetter environments than *P. leptocoma*, the opposite of our observations at the other three sites. Soil moisture was overall much lower at Site 3, and differences between *P. leptocoma* and *P. reflexa* habitats were smaller than at other sites. These data suggest that there is some niche overlap between the two grass species and that soil moisture is likely not the sole factor determining where each species is able to grow.

When choosing plants in sites 1, 2, and 3, we used the stream as a transect and found the plant of each species closest to the stream. This method allowed us to find the edge of the habitat of both species of grass, but did not show how far that habitat extends or the extent to which the distributions of the two species overlap. However, in sites 1 and 2, *Poa leptocoma* grew much closer to the stream than *Poa reflexa*. This means that there was a fairly large area where *P. leptocoma*'s niche did not overlap with that of *P. reflexa*, and so in that area interspecific competition between the two would not occur.

Our results suggest that endophyte presence may help the two species coexist by shifting the early niche requirement of *P. leptocoma* seeds and seedlings. In the seed germination experiment *P. leptocoma* without endophyte had higher germination at *P. reflexa* sites than *P. leptocoma* with the endophyte. This result suggests that without the endophyte, *P. leptocoma* would have greater overlap with the niche of *P. reflexa*, resulting in stronger interspecific competition.

From our data so far, it appears that the endophyte acts more strongly in the seed germination/seedling stage of host plant development than in the adult stage. While there was a significant decline in growth of *P. leptocoma* with distance from the stream, this was not significantly modified by the presence of the endophyte or even by the watering treatment. To further investigate this, we will continue the watering treatments until the end of the season this year and start them again after the snow melts next year. We suspect that plants have not been in the ground long enough to see a strong effect of our treatments yet. By observing the plants for a full growing season next year, we will get a more complete picture of what endophyte presence and water addition mean for adult plants.

*Neotyphodium* sp., the endophyte of *Poa leptocoma*, appears to allow the host grass to coexist with the similar species *Poa reflexa*. This is interesting because it identifies a symbiosis with a positive interaction as a mechanism that allows species to coexist. Often we think of negative species interactions as the driving force behind ecological processes. However, positive interactions can have a strong effect as well. Endophyte symbiosis can have more effects on host plants than direct benefits associated with gaining drought, flood, or herbivory resistance; it can change the distribution of a host, allowing it to survive where otherwise it would not be able to.

## Figures & Tables

| Species             | Site               | Latitude  | Longitude  | Elevation (m) | %Symbiotic | n   | Date            |
|---------------------|--------------------|-----------|------------|---------------|------------|-----|-----------------|
| <i>P. leptocoma</i> | Cottonwood Pass    | 38°48.764 | 106°24.136 | 3541          | 67         | 6   | 8/17/08         |
|                     | Snodgrass Mountain | 38°55.083 | 106°59.175 | 3357          | 100        | 11  | 8/10/11         |
|                     | Copper Creek1      | 38°57.586 | 106°58.637 | 2986          | 100        | 13  | 8/15/08         |
|                     | Copper Creek2      | 38°58.112 | 106°58.048 | 3066          | 100        | 3   | 8/16/08         |
|                     | Virginia Basin     | 38°58.314 | 106°58.942 | 3229          | 98         | 110 | 8/15 - 9/2/2008 |
|                     | Scofield Pass 1    | 39°01.329 | 107°02.938 | 3218          | 100        | 10  | 8/9/11          |
|                     | Scofield Pass 2    | 39°01.461 | 107°02.960 | 3193          | 80         | 10  | 8/9/11          |
|                     | Scofield Pass 4    | 39°01.489 | 107°02.851 | 3189          | 71         | 28  | 8/17/08         |
|                     | Scofield Pass3     | 39°01.497 | 107°02.850 | 3200          | 90         | 10  | 8/10/08         |
|                     | Niwot Ridge        | 40°02.540 | 105°34.297 | 3275          | 100        | 10  | 8/28/08         |
|                     | St. Vrain1         | 40°10.160 | 105°32.785 | 2893          | 100        | 21  | 8/26/08         |
|                     | Black Lake         | 40°16.103 | 105°38.373 | 3324          | 100        | 8   | 8/20/08         |
|                     | St. Vrain2         | 40°16.279 | 105°33.222 | 2907          | 90         | 10  | 8/26/08         |
|                     |                    |           |            |               |            |     |                 |
| <i>P. reflexa</i>   | Cottonwood Pass    | 38°48.764 | 106°24.136 | 3541          | 0          | 16  | 8/17/08         |
|                     | Snodgrass Mountain | 38°55.083 | 106°59.175 | 3357          | 0          | 13  | 8/10/11         |
|                     | Virginia Basin3    | 38°58.187 | 106°59.001 | 3205          | 0          | 6   | 8/9/08          |
|                     | Scofield Pass      | 38°58.314 | 106°58.942 | 3229          | 0          | 10  | 9/1/08          |
|                     | Virginia Basin1    | 38°58.314 | 106°58.942 | 3229          | 0          | 10  | 9/2/08          |
|                     | Virginia Basin2    | 38°58.913 | 106°58.249 | 3630          | 0          | 16  | 8/11/08         |
|                     | Mt. Bellview       | 39°01.023 | 107°01.742 | 3630          | 0          | 9   | 8/12/08         |
|                     | Scofield Pass 1    | 39°01.329 | 107°02.938 | 3218          | 0          | 10  | 8/9/11          |
|                     | Scofield Pass 2    | 39°01.461 | 107°02.960 | 3193          | 0          | 10  | 8/9/11          |
|                     | Niwot              | 40°02.876 | 105°34.307 | 3373          | 0          | 9   | 8/28/08         |
|                     | Mitchell Lake      | 40°04.994 | 105°34.915 | 3214          | 0          | 10  | 8/28/08         |
|                     | Black Lake         | 40°16.103 | 105°38.373 | 3324          | 0          | 3   | 8/20/08         |
|                     |                    |           |            |               |            |     |                 |
|                     |                    |           |            |               |            |     |                 |
|                     |                    |           |            |               |            |     |                 |

