

How do above and belowground grass-fungus symbioses change over elevational gradients in mountainous Colorado?

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ABSTRACT

Plants and fungi are closely associated and likely have been since terrestrial ecosystems were first inhabited. These symbiotic relationships have taken two main forms: internal aboveground symbionts known as endophytes, and belowground root associated fungi, the most common of which are mycorrhizal fungi. Fungal endophytes produce alkaloids that help the grass defend against herbivory, and have been shown to increase drought tolerance and overall production; mycorrhizal fungi help the plant obtain nutrients and water from the soil. Current data on fungal symbionts do not clearly predict how fungal abundance varies with elevation. Our survey, the first broad grass-endophyte survey in Colorado, spans greater host diversity and steeper gradients than have been examined in previous research and has potential to increase the ability to predict how fungal symbionts will vary along environmental gradients and with important abiotic factors. We surveyed 7 elevational gradients—7 peaks and every vertical 200 m below to an endpoint of about 8600m—in the Colorado Rocky Mountains (Elk Mountain range) and evaluated present grass and fungus species. We examined leaf and root samples for presence of fungal endophytes and mycorrhizae. We likely discovered a novel species of systemic fungal endophyte, and found a number of significant correlations with fungal frequency as related to elevation and soil moisture and also among fungal groups.

INTRODUCTION

Plants and fungi have likely been closely associated for greater than 400 million years, perhaps since the earliest colonization events of terrestrial ecosystems (Krings et al. 2007). These symbioses have primarily taken two forms: internal aboveground symbionts known as endophytes, and belowground root associated fungi, the most common of which are mycorrhizal

fungi. Such close symbioses suggest a co-evolutionary history between plants and their fungal associates (Krings et al. 2007), and in some cases have been shown to confer strong influences on the community composition of plants and animals (Rudgers et al. 2007, Rudgers and Clay 2008, van der Heijden et al. 1998).

Elevational gradients are useful tools for surveys because they provide a natural gradient of variation for abiotic factors like climate and soil properties. At high elevations, temperature and soil nutrients amounts are generally lower, while precipitation is greater (Gardes and Dahlberg 1996, Laughlin and Abella 2007). Other documented abiotic factors that vary with increasing elevation include lower pH and increased organic matter in the soil (Laughlin and Abella 2007, Lugo et al. 2008). There has been relatively little research to date concerning the distribution and abundance of communities of fungal symbionts of plants, including both endophytes and mycorrhizal fungi along elevational gradients.

Current data on fungal symbionts do not clearly predict how fungal abundance varies with elevation. The most recent data from a study at RMBL showed a significant negative correlation between elevation and the frequency of the *Neotyphodium* endophyte in its host plant, *Festuca thurberi* (Jennifer Rudgers, unpublished data). However, scientists conducting a survey in Sweden that focused on a minimal number of grass species (Granath et al. 2007) observed decreasing endophyte frequency with elevation in *Festuca rubra* and increasing frequency with elevation in *Festuca ovina*.

Results from surveys of mycorrhizal fungi are equally equivocal. One study, utilizing an elevation gradient in Arizona, found that mycorrhizal colonization increased with elevation for one grass species (*Muhlenbergia montana*) but elevation had no impact on mycorrhizae for two other grass species (Fisher et al. 2004). In contrast, Lugo et al. (2008) observed fewer arbuscular

mycorrhizal fungi species and decreased spore production at higher elevations (3700 and 3870 m) in the South American Puna Grasslands. Although there are more studies evaluating the response of bacterial colonization to elevation, the predictive power of these studies is limited because of conflicting results. In one study bacterial diversity was found to decrease with elevation (Bryant et al. 2008) while in another study bacterial diversity was unrelated to elevation (Fierer and Jackson 2006, Fierer et al. 2010). Surveys spanning greater host diversity and larger gradients have potential to increase our ability to predict how fungal symbionts will vary along environmental gradients.

Prior to our efforts, a survey of grass endophytes had not been completed in Colorado, although Dr. Rudgers began a survey of graminoid plants in this region in 2008. While Dr. Rudgers' survey uncovered new grass-fungal symbioses, we predicted a more extensive and methodologically consistent survey would find additional fungal diversity. Also, there is a high likelihood that we will discover new grass species as hosts considering the dearth of data concerning endophyte frequency in endemic grass species typically of little commercial importance or value (Cheplick and Faeth 2009). Additionally, our study provides novel and valuable information about how soil nutrients and moisture changes on an elevational gradient in the Elk Mountain region (Ian Billick, personal communication, 2012). Currently, all that is known specific to the Rocky Mountain Biological Laboratory (RMBL) is that soil moisture was significantly lower at a lower elevation site (2770 m) than it was in and above the Gothic townsite—2940 m and 3190 m, respectively—(Dunne et al. 2003). Finally, in our review of the literature, we found no studies that simultaneously examined distributions of above and belowground fungal symbionts across environmental gradients, and therefore we examined the grasses we sampled for both above and belowground fungi to address this question also. Such

patterns are particularly interesting given experimental work indicating such interactions between spatially separated fungal groups are potentially antagonistic (e.g. Mack and Rudgers 2008, Omacini et al. 2006).

