

Can fungal symbionts shift host niche dimensions to promote species coexistence?

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Abstract

Defining the mechanisms that enable species to coexist is a fundamental goal of community ecology. Attention to this question has traditionally focused on antagonistic species interactions, namely competition and predation, as controls on species coexistence. However, positive species interactions may rival antagonistic interactions in their importance. We examined the potential role of endophytic fungi in enabling species coexistence, considering whether the presence of the fungal endophyte *Neotyphodium* sp. may alter the niche of marsh bluegrass, *Poa leptocoma*, and affect its coexistence with a closely related but endophyte-free grass species, nodding bluegrass, *Poa reflexa*, in subalpine meadows of the Rocky Mountains, USA. We tested two questions: 1) Do *P. leptocoma* and *P. reflexa* occupy different ecological niches? and 2) Does endophyte presence affect *P. leptocoma* and *P. reflexa* seed germination and seedling growth in different putative niches? *Poa leptocoma* grew closer to water sources and in wetter soils than *P. reflexa*, although the two species can be found intermixed in certain environments. Endophyte-symbiotic *P. leptocoma* seeds germinated with greater frequency in *P. leptocoma* niches relative to *P. reflexa* niches, whereas there was no difference in percent germination of endophyte-free (experimentally removed) *P. leptocoma* seeds or *P. reflexa* seeds between the two habitats. Endophyte-symbiotic *P. leptocoma* seeds germinated marginally more quickly in *P. leptocoma* niches relative to *P. reflexa* niches, and *P. reflexa* seeds germinated more quickly in *P. reflexa* niches. Our study indicates that *P. leptocoma* and *P. reflexa* occupy different ecological niches in the habitats that we examined, but that niche overlap does exist between the two species. Our results suggest that the fungal endophyte *Neotyphodium* sp. constrains the niche of *Poa leptocoma* to wetter microsites during early life history stages, thus contributing to partitioning the niches of the two species by limiting its host rather than providing it with greater niche opportunities. While endophyte symbiosis has been documented to convey tolerance to abiotic and biotic stresses, it may also play a more complicated role in influencing community composition in certain systems.

Introduction

Defining the mechanisms that enable species to coexist is a fundamental goal of community ecology. Attention to this question has traditionally focused on antagonistic species interactions, namely competition and predation, as controls on species coexistence (Bruno et al. 2003, Stanton 2003, Begon, Townsend, and Harper 2006). However, positive species interactions have gained increased attention and may rival antagonistic interactions in their importance (Bertness and Callaway 1994, Bruno et al. 2003, Agrawal et al. 2007, Kiers et al. 2010).

Mutualisms have been suggested to play important roles in influencing species coexistence and determining community composition (Palmer, Stanton, and Young 2003, Umbanhower and McCann 2005). Such positive interactions may give species larger ranges by expanding or shifting the niches of individual partners (Bruno et al. 2003, Goodacre et al. 2009). Mutualists may increase the competitive dominance of host species, thereby decreasing species coexistence and the species richness of competitors in the community (Harnett and Wilson 1999, Rudgers et al. 2007). In other systems, mutualisms appear to increase species diversity and coexistence, often when benefits are provided to multiple species in the community (Müller and Godfrey 1999, Hart et al. 2003).

A small number of studies have directly tested whether mutualisms have the potential to promote species coexistence by altering the niches of host organisms. For instance, ants that distribute seeds of *Hexastylis arifolia* were not found to affect the niche of their hosts (Warren et al. 2010). In contrast, mutualistic ants were found to shift the niche of *Lycaeides melissa* caterpillars, enabling them to expand to a new host (Forister et al. 2010). Similarly, arbuscular mycorrhizal fungi can increase species coexistence by reducing the ability of one plant species to repress the other via competition for shared resources (Wagg et al. 2011).

This study focuses on the potential role of endophytic fungi in enabling species coexistence. Endophytic fungi and bacteria are commonly found in the tissues of plants (Bacon and White 2000), and range from mutualists to parasites. Endophytes are responsible for certain secondary chemicals previously thought to be produced by plants (Strobel 2002), and can buffer plants against damage by pathogens and herbivores (Clay and Schardl 2002) as well as abiotic stressors (Rodriguez et al. 2008). Such effects on host plants can in turn have community (Rudgers et al. 2007) and ecosystem-level consequences (Lemons et al. 2005, Omacini et al. 2012).

In this study, we examined grasses that form symbioses with systemic, vertically transmitted fungal endophytes. These Class 1 endophytes (family Clavicipitaceae) occur in 20-30% of all grass species and have also been reported in sedges and morning glories (Leuchtmann 1992, Rodriguez et al. 2009). Fungal endophytes are typically found in all aboveground tissues of the plant and are commonly passed from mother plant to daughter seed (Clay and Schardl 2002). In some host grasses, endophytes are known to improve host tolerance to drought, increase nitrogen and phosphorus content, and produce alkaloids that deter herbivores (reviewed by Clay and Schardl 2002, Malinowski and Belesky 2000, Cheplick and Faeth 2009, Schardl 2011). However, whether endophytes can shift the niche of their hosts and thereby promote coexistence with closely related competitor plants remains undocumented.

