

The future of plant-fungal symbioses along elevational gradients

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Abstract

Plant-fungal symbioses affect the growth and fitness of most plants on earth. Moreover, they can structure plant community composition and feedback to affect ecosystem-level properties such as carbon storage. As climate change drives species distribution shifts in plants, the fungi may or may not co-disperse along with their symbiotic plant species. Thus, the future of these relationships could look very different, and owing to the potential community and ecosystem-level ramifications, it is important to understand the consequences of these potential plant-symbiont mismatches. We simulated the species distribution shift of *Festuca thurberi* and *Festuca saximontana* expected under climate warming in the next century and manipulated the fungal communities of plants at high and low elevation. We found that broadly distributed *Festuca saximontana* will perform poorly if associated with fungi from low elevation, regardless of which elevation it is grown in. *Festuca thurberi* on the other hand, will perform better at all elevations if they co-disperse with their fungi from lower elevations. This difference in performance could disrupt current montane plant communities and lead to a change in carbon storage and nutrient cycling in these ecosystems.

Introduction

Some of the most influential changes in climate by the year 2100 include a 2–4.5°C increase in mean annual temperature and shifting precipitation patterns (Soloman et al. 2007). These changing conditions drive independent species range shifts that can create communities without contemporary analogs (van der Putten

2012). Because symbiont partners may disperse at different rates, the interactions between species may also change (Walther 2010). For example, the consortia of symbiotic microbes in host organisms can be disturbed by climate change (Hughes et al. 2003). Vertically transmitted symbionts can accompany their host organism to novel environments (Goodacre et al. 2009). However, plenty of factors affected by climate change have potential to disrupt the existing symbioses; differences in environmental tolerance, dispersal, and phenology between the host and the symbiont can lead to the decoupling of species (Compant et al. 2010, Pritchard 2011).

Around the world, plants have been moving up mountain slopes (Walther et al. 2005, Lenoir et al. 2008, Gottfried et al. 2012, Pauli et al. 2012). Predicting realized range shifts of these plants requires the incorporation of factors other than climate (Davis et al. 1998), such as their interactions with mutualistic and pathogenic symbionts (van der Putten et al. 2010). Belowground fungal symbionts such as arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE) colonize the roots of up to 80% of plant species, heavily influencing nutrient uptake and stress tolerance (Smith and Read 2008, Porras-Alfaro and Bayman 2011). Beyond that, fungal symbionts can affect plant population dynamics (Rudgers et al. 2012), community composition (van der Heijden et al. 1998, Rudgers et al. 2007), and biogeochemical process rates that influence water, carbon, and nitrogen dynamics (Bever et al. 2012). The effects of warming and drought can even be buffered by fungal symbionts (Kivlin et al. 2013).

Considering the extensive relationships that fungal symbionts have with their plant hosts, it is important to study the effect that climate change has on future plant-fungal symbioses. We conducted a mismatch experiment simulating plant species experiencing novel environments due to climate change. Individual plants were grown with fungi from their local, low elevation site, or fungi from a novel, higher elevation and placed in the field at both low elevation (ave. 2880m) and high elevation (ave. 3300m) sites. We monitored the growth of the plants in the field to estimate plant fitness. The experiment was designed to refine our knowledge of symbiotic mismatches that may occur due to the changing climate.

We predicted that if plants grew better with low elevation symbionts at every site, this would be evidence that plant and fungal symbiont benefit from co-dispersing (i.e. plant-fungal symbiont adaptation). Alternatively, if plants grew better with low elevation symbionts at low elevation sites and high elevation symbionts at high elevation sites, this would be evidence for habitat matching or local adaption of fungi to the environment. If plants grew better (worse) without fungal symbionts at high or low elevation, this would be evidence that fungi have a net parasitic (mutualistic) effect on the plant. Finally, if plants grew better with fungi at high elevation, but without fungi at low elevation, this would be evidence for increased symbiont benefits in more stressful environments (e.g., the stress gradient hypothesis, Maestre 2009).