Table 1- *Poa leptocoma* grasses are infected by the systemic endophyte *Neotyphodium* sp. at high frequencies, often approaching 100%, while *Poa reflexa* is never infected.



Figure 1- Seeds were planted around plants in random order in a circle with radius ~5cm.

|                                |         | Distance from Stream |     |     |     |
|--------------------------------|---------|----------------------|-----|-----|-----|
|                                |         | 0m                   | 1m  | 4m  | 8m  |
| <i>P. leptocoma</i><br>E+      | Control | n=5                  | n=5 | n=5 | n=5 |
|                                | Water   | n=5                  | n=5 | n=5 | n=5 |
| <i>P. leptocoma</i><br>E-      | Control | n=5                  | n=5 | n=5 | n=5 |
|                                | Water   | n=5                  | n=5 | n=5 | n=5 |
| <i>P. reflexa</i><br>Control   | Control | n=5                  | n=5 | n=5 | n=5 |
|                                | Water   | n=5                  | n=5 | n=5 | n=5 |
| <i>P. reflexa</i><br>Fungicide | Control | n=5                  | n=5 | n=5 | n=5 |
|                                | Water   | n=5                  | n=5 | n=5 | n=5 |

Figure 2- Adult plants were planted at four distances from the stream, with 10 genotypes of each plant type planted at each distance, and half of those plants treated with water twice a week.