We considered whether the presence of a fungal endophyte may alter the niche of marsh bluegrass, *Poa leptocoma*, and affect its coexistence with a similar but endophyte-free grass species, nodding bluegrass, *Poa reflexa*, in subalpine meadows of the Rocky Mountains, USA. *P. leptocoma* and *P. reflexa* 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 excess water -- although this hypothesis has never been tested. Specifically, we tested two 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? and 2) Does endophyte presence affect *P. leptocoma* and *P. reflexa* seed germination and seedling growth in different putative niches?

Methods

Study organisms

Both *Poa leptocoma* and *Poa reflexa* 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 collected samples from *P. leptocoma* and *P. reflexa* populations in the Rocky Mountains of Colorado, USA, sampling from the Rocky Mountain National Park (Larimer, Grand, and Boulder counties) south to Gunnison County. Locality information and samples sizes are provided in Table 1. We took thin sections from the inner leaf sheaths of collected samples and stained them with aniline blue lactic acid dye following methods in Bacon and White (1994) to check for endophyte presence or absence at 200X magnification.

In our surveys, *P. leptocoma* was symbiotic with the systemic, asexual, vertically transmitted fungal endophyte *Neotyphodium* sp. (we never observed stromata), approaching 100% frequency of infection, while no systemic endophyte was associated with *P. reflexa*. *Neotyphodium* sp. is a Class 1 endophyte that appears to reproduce exclusively asexually, growing into the seeds of its host. In greenhouse studies, the endophyte was passed on to daughter seedlings approximately 95% of the time, but it remains at very high frequencies in the natural populations surveyed.

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) (Figure 9). Plants were marked approximately every 2 m along transects in three regions of the watershed: directly in and along a shady streambed (where 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 (39°01.329N, 107°02.938W, elevation 3218 m) and at one site on Snodgrass Mountain (38°55.083N, 106°59.175W, elevation 3357 m). Since anecdotal data suggested that *P. leptocoma* prefers wetter environments than *P. reflexa* (Barkworth et al. 2007), we examined 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 12 cm 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).

At the Virginia Basin site, we measured soil moisture at all marked plants, 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 a transect line and recorded data on the individual plant of each species that was found closest to the stream. At the Schofield and Snodgrass sites, each examined plant was located at least 3 m away from the next plant of the same species.

We analyzed data with ANOVA, and included the fixed factors of plant species identity, site, and the site×species interaction (SAS version 9.2, SAS Institute, Inc., Cary, NC). A significant interaction would indicate that differences between the two species in their niche characteristics were not consistent across sites. We log-transformed the responses of distance and VWC to improve both the normality of residuals and homogeneity of variances.

2) Does endophyte presence affect *P. leptocoma* and *P. reflexa* seed germination and seedling growth in different putative niches?

Experimental approach. We used the marked plants at the Virginia Basin site to define 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 one *P. leptocoma* endophyte-symbiotic (E+) control seed, three *P. leptocoma* endophyte-free (E-) disinfected seeds, two *P. leptocoma* naturally collected (and naturally E+) seeds, and three *P. reflexa* naturally collected (and naturally E-) seeds. All seeds were originally collected from the Virginia Basin site. *Poa 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- control 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 to 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 (Premier Horticulture, Quakertown, PA) and 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 planted the toothpicks in the ground to position the seeds just at the soil surface, providing exposure to light for germination. The seeds were planted on 9 July 2011 in randomized order in a circle with a radius of 5 cm around each original, naturally occurring plant. In 2011, we scored germination of the seeds weekly for 7 weeks. We also measured volumetric water content at each niche site on 25 July and 1, 8, 15, 22, and 29 August 2011, to gain a season-long perspective on differences in water availability. We resumed measurements in the summer of 2012, and scored germination, measured height to the tallest leaf (cm), and counted the number of leaves and tillers on each plant on 14 June and 20 August of that year. We repeated these measurements on 20 June 2013.

Expectations and data analysis. We expected that endophyte presence would shift the niche of *P. leptocoma*, and predicted that *P. leptocoma* E+ seeds would germinate best in *P. leptocoma* habitats, *P. reflexa* seeds would germinate best in *P. reflexa* habitats, and *P. leptocoma* seeds without the endophyte would germinate best in *P. reflexa* habitats. This prediction would result in a statistically significant interaction between the seed identity and the site (niche) identity. We analyzed data using a log-linear model, including the binomial response of germination (0/1) with a logit link function (Proc GLIMMIX, SAS version 9.2, SAS Institute, Inc., Cary, NC). The model included the fixed effects of seed type, site type (*P. leptocoma* or *P. reflexa* niche) and their interaction, as well as the random effect of plant identity (nested within

site type) to account for the non-independence of seeds placed around the same naturally-occurring plant. 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. We also specifically compared the responses of E+ versus E- *P. leptocoma* seeds within each niche type. For seeds that germinated, we used a proportional hazards models to examine the duration of time to germination and the amount of time that seedlings survived (irrespective of when they germinated) (Proc PHREG, SAS version 9.2).

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 1). We thus found that *P. reflexa* typically occupied 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% at *P. reflexa* sites (Figure 2). This trend was present on all dates that VWC measurements were taken, with soil moisture seen to be higher at *P. leptocoma* sites (Figure 3).

Similar differences were observed at two of the three additional locations surveyed (Figures 1 and 2). At site 1 and site 2, both at Schofield Pass, *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 Snodgrass Mountain, there were no significant differences between the two plant species in terms of mean distance from a water source or mean VWC (Figures 1 and 2). Soil moisture was lower overall at Snodgrass Mountain than at the other sites.