Methods

Study Site

We studied two species of grasses: *Festuca saximontana*, a broad altitudinal range species, and *Festuca thurberi*, a low elevation-restricted species. Grasses were planted in fungal exclusion cylinders along three independent elevational gradients that followed major watersheds in the Upper Gunnison Valley Basin: East River (ER) valley, Washington Gulch (WG) valley, and Slate River (SR) valley. For each elevational gradient, there is a high site (~3300m) and a low site (~2880m). The average of 420 meter difference in altitude in the Rocky Mountains represents the average range shift plants are expected to undergo in a ~3°C annual mean temperature increase due to climate change (Peplin and Losleben 2002). Seeds for the plants were taken from the low site in order to simulate the predicted plant range shifts upslope.

Each site had nine blocks of plants. Within each block, there were 6 cylinders, 3 of each species randomly assigned to a position within the block. There were three separate fungal treatments: one plant with no fungi in sterile soil, low fungi, inoculated from spores at 2880m, and high fungi, inoculated from spores at 3300m. Our 3 gradients × 9 blocks = 27 complete replicates, and with 2 elevations and with 6 cylinders per block, we had 324 total cylinders.

Fungal Treatments

The cylinders were filled with sterilized soil from the local site mixed 1/3 with sterile sand and dug into the ground at a depth of ~15cm. The cylinders exclude exterior fungi using a 0.45µm mesh secured to a 5cm diameter ring. The mesh allows unimpeded water and nutrient flow while excluding hyphae and roots from penetrating the cylinder (Reed and Martiny 2007). Cylinders were additionally protected from pocket gophers and insects with an outer layer of aluminum window screen. Fungal spores extracted from rhizospheric soil and roots via centrifugation and sedimentation (Faegri et al. 1977, Klironomos 2002) were inoculated into the cylinders during planting in September 2014. Spore treatment into a given cylinder represented combined spores from 3 plant rhizospheres at either the low elevation or high elevation site on a gradient, with 3 independent replicates of 3 rhizospheres for each focal plant species. Thus, during August 2014, we collected 18 rhizospheres each site for spore extraction.

In order to germinate seedlings, 10-20 seeds of the species were planted into the cylinder during the natural seed dispersal period in September 2014. Seed numbers were adjusted for the germination rate of each population with the goal of at least 5 germinated seedlings per cylinder. Additional seedlings were removed once one plant was established during the summer of 2015.

Data Analyses

Plant fitness was estimated using the number of tillers, number of leaves, and height, measured to the nearest 0.1 cm. These measurements were taken twice

throughout the summer of 2016, and analyzed independently. Number of plants and inflorescences were recorded once per week in order to keep track of mortality. The raw data were analyzed with a mixed effects model in R that included the effects of elevation, plant species identity and fungal treatment with all interactions and the random effects of gradient (ER, WG, or SR) and block nested within gradient.

Results

At the low elevation sites, plants did better with no fungi. Both *Festuca saximontana* (Fig. 1, $p=0.001$) and *Festuca thurberi* (Fig. 2, $p=0.04$) had a higher number of tillers when paired with no fungi at low elevation. *F. saximontana* had a higher change in the number of tillers since this year's snowmelt when paired the same way (Fig. 5, $p=0.03$). *F. thurberi* also had a higher number of live leaves when paired with no fungi at low elevation (Fig. 7, $p=0.08$).

Height of plant was not affected by fungal treatment. *Festuca saximontana* had no statistically significant differences in height (Fig. 3, $p=0.87$). *Festuca thurberi*, similarly, did not differ significantly in height of the plants (Fig. 4, $p=0.63$).

At the high elevation sites, a fungal mismatch caused poorer plant fitness. *Festuca saximontana* had a significantly lesser change in the number of tiller since snowmelt (Fig. 5, $p=0.09$). *F. saximontana* also had less live leaves when paired with low fungi at the high elevation (Fig. 6, $p=0.09$).

Discussion

Fungal communities associated with plants could influence the structure and function of ecosystems in the wake of climate change. If the plant has a broad geographic range, then the expanding range of pathogenic fungi from lower elevations could hinder the growth of these plants. The plants are adapted to the higher elevation fungi, and perhaps they cannot readily fight off novel pathogenic fungi. If the lower elevation fungi do not keep up with the upward movements of their symbionts, the broad-range species will be healthier. Low elevation-restricted plant species, on the other hand, will benefit from bringing their low elevation fungi with them. If these species co-disperse as climate changes, the plants will benefit from maintaining their relationships. If the fungi from lower elevations cannot meet the expanding ranges of their plant counterparts, the plants will suffer. Thus, depending on the evolutionary history of the plant species, fungal dispersion up the mountain in the same manner as plants will either help or harm the plant. This distinction is important to understand the future of plant communities in montane environments. The success of one plant species or the other may depend on whether or not the fungi from lower elevations are able to co-disperse with their plant symbionts.