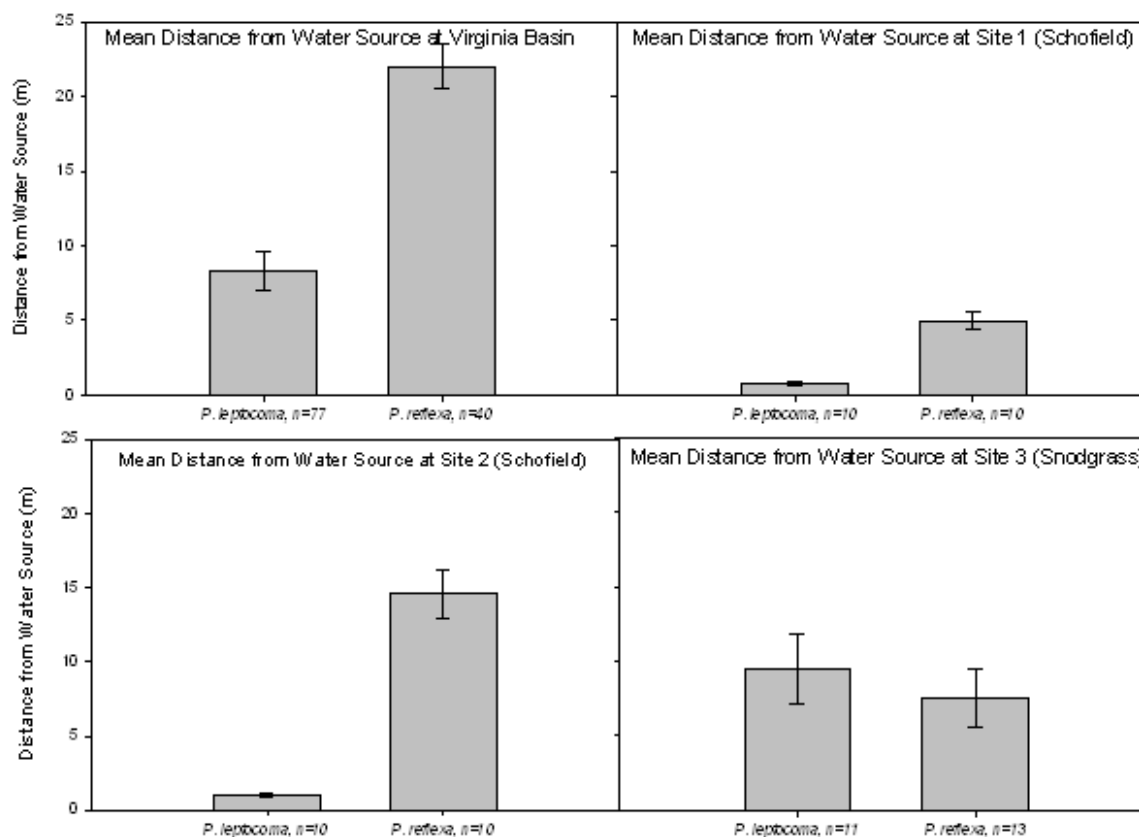


Figure 3- Mean distance ( $\pm$  s.e.) of *Poa leptocoma* and *Poa reflexa* plants from a water source, either a stream or a snow bank (Virginia Basin:  $F_{1,115}=43.97$ ,  $p<0.0001$ ; Site 1:  $F_{1,20}=12.41$ ,  $p=0.0005$ ; Site 2:  $F_{1,19}=21.34$ ,  $p<0.0001$ ; Site 3:  $F_{1,23}=1.67$ ,  $p=0.1973$ ).