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

The percentage of seeds that germinated depended upon both upon seed type and site type (Table 2). In accordance with predictions, a significantly greater percentage of endophyte-symbiotic *Poa leptocoma* seeds germinated in *Poa leptocoma* habitats relative to *Poa reflexa* habitats (Figure 4). This was the case for both control and natural *P. leptocoma* seeds. For both endophyte-free *P. leptocoma* seeds and *P. reflexa* seeds, however, there was no significant difference in percentage germination between *P. leptocoma* and *P. reflexa* niches (Figure 4). *P. leptocoma* E- plants had the highest overall germination of any of the seed type/site type combinations examined (Figure 4), suggestive of a potential cost to endophyte symbiosis in *P. leptocoma* during the seed germination stage.

Similarly, the number of weeks in which seeds germinated differed by seed type and site type (Table 3). E+ control *P. leptocoma* seeds germinated marginally more quickly in *P. leptocoma* niches relative to *P. reflexa* niches, and *P. reflexa* seeds germinated significantly more quickly in *P. reflexa* niches (Figure 5). There was no significant difference in weeks to germination between the two niche types for *P. leptocoma* E- seeds (Figure 5). This was also the case for natural *P. leptocoma* E+ seeds, although sample sizes for these naturally grown seeds

were small because the germination percentage was low (Figures 4 and 5). Greenhouse-grown seeds germinated in fewer weeks than did naturally-occurring seeds (Figure 5).

Length of survival of seedlings differed significantly by site type (Table 3), but not by seed type. On average, most seed types survived for greater lengths of time in the *P. leptocoma* niche relative to the *P. reflexa* niche (Figure 6). However, surprisingly, this trend was only statistically significant for *P. reflexa* plants, for which we expected higher survival in the *P. reflexa* niche.

Plant height, as measured in June 2013, did not differ significantly between seed types and site types (Table 4, Figure 7). Site type marginally affected the number of leaves on each plant in June 2013 (Table 4), with *P. leptocoma* E+ plants having a higher number of leaves in *P. leptocoma* niches (Figure 8). The number of leaves on *P. leptocoma* E- plants and *P. reflexa* plants did not differ significantly between niches.

Discussion

In three of the four sites examined, *Poa leptocoma* grew significantly closer to water sources and in wetter soils than did *Poa reflexa* (Figures 1 and 2), supporting the hypothesis that the two grass species occupy different ecological niches in the habitats that we examined. This result is consistent with anecdotal observations that *P. leptocoma* inhabits wetter environments than *P. reflexa* (Barkworth et al. 2007). We also found that *P. reflexa* seeds germinated more quickly in *P. reflexa* niches relative to *P. leptocoma* niches (Figure 5), while E+ control *P. leptocoma* seeds germinated more quickly in *P. leptocoma* niches, providing some experimental evidence for niche differentiation between the two species.

It is possible that the niche differences observed between *Poa leptocoma* and *Poa reflexa* are not based on water availability alone but are in fact linked to other factors. Shade effects, soil nutrient effects, or other factors, such as soil microbe composition, could be correlated with soil moisture or distance from a water source, and may be the true source of niche divergence. The two factors we measured are not necessarily the only ones that contribute to differences in the two species' niches. However, our finding that differences in soil water content were generally stronger than differences in distance from the nearest water source (e.g., 165% difference vs. 77% at Virginia Basin) suggests that water availability may be an important niche differentiator for *P. leptocoma* and *P. reflexa*.

Poa leptocoma and *Poa reflexa* can also inhabit similar environments, as indicated by the results obtained at the Snodgrass Mountain site. At that site, significant differences were not observed between the two grass species in terms of mean distance from the closest water source or soil water content (Figures 1 and 2). However, at Snodgrass Mountain, soil moisture was overall much lower than at the other sites, and differences between *P. leptocoma* and *P. reflexa* habitats were smaller than at the other sites. These findings 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 grows.

In the seed germination experiment, *P. leptocoma* without the endophyte had higher germination at *P. reflexa* sites than did *P. leptocoma* with the endophyte (Figure 4). This result suggests that, without the endophyte, *P. leptocoma* would have greater overlap with the niche of *P. reflexa*, resulting in stronger interspecific competition. The presence of the fungal endophyte may therefore help the two grass species coexist by shifting the early niche requirement of *P. leptocoma* seeds and seedlings.

However, while there was greater germination of *P. leptocoma* E+ seeds in *P. leptocoma* habitats relative to *P. reflexa* habitats, there was no significant difference in percentage germination of either *P. leptocoma* E- or *P. reflexa* seeds between niches (Figure 4). *P. reflexa* thus appears to be the superior competitor in the environments we examined, as it germinated equally well in both *P. leptocoma* and *P. reflexa* niches. Our results therefore suggest that, in this system, the fungal endophyte does not convey a benefit during the early life history stages of its host grass, but may in fact be constraining *P. leptocoma* to a unique niche.

The observation that all seed types had better survival in the *P. leptocoma* niche relative to the *P. reflexa* niche (Figure 6) can possibly be explained by the fact that 2012 was an exceptionally dry year in the study area. The greater availability of water in the *P. leptocoma* habitat may have enabled all seed types to survive better in that habitat during a year of little precipitation. Additional survival data will be collected at the end of the summer of 2013, and analyses will be conducted to examine whether the observed survival trend continues to hold.