In surveying a wide range of grass species for above and belowground fungal symbionts, we compiled some of the first holistic data on relationships among abiotic factors and fungal symbionts on elevational gradients. Specifically, we evaluated (1) trends in abiotic factors over elevational gradients, (2) influence of abiotic factors on fungal frequency, (3) impact of elevation on fungal frequency, and (4) interactions between fungal groups.

METHODS

Study System

Only grasses in the subfamily Pooideae, which contains 9 tribes and 618 species in North America (Barkworth et al. 2007), are known to be associated with systemic Clavicipitaceous fungal endophytes. This survey was limited to grasses in this subfamily and consists of grasses in the tribes Poeae, Triticeae, Bromeae, Stipeae, and Aveneae.

There are two main groups of grass-associated Clavicipitaceous endophytic fungi and they are organized by life history rather than by phylogenetic relatedness. The genus *Epichloë* reproduces sexually and the genus *Neotyphodium* reproduces asexually through vertical transmission in the seeds of their host (Clay and Shardl 2002). *Epichloae* refers to the group containing both genera. Along the continuum of symbioses, individuals in the genus *Epichloë* spread contagiously via spores, tend to be parasitic, and are typically generalists in terms of host preference (Rudgers et al. 2009, White 1988). *Neotyphodium* species on the other hand, being vertically transmitted, have been shown to confer numerous benefits to their hosts including

drought resistance (Bacon 1993), herbivore resistance (Cheplick and Clay 1988), and increased aboveground growth (Groppe et al. 1999). Because of their vertical transmission, species of the genus *Neotyphodium* are often specific to a single host plant species (Schardl 2010), which suggests that surveys of endemic grass species in new areas, such as the Rocky Mountains, could yield novel fungal biodiversity.

Mycorrhizal fungi in the phylum Glomeromycota are usually mutualists associated with plant roots. The fungus penetrates the plant root with intraradical mycelium and extends into the soil as extraradical mycelium, thus forming an interface for resource exchange. In this system, the fungus can provide nitrogen, phosphorous, water, and other nutrient minerals to the plant in exchange for excess carbon from the plant (Smith and Smith 2011). There are different types of mycorrhizal fungi, but the oldest and most common is arbuscular mycorrhizal fungi (AMF). Ectomycorrhizal fungi tend to dominate trees in forest ecosystems (Allen et al. 2003); because we primarily limited sampling to grasses in open treeless meadows we expected to encounter mainly the AMF. Although mycorrhizae typically confer benefits to the plant, the exact response of the plant is hard to predict because it is based on the growth of fungal hyphae, root characteristics, functionality of resource exchange interfaces, and the overall ambient environment (Smith and Smith 2011).

Finally, we made note of non-AM fungi in the roots, some of which could be dark septate endophytes, a polyphletic group of poorly understood soil fungi which also occur in plant roots and may be beneficial to their hosts (Porrás-Alfaro and Bayman 2011). These fungi can be easily distinguished morphologically from the arbuscular mycorrhizal fungi by their dark pigmentation and visible septae.

Site Description

We surveyed 7 total elevational gradients in the Elk Mountains Region of Rocky Mountains of Colorado (Fig. 1). Three of these gradients have their highest site near to existing Global Observation Research Initiative in Alpine Environments (G.L.O.R.I.A) sites. We used portable GPS units (Trimble, Location; Garmin, Location) to record map our sample sites along each transect.

Field Survey

The first site of each gradient was determined by the highest point we could sample in a given valley, usually near 3800 m above sea level. The remaining sites were sampled descending from the high point in increments of 200 m vertical elevation. At each site we selected and recorded a point using our portable GPS units (Trimble or Garmin) and from that point ran a linear 50 m transect in a direction we recorded. Transects, when possible, followed established hiking trails or roads thereby minimizing disturbance to pristine environments. To buffer against the possible confounding variables associated with proximity to established roads—dust, soil disturbance, and chemical additions—we choose sites a minimum of 30 m from the roads. At each site we sampled 5-9 grass species and 6 individuals of each species, collecting individuals closest to the flags placed every five meters along the transect line to ensure they were 5 m apart and separate plants. We collected soil moisture data as well as soil samples at each flag to calculate a mean of nutrients and moisture for each site.