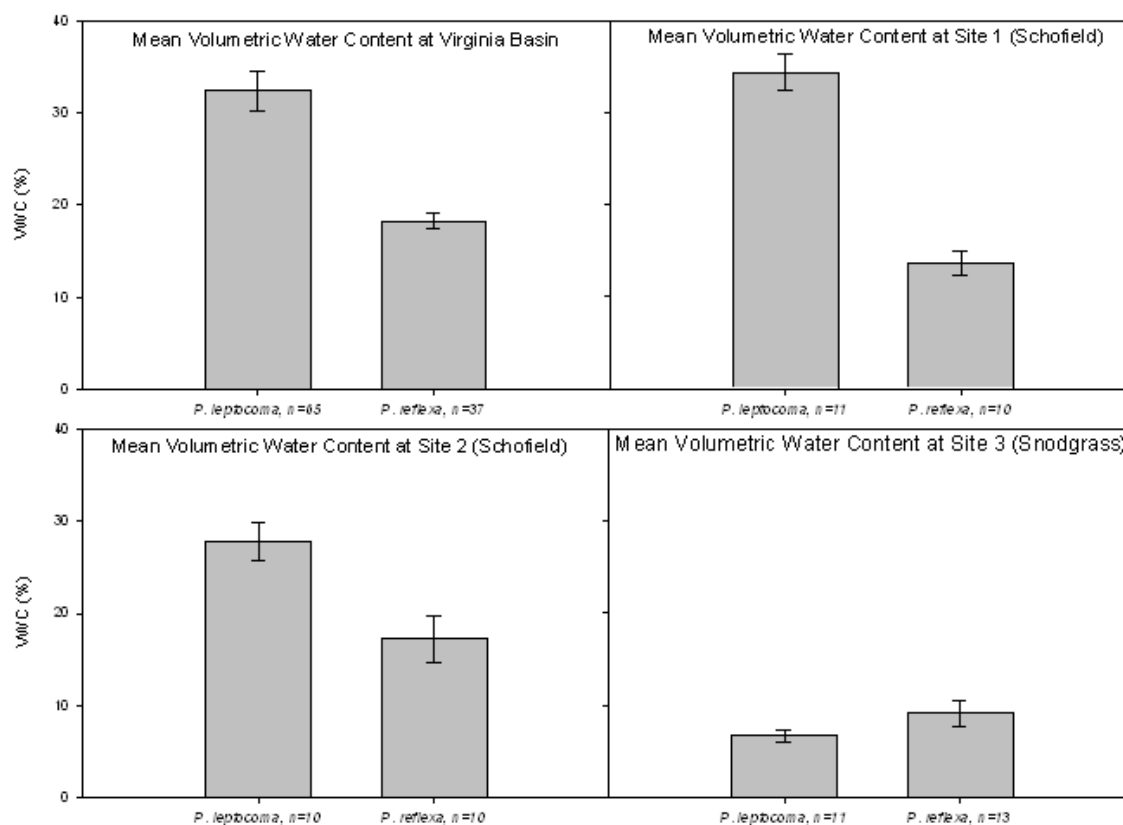


Figure 4-Mean Volumetric Water Content (VWC) ( $\pm$  s.e) of *Poa leptocoma* and *Poa reflexa* (Virginia Basin:  $F_{1,100}=37.67$ ,  $p<0.0001$ ; Site 1:  $F_{1,19}=22.46$ ,  $p<0.0001$ ; Site 2:  $F_{1,19}=7.46$ ,  $p=0.0071$ ; Site 3:  $F_{1,23}=0.77$ ,  $p=0.3826$ ).

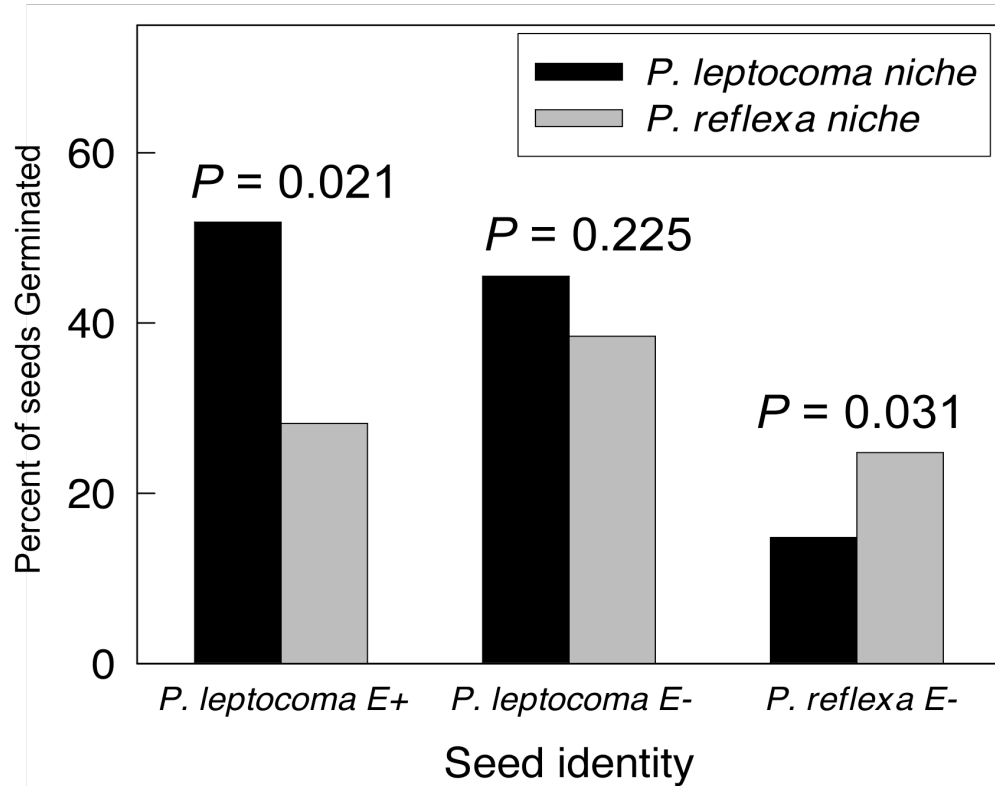


Figure 5- Percent of seeds germinated by five weeks after planting (SeedXSite interaction:  $p=0.0039$ ).