It is worthwhile to note that this study examined only the early life history stages of *P. leptocoma* and *P. reflexa* when considering the effects of fungal endophyte presence or absence. It is possible that the examination of adult grasses would yield different results regarding how the fungal endophyte contributes to niche partitioning between the two bluegrass species. An ongoing experiment is considering adult grasses and their growth and survival in *P. leptocoma* and *P. reflexa* niches.

Our study provides certain evidence that *P. leptocoma* and *P. reflexa* occupy different ecological niches, while also suggesting that niche overlap can occur between the two species. *Neotyphodium* sp., the endophyte of *Poa leptocoma*, appears to play a role in partitioning the niches of the two species, but perhaps mainly by restricting its host's distribution as opposed to providing it with greater niche opportunities. Our study therefore identifies a symbiotic relationship as a mechanism influencing the coexistence of two species. While endophyte symbiosis has been documented to convey tolerance to abiotic and biotic stresses, it may also play a more complicated role in influencing community composition in certain systems. While negative species interactions are often considered the driving force behind ecological processes, symbiotic relationships can have important effects as well and should be further examined.

Acknowledgments

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Figures and Tables

Table 1. Locality information of sites at which *Poa leptocoma* and *Poa reflexa* plants were collected and/or marked, along with date of sampling, sample size, and percent of plants at each site that were symbiotic with the fungal endophyte *Neotyphodium* sp. *n* indicates the number of individual plants sampled per site.

<u>Species</u>	<u>Site</u>	<u>Latitude</u>	<u>Longitude</u>	<u>Elevation (m)</u>	<u>% Symbiotic</u>	<u>n</u>	<u>Date</u>
<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 Creek 1	38°57.586	106°58.637	2986	100	13	8/15/08
	Copper Creek 2	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 - 9/2/08
	Schofield Pass 1	39°01.329	107°02.938	3218	100	10	8/9/11
	Schofield Pass 2	39°01.461	107°02.960	3193	80	10	8/9/11
	Schofield Pass 4	39°01.489	107°02.851	3189	71	28	8/17/08
	Schofield Pass 3	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. Vrain 1	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. Vrain 2	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 Basin 3	38°58.187	106°59.001	3205	0	6	8/9/08
	Schofield Pass	38°58.314	106°58.942	3229	0	10	9/1/08
	Virginia Basin 1	38°58.314	106°58.942	3229	0	10	9/2/08
	Virginia Basin 2	38°58.913	106°58.249	3630	0	16	8/11/08
	Mt. Bellevue	39°01.023	107°01.742	3630	0	9	8/12/08
	Schofield Pass 1	39°01.329	107°02.938	3218	0	10	8/9/11
	Schofield 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

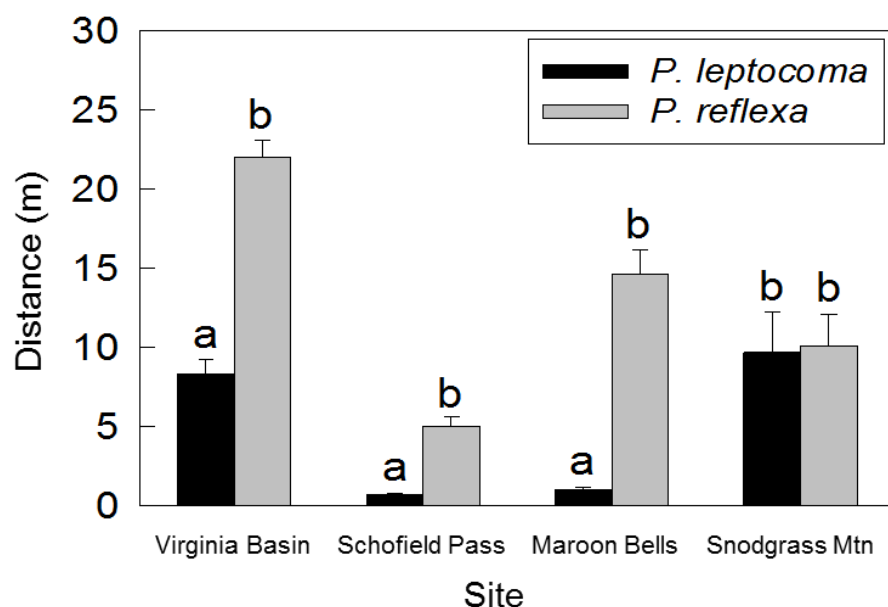


Figure 1. Mean distance from the nearest water source (± 1 standard error) of *Poa leptocoma* and *Poa reflexa* plants at each site examined. Pairs of means annotated with different letters differed significantly from one another.