The plants at lower elevations performed more poorly when associated with any fungi at all compared to our control with sterile soil. This suggests that the fungi are, overall, playing a pathogenic role on the plants at this elevation. Since there are no sterile soils in the environment we observed, this result hints at the parasitic nature of the symbiotic relationship under these more stressful environmental

conditions. If temperature drives pathogenicity, however, then the fungi may become more parasitic over all elevations.

Our results have broader implications when applied to entire mountain ranges. Since these results were obtained while manipulating three independent altitudinal gradients, we can apply our data to the entire system with much more confidence than if only a single gradient were studied. If encroaching fungi from low elevation hinder the plant species that currently outcompete other species, we may see a shift of entire plant communities towards some unknown community composition with unknown effects at the ecosystem scale. This impacts carbon storage levels in montane systems, as well as nutrient cycling, and could even feed back into affecting climate change. These results call for a deeper understanding of how fungal communities are affected by climate change, as it may control the outlook of our future montane plant communities.

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Tables and Figures

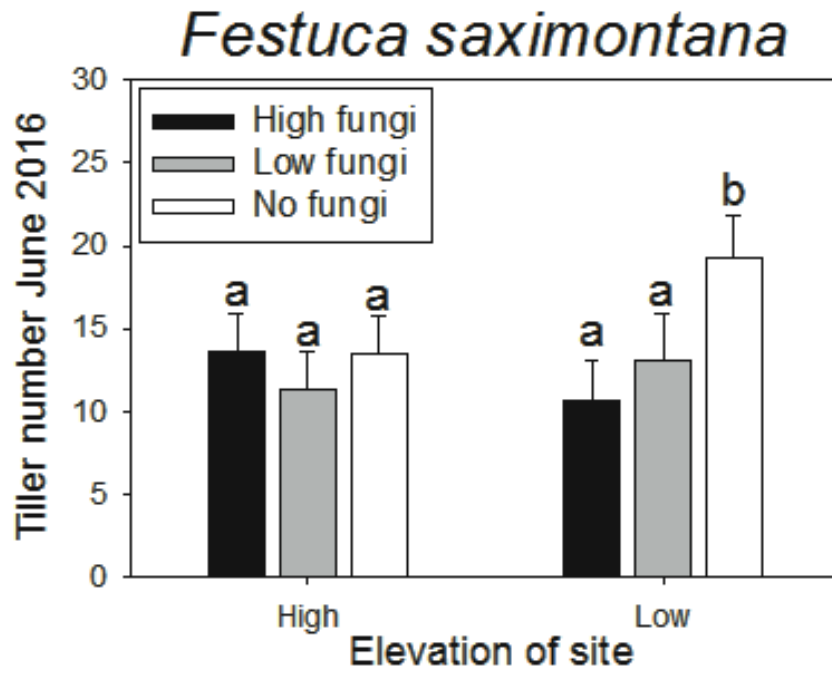


Figure 1 Broad altitudinal range species *F. saximontana* mean number of tillers in June 2016, $p=0.001$