| <i>P. leptocoma</i>         |      | Dependent Variable: deltatillers |             |      |               |
|-----------------------------|------|----------------------------------|-------------|------|---------------|
| Source                      | df   | Sum of Squares                   | Mean Square | F    | P             |
| Model                       | 9    | 40.4139216                       | 4.4904357   | 3.05 | 0.0039        |
| Error                       | 69   | 101.4848125                      | 1.4707944   |      |               |
| <i>R-Square</i>             | 0.28 |                                  |             |      |               |
| Effect                      | df   | Type III SS                      | Mean Square | F    | P             |
| Dist_Stream                 | 1    | 9.54399004                       | 9.54399004  | 6.49 | <b>0.0131</b> |
| Fungicide                   | 1    | 0.54349156                       | 0.54349156  | 0.37 | 0.5453        |
| Dist_Stream*Fungicide       | 1    | 0.10272078                       | 0.10272078  | 0.07 | 0.7924        |
| Water                       | 1    | 3.92844964                       | 3.92844964  | 2.67 | 0.1067        |
| Dist_Stream*Water           | 1    | 1.69520574                       | 1.69520574  | 1.15 | 0.2868        |
| Fungicide*Water             | 1    | 4.78381158                       | 4.78381158  | 3.25 | 0.0757        |
| Dist_Stream*Fungicide*Water | 1    | 1.48823163                       | 1.48823163  | 1.01 | 0.3180        |
| Genotype(Treatment)         | 2    | 17.46917727                      | 8.73458863  | 5.94 | <b>0.0042</b> |

| <i>P. reflexa</i>           |      | Dependent Variable: deltatillers |             |      |               |
|-----------------------------|------|----------------------------------|-------------|------|---------------|
| Source                      | df   | Sum of Squares                   | Mean Square | F    | P             |
| Model                       | 9    | 15.26959677                      | 1.69662186  | 1.42 | 0.1956        |
| Error                       | 70   | 83.53040323                      | 1.19329147  |      |               |
| <i>R-Square</i>             | 0.15 |                                  |             |      |               |
| Effect                      | df   | Type III SS                      | Mean Square | F    | P             |
| Dist_Stream                 | 1    | 0.08258065                       | 0.08258065  | 0.07 | 0.7933        |
| Fungicide                   | 1    | 4.46135152                       | 4.46135152  | 3.74 | <u>0.0572</u> |
| Dist_Stream*Fungicide       | 1    | 0.0116129                        | 0.0116129   | 0.01 | 0.9217        |
| Water                       | 1    | 0.94064516                       | 0.94064516  | 0.79 | 0.3777        |
| Dist_Stream*Water           | 1    | 0.49064516                       | 0.49064516  | 0.41 | 0.5235        |
| Fungicide*Water             | 1    | 1.39571485                       | 1.39571485  | 1.17 | 0.2832        |
| Dist_Stream*Fungicide*Water | 1    | 3.42225806                       | 3.42225806  | 2.87 | 0.0948        |
| Genotype(Treatment)         | 2    | 10.5625                          | 5.28125     | 4.43 | <b>0.0155</b> |

Table 2- Statistics for the results of the change in tillers of adult *P. reflexa* and *P. leptocoma* plants