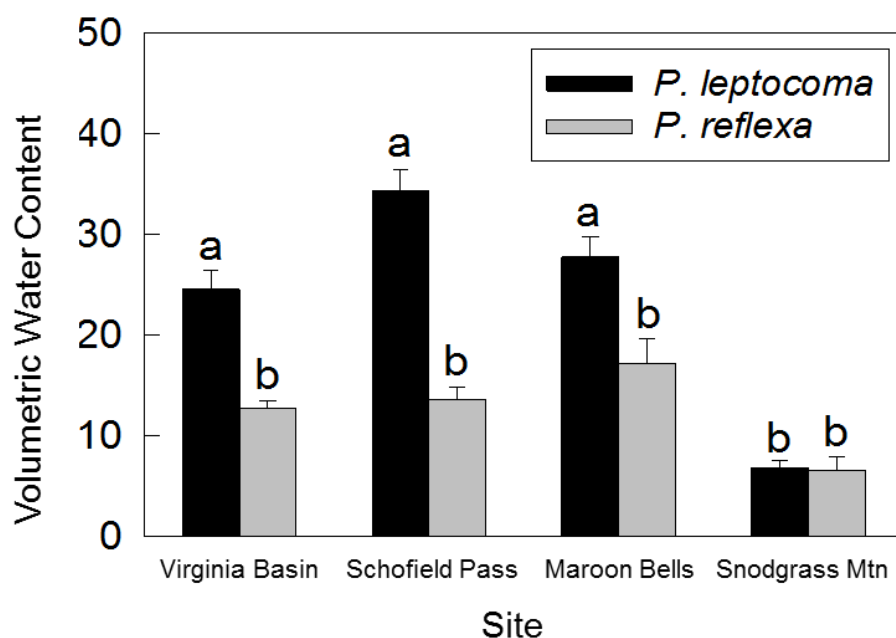


Figure 2. Mean volumetric water content (VWC) (± 1 standard error) of the soil at *Poa leptocoma* and *Poa reflexa* plants at each site examined. Pairs of means annotated with different letters differed significantly from one another.

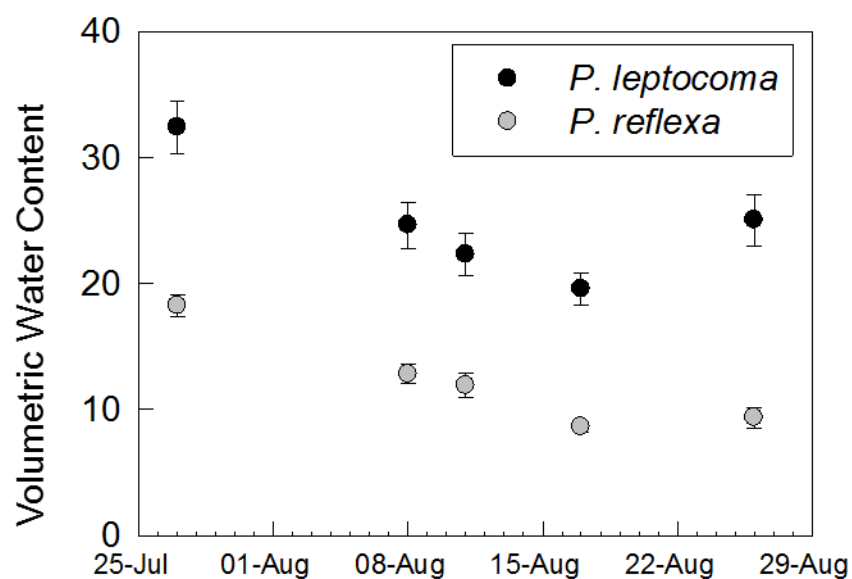


Figure 3. Mean volumetric water content (VWC) (± 1 standard error) of the soil at *Poa leptocoma* and *Poa reflexa* plants at the Virginia Basin site by date in 2011.

Table 2. Results of a log-linear model testing the effects of seed type, site type, and the interaction between seed type and site type on percentage germination of seeds.

<u>Effect</u>	<u>Numerator Degrees of Freedom</u>	<u>Denominator Degrees of Freedom</u>	<u>F-value</u>	<u>P-value</u>
Seed Type	3	808	17.18	<.0001
Site Type	1	100	5.52	0.0208
Seed Type*Site Type	3	808	3.46	0.0161

Table 3. Results of proportional hazard models testing the effects of seed type, site type, and the interaction between seed type and site type on number of weeks taken for seeds to germinate and length of time in weeks that germinated seedlings survived.

<u>Effect</u>	<u>Weeks to Germination</u>		<u>Length of Survival</u>	
	<u>Degrees of Freedom</u>	<u>P-value</u>	<u>Degrees of Freedom</u>	<u>P-value</u>
Seed Type	2	0.0024	3	0.0937
Site Type	1	0.0519	1	0.0412
Seed Type*Site Type	3	0.0157	3	0.3018

Table 4. Results of statistical analyses of the effects of seed type, site type, and the interaction between seed type and site type on height of each plant and number of leaves on each plant in June 2013.