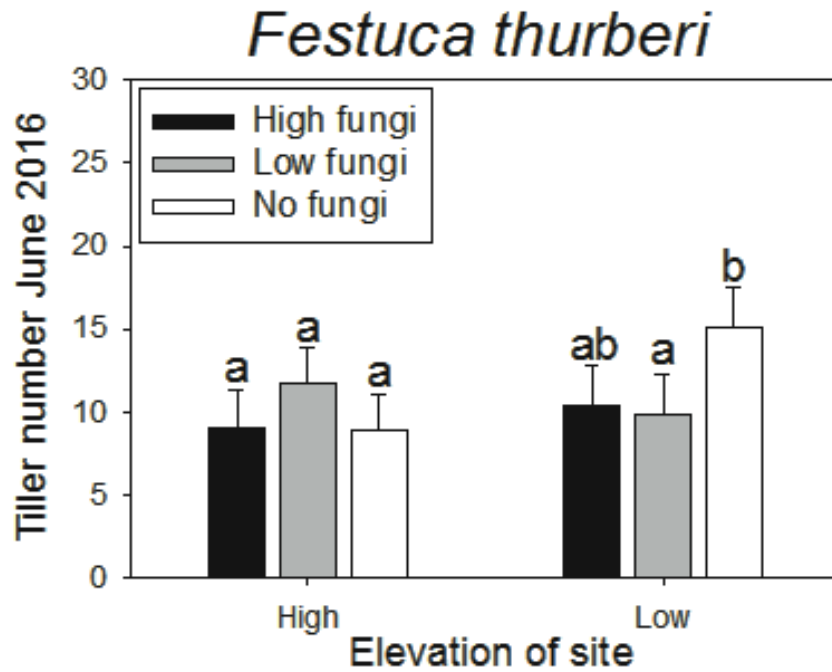


Figure 2 Low elevation restricted species *F. thurberi* mean number of tillers in June 2016, $p=0.04$

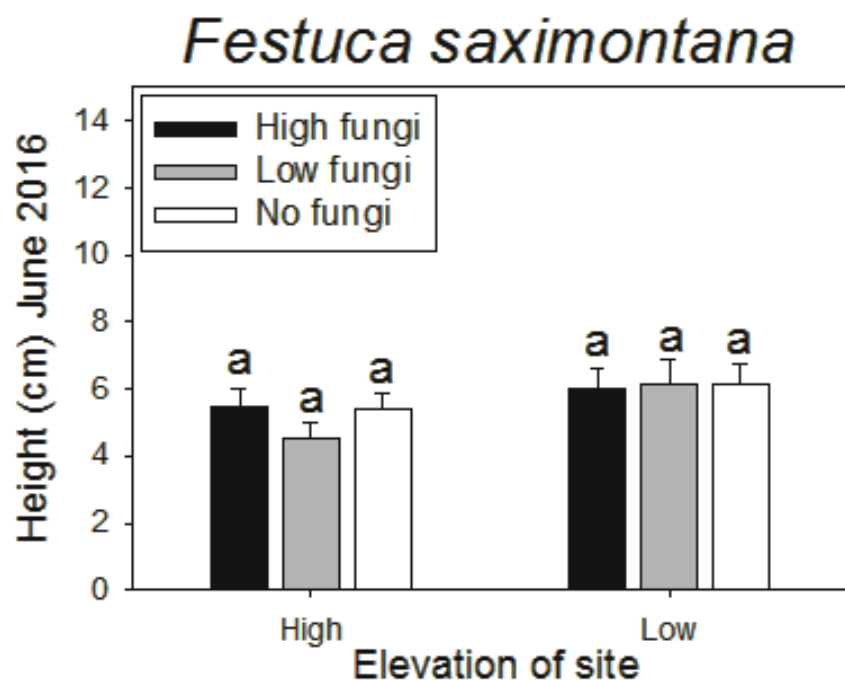


Figure 3 Broad altitudinal range species *F. saximontana* mean height of plant in June 2016, $p=0.87$