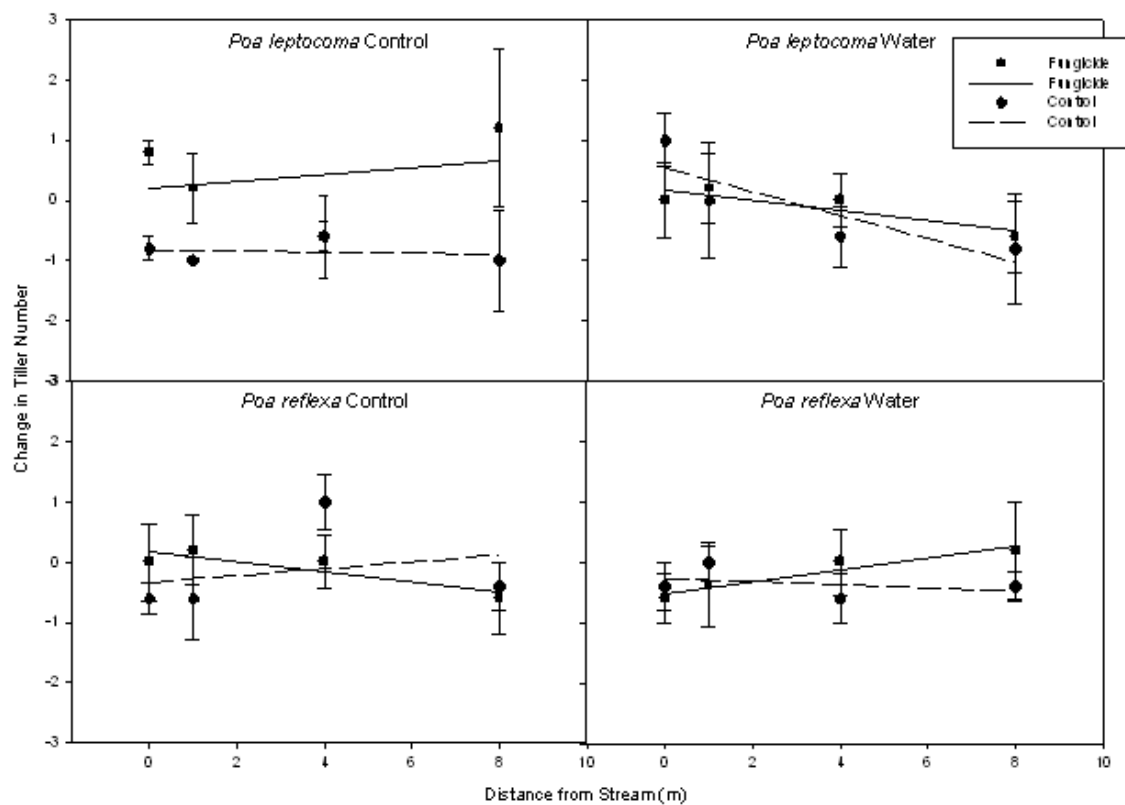


Figure 6- Change in number of tillers for plants grown at 0m, 1m, 4m, and 8m from a stream. Control treatments had no water added, while Water treatments had 0.5L of water added twice a week.

## References

- Agrawal, A. A., D. D. Ackerly, F. Adler, A. E. Arnold, C. Caceres, D. F. Doak, E. Post, P. J. Hudson, J. Maron, K. A. Mooney, M. Power, D. Schemske, J. Stachowicz, S. Strauss, M. G. Turner, and E. Werner. 2007. Filling key gaps in population and community ecology. *Frontiers in Ecology and the Environment* **5**:145-152
- Bacon, C. W. and J. F. White, Jr. 1994. Stains, media, and procedures for analyzing endophytes. Pages 47-56 in C. W. Bacon and J. F. White, Jr., editors. *Biotechnology of Endophytic Fungi of Grasses*. CRC Press, Boca Raton, Florida, USA.
- Bacon, C. W. and J. F. White, editors. 2000. *Microbial Endophytes*. Marcel Dekker, Inc., New York, New York.
- Barkworth, M. E., K. M. Capels, S. Long, L. K. Anderton, and M. B. Piep, editors. 2007. *Flora of North America Volume 24: North of Mexico: Magnoliophyta: Commelinidae (in part): Poaceae, part 1*. Oxford University Press, New York, NY.
- Begon, M., C. R. Towsend, and J. L. Harper. 2006. *Ecology: From Individuals to Ecosystems*. Blackwell, Malden, MA.
- Bertness, M. D. and R. Callaway. 1994. Positive interactions in communities. *Trends in Ecology & Evolution* **9**:191-193.
- Bruno, JF, Stachowicz, JJ, and Bertness, MD. 2003. Inclusion of facilitation into ecological theory. *Trends in Ecology and Evolution* **18**:119-125.
- Cheplick, G. P. and S. H. Faeth. 2009. *Ecology and evolution of grass-endophyte symbiosis*. Oxford University Press, Oxford.
- Clay, K. and C. Schardl. 2002. Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. *American Naturalist* **160**:S99-S127.
- Davitt, A. J., M. Stansberry, and J. A. Rudgers. 2010. Do the costs and benefits of fungal endophyte symbiosis vary with light availability? *New Phytologist* **188**:824-834
- Forister, ML, Gompert, Z, Nice, CC, Forister, GW, and Fordyce, JA. 2010. Ant association facilitates the evolution of diet breadth in a lycaenid butterfly. *Proceedings of the Royal Society B* **278**:1529-1547.
- Gause, G.F. (1934). *The struggle for existence*. Baltimore, MD: Williams & Wilkins.