<u>Effect</u>	<u>Numerator</u>	<u>Denominator</u>	<u>Height</u>		<u>Number of Leaves</u>	
	<u>DF</u>	<u>DF</u>	<u>F-value</u>	<u>P-value</u>	<u>F-value</u>	<u>P-value</u>
Seed Type	2	23	0.42	0.6592	0.31	0.7362
Site Type	1	51	0.03	0.8654	3.87	0.0545
Seed Type*Site Type	2	23	0.64	0.5351	0.46	0.6339

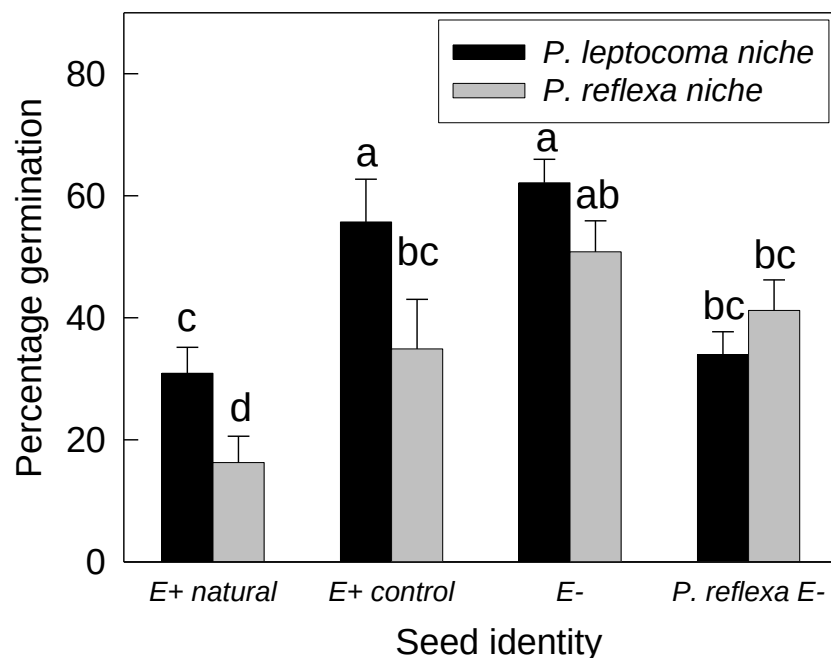


Figure 4. Mean percentage germination (± 1 standard error) of *Poa leptocoma* and *Poa reflexa* seeds in *Poa leptocoma* and *Poa reflexa* presumed niches. Means annotated with different letters differed significantly from one another. E+ natural signifies *Poa leptocoma* seeds from naturally occurring plants, E+ control signifies greenhouse-grown *Poa leptocoma* seeds, E- signifies endophyte-free *Poa leptocoma* seeds, and *P. reflexa* E- signifies *Poa reflexa* seeds.

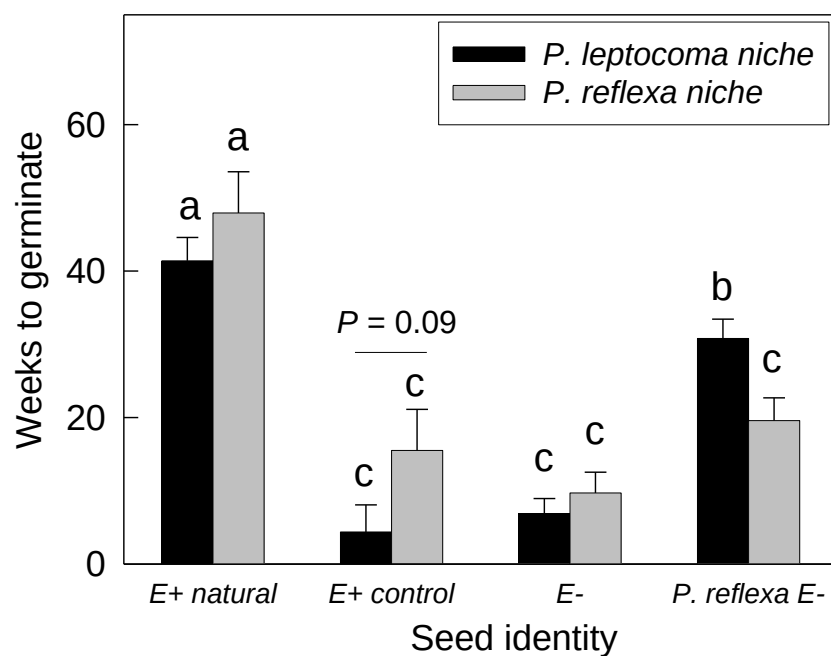


Figure 5. Mean number of weeks taken for *Poa leptocoma* and *Poa reflexa* seeds to germinate in *Poa leptocoma* and *Poa reflexa* presumed niches (± 1 standard error). Means annotated with different letters differed significantly from one another. E+ natural signifies *Poa leptocoma* seeds from naturally occurring plants, E+ control signifies greenhouse-grown *Poa leptocoma* seeds, E- signifies endophyte-free *Poa leptocoma* seeds, and *P. reflexa* E- signifies *Poa reflexa* seeds.