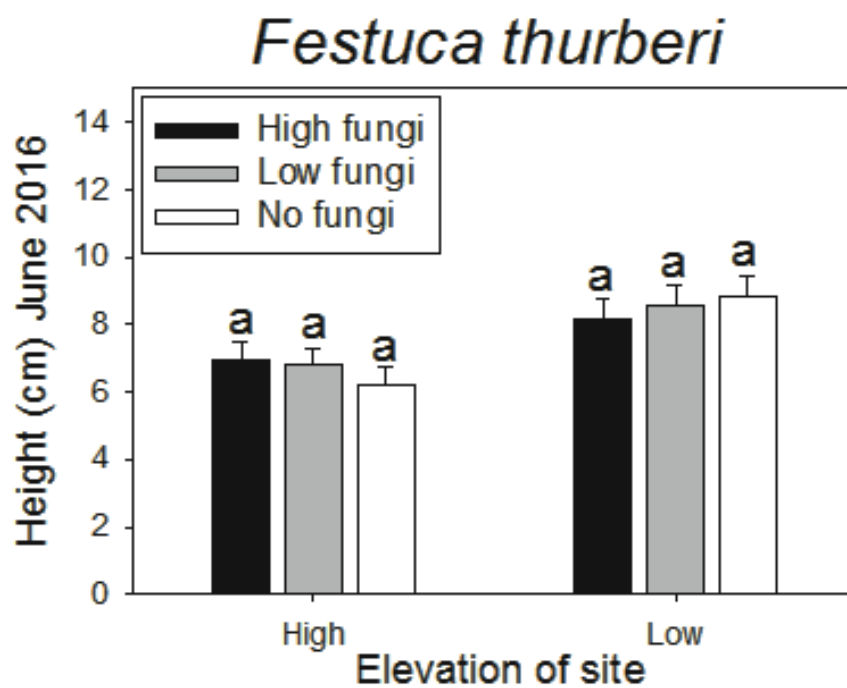


Figure 4 Low elevation restricted species *F. thurberi* mean height of plant in June 2016, $p=0.63$

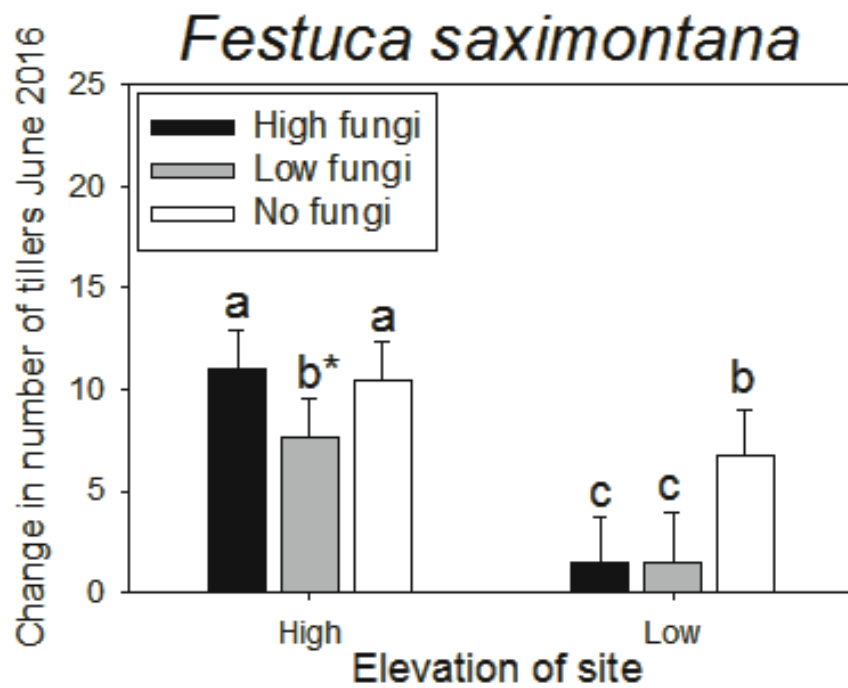


Figure 5 Broad altitudinal range species *F. saximontana* mean change in tillers since snowmelt until June 2016, *p=0.09, p=0.03

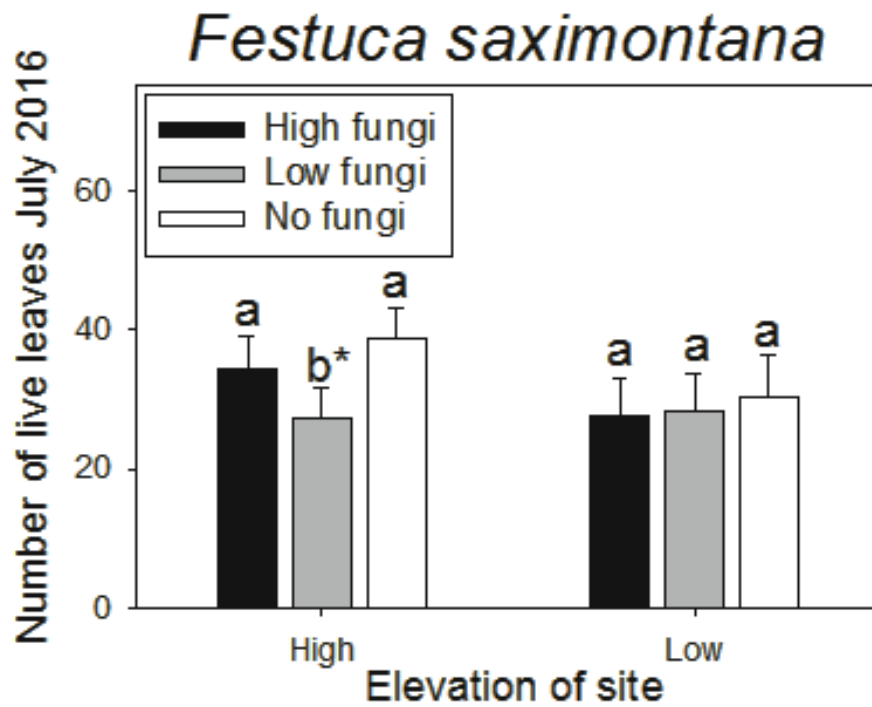
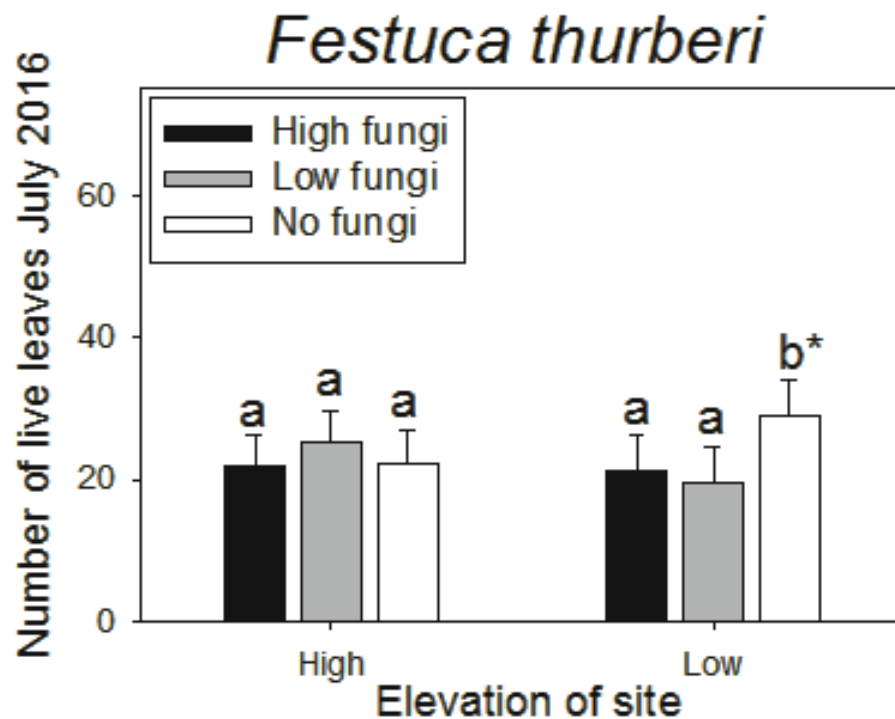


Figure 6 Broad altitudinal range species *F. saximontana* mean number of live leaves in July 2016, *p=0.09



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Figure 7 Low elevation restricted species *F. thurberi* mean number of live leaves in July 2016, *p=0.08

Figure 8 Vicinity map of all 6 sites at three different elevational gradients. Each point shown is a 200m area around the center of the site. Three town areas are shown along with major roads in the area.

Figure 9 East River Valley high site is shown. Each point on the map represents one block of plants. This site is in the Virginia Basin area.

Figure 10 East River Valley low site is shown. Each point on the map represents one block of plants. This site is within the town of Gothic along Gothic Rd.

Figure 11 Washington Gulch Valley high site is shown. Each point on the map represents one block of plants. This site is between Gothic Mt. and Mt. Baldy.

Figure 12 Washington Gulch Valley low site is shown. Each point on the map represents one block of plants. This site is along Washington Gulch Rd.

Figure 13 Slate River Valley high site is shown. Each point on the map represents one block of plants. This site is along Slate Rd.

Figure 14 Slate River Valley low site is shown. Each point on the map represents one block of plants. This site is along Slate Rd.