Lab Analysis

Plant identifications were verified with a dichotomous key (Shaw 2008). For each individual plant, two peels from two leaf sheaths of separate culms were mounted on microscope slides and stained with aniline blue lactic acid (Bacon and White 1994). We used light microscopy to score slides for the systemic *Epichloae* endophytes and also noted if localized

foliar endophytic fungi were present. Plant material was refrigerated until the leaf tissue slides were prepared at which point samples were frozen.

We evaluated root samples on the scale of species per site for colonization by AMF and other dark septate fungi. When the endophyte was absent in a species at a site, we combined fine roots from individual plants for analysis. When the endophyte was present in a species, we evaluated the roots of all endophyte positive plants and the roots of all endophyte negative plants separately. To clear roots we packed them in tissue cassettes, refrigerated them in 10% w:v KOH overnight, brought them to a boil, and let them cool to room temperature. For staining we poured off the KOH, rinsed the cassettes in water (4 x 5 min), soaked them in 1% HCl for 20 min, soaked them in preheated 5% ink:vinegar stain for 20 min, and finally rinsed cassettes in water (4 x 5 min) (McGonigle et al. 1990, Vierheilig et al. 1998). To score root slides we counted total views and views of AMF, arbuscules, vesicles, and non-AMF saprophytic fungi. Roots that did not get stained this summer were preserved in 70% ethanol.

To prepare plant tissue samples for future genetic analysis we kept endophyte negative and endophyte positive samples of each species per site separate. Roots and leaves were surface-sterilized in a hypochlorite solution, placed in sterile eppendorf tubes, and frozen. The excess soil attached to the roots of each plant was collected and placed in an eppendorf tube and frozen for future evaluation of microbes in the soil immediately around the grass so that the macrosite- and microsite-level soil microbial communities can be compared. We will send soil samples to the Texas A&M extension (Texas AgriLife Extension Service) for information on pH and Nitrogen, Phosphorous, and Carbon content. We anticipate sending leaf samples to Carolyn Young for sequencing and species-level identification of foliar endophytes (The Samuel Young Noble Foundation).

In the process of using existing grass species collections in the RMBL herbarium to identify our samples, we will likely augment the collection and press some of our specimens.

Data Analysis

We used SAS, Microsoft Excel, and SigmaPlot for statistical analysis. To characterize abiotic changes with elevation, we used regression analysis models and Spearman rank correlations to relate average soil moisture (VWC) per site and variation among soil moisture readings (covariate of VWC) at each site with elevation. We used regression analysis and Spearman ranks to relate *Epichloae* endophyte frequency, other localized endophyte frequency, and percentages of fungi colonizing roots (both AMF and septate/non-AM fungi) to elevation and soil moisture. Finally, we used similar methods to evaluate relationships between *Epichloae* endophytes, localized endophytes, and root colonizers. Because we hope to incorporate multiple variables in our analyses (soil moisture, soil nutrients, pH, elevation, endophyte, mycorrhizae, and host species), we may use model selection procedures (Burnham and Anderson 2002) to determine the best predictors of fungal symbiont frequencies.

RESULTS & DISCUSSION

We sampled more than 1500 individual grass plants primarily in the Elk Mountain range of Colorado and evaluated the presence of fungal symbionts in both above and below ground associations. Within those individuals, 46 grass species were represented, 45 of which were endemic (Appendix B). Only 8.7% of the sampled grass species—4 species—were infected with an *Epichloae* endophyte. In one of those host species, *Trisetum spicatum*, we think we found a new species of systemic fungal endophyte because these fungi are host specific and no

endophytes have been reported in the genus *Trisetum*. However, we still need to pursue molecular analysis to confirm that it is a novel species.

1. Trends in abiotic factors over elevational gradients

The original goal of our survey was to evaluate abundance of soil nutrients, soil organic matter, and soil moisture at every site we sampled. The soil samples we took from each site have not been analyzed for nutrient content yet, and the soil moisture values are almost complete (except for two summits and a few mid-elevation sites). The limited soil moisture data we obtained was positively correlated with elevation but not significantly so ($R=0.18208$, $p=0.2808$, $n=37$). We believe this relationship might become statistically significant when we obtain soil moisture data from the remaining sites.

2. Influence of abiotic factors on fungal frequency

We found some statistically significant correlations between the abiotic factor we have data on, soil moisture, and a couple groups of fungal symbionts. Firstly, we observed a statistically significant positive relationship between soil moisture and the frequency of endophyte infection in the grass genus *Festuca* (Fig. 2). In the localized other aboveground fungi, we observed the same significant trend in the genus *Poa* between soil moisture and the infection frequency of the non-systemic foliar fungal endophytes we called “other fungi” (Fig 3). Increasing infection frequencies of systemic endophytes with moisture could indicate a positive selective pressure for the mutualism because the endophyte could be conferring flood tolerance to their hosts. Finally, we observed a significant *negative* trend in the grass genus *Elymus* for