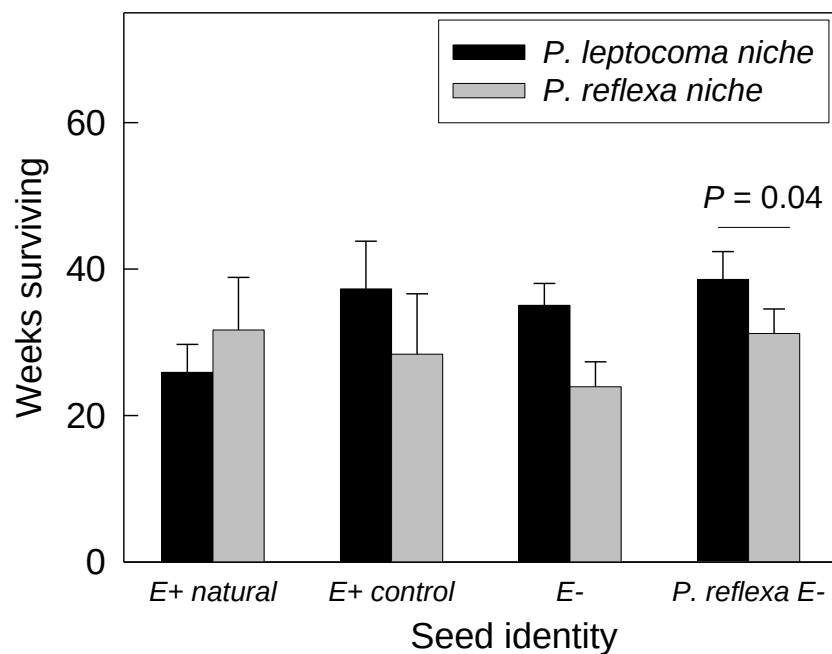


Figure 6. Length of time in weeks that germinated seedlings survived (± 1 standard error). E+ natural signifies *Poa leptocoma* seeds from naturally occurring plants, E+ control signifies greenhouse-grown *Poa leptocoma* seeds, E- signifies endophyte-free *Poa leptocoma* seeds, and *P. reflexa* E- signifies *Poa reflexa* seeds.

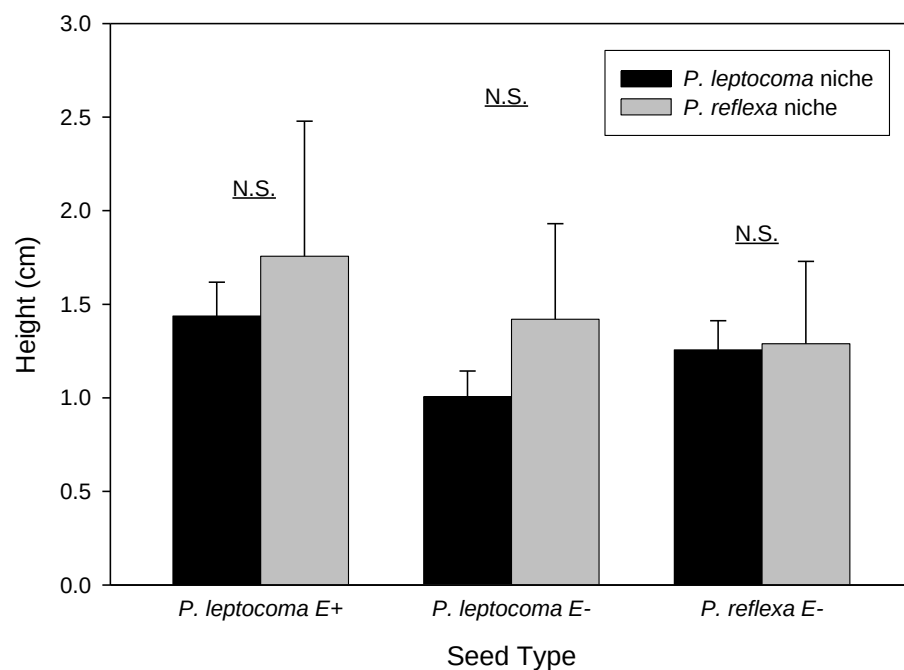


Figure 7. Mean height in cm of *Poa leptocoma* and *Poa reflexa* plants in June 2013 (± 1 standard error).

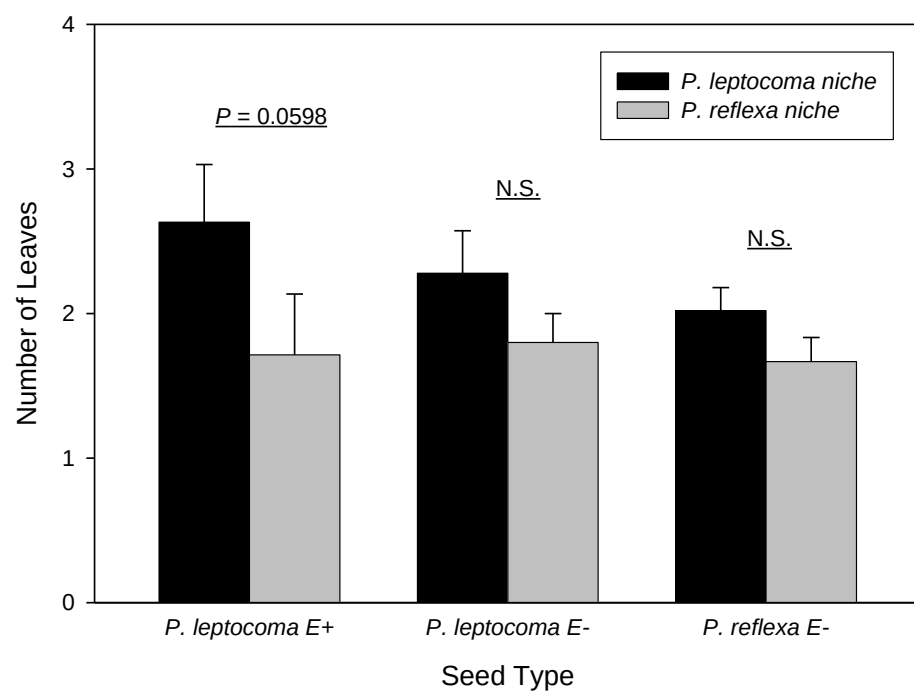


Figure 8. Mean number of leaves on *Poa leptocoma* and *Poa reflexa* plants in June 2013 (± 1 standard error).