- Harnett, DC and Wilson, GWT. 1999. Mycorrhizae influence plant community structure and diversity in tallgrass prairie. *Ecology* **80**:1187-1195.
- Hart, MM, Reader, RJ, and Klironomos, JN. 2003. Plant coexistence mediated by arbuscular mycorrhizal fungi. *Trends in Ecology and Evolution* **18**:418-423.
- Kearns, CA and Inouye, DW. 1993. *Techniques for Pollination Biologists*. University Press of Colorado, Niwot, CO.
- Lemons, A., K. Clay, and J. A. Rudgers. 2005. Connecting plant-microbial interactions above and belowground: a fungal endophyte affects decomposition. *Oecologia* **in press**.
- Leuchtmann, A. 1992. Systematics, distribution, and host specificity of grass endophytes. *Natural Toxins* **1**:150-162.
- Malinowski, D. P. and D. P. Belesky. 2000. Adaptations of endophyte-infected cool-season grasses to environmental stresses: Mechanisms of drought and mineral stress tolerance. *Crop Science* **40**:923-940.
- Müller, CB and Godfray, HCJ. 1999. Predators and mutualists influence the exclusion of aphid species from natural communities. *Oecologia* **119**:120-125.
- Rodriguez, R. J., J. F. White, A. E. Arnold, and R. S. Redman. 2009. Fungal endophytes: diversity and functional roles. *New Phytologist* **182**:314-330.
- Rudgers, JA, Holah, J, Orr, SP, and Clay, K. 2007. Forest succession suppressed by an introduced plant-fungal symbiosis. *Ecology* **88**:18-25.
- Rudgers, JA, Afkhami, ME, Rúa, MA, Davitt, AJ, Hammer, S, and Huguet, VM. 2009. A fungus among us: broad patterns of endophyte distribution in the grasses. *Ecology* **90**:1531-1539.
- Sachs, J. L., U. G. Mueller, T. P. Wilcox, and J. J. Bull. 2004. The evolution of cooperation. *Quarterly Review of Biology* **79**:135-160.
- Schardl, CL. 2010. The Epichloae, symbionts of the grass subfamily Pooideae. *Annals of the Missouri Botanical Garden* **97**:646-665.
- Schardl, C. L. 2011. The Epichloae, symbionts of the grass subfamily Pooideae. *Annals of the Missouri Botanical Garden* **97**:646-665.
- Sharon, G, Segal, D, Ringo, JM, Hefetz, A, Zilber-Rosenberg, I, and Rosenberg, E. 2010. Commensal bacteria play a role in mating preference of *Drosophila melanogaster*. *PNAS* **107**:20051-20056.

- Smith, S. E. and D. J. Read. 1997. Mycorrhizal symbiosis. 2nd edition. Academic Press, San Diego.
- Strobel, GA. 2002. Rainforest Endophytes and Bioactive Products. Critical Reviews in Biotechnology **22**:315-333.
- Umbanhower, J and McCann, K. 2005. Simple rules for the coexistence and competitive dominance of plants mediated by mycorrhizal fungi. Ecology Letters **8**:247-252.
- Wagg, C, Jansa, J, Stadler, M, Schmid, B, and Van der Heijden, MGA. 2011. Mycorrhizal fungal identity and diversity relaxes plant-plant competition. Ecology **92**:1303-1313.
- Warren, RJ, Giladi, I, and Bradford, MA. 2010. Ant-mediated seed dispersal does not facilitate niche expansion. Journal of Ecology **98**:1178-1185.