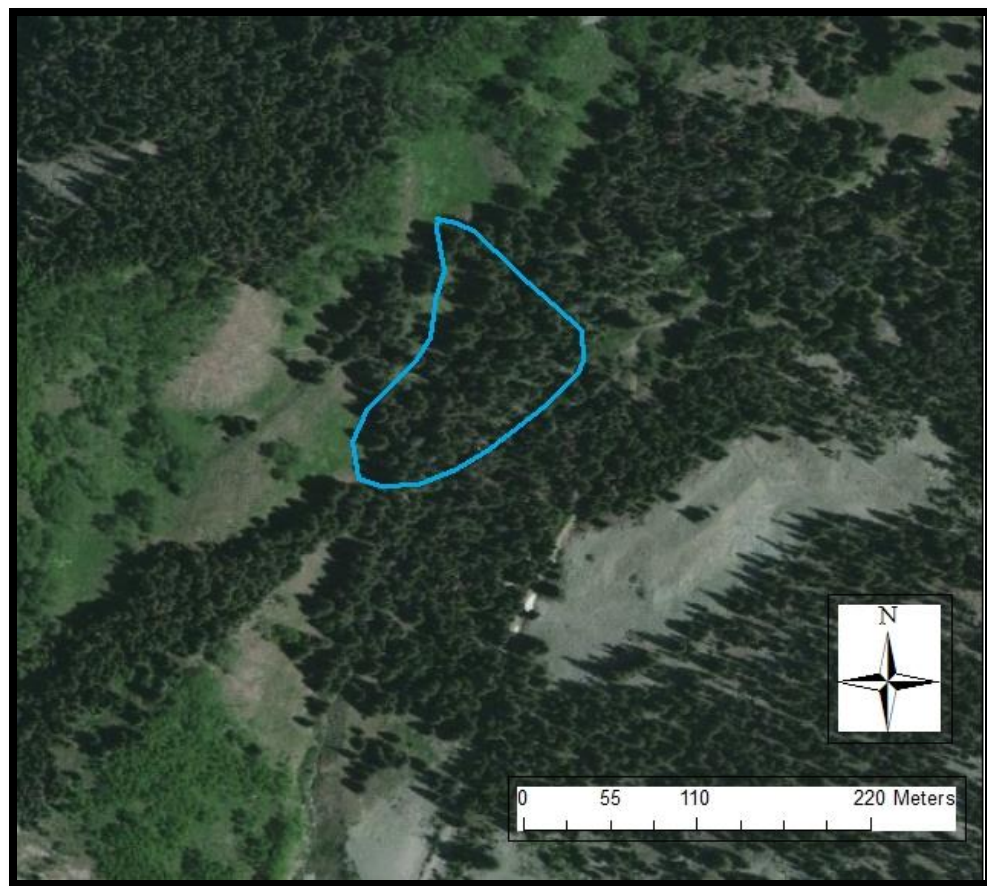


Figure 9. Map of the site in Virginia Basin, Gunnison County, Colorado ($38^{\circ}58.314\text{N}$, $106^{\circ}58.942\text{W}$, elevation 3229 m) at which *Poa leptocoma* and *Poa reflexa* plants were marked.

Literature Cited

- 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. Townsend, 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.
- 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.
- Goodacre, S. L., O. Y. Martin, D. Bonte, L. Hutchings, C. Woolley, K. Ibrahim, C. F. G. Thomas, and G. M. Hewitt. 2009. Microbial modification of host long-distance dispersal capacity. *BMC Biology* 7:1741-7007.
- 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.

- Kiers, E. T., T. M. Palmer, A. R. Ives, J. F. Bruno, and J. L. Bronstein. 2010. Mutualisms in a changing world: an evolutionary perspective. *Ecology Letters* **13**:1459-1474.
- Lemons, A., K. Clay, and J. A. Rudgers. 2005. Connecting plant-microbial interactions above and belowground: a fungal endophyte affects decomposition. *Oecologia* **145**:595-604.
- Leuchtman, 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.
- Omacini, M., M. Semmartin, L. I. Perez, and P. E. Gundel. 2012. Grass-endophyte symbiosis: A neglected aboveground interaction with multiple belowground consequences. *Applied Soil Ecology* **61**:273-279.
- Palmer, T. M., M. L. Stanton, and T. P. Young. 2003. Competition and coexistence: Exploring mechanisms that restrict and maintain diversity within mutualist guilds. *American Naturalist* **162**:S63-S79.
- Rodriguez, R. J., J. Henson, E. Van Volkenburgh, M. Hoy, L. Wright, F. Beckwith, Y. O. Kim, and R. S. Redman. 2008. Stress tolerance in plants via habitat-adapted symbiosis. *Isme Journal* **2**:404-416.
- 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.
- 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.
- Stanton, M. L. 2003. Interacting guilds: Moving beyond the pairwise perspective on mutualisms. *American Naturalist* **162**:S10-S23.
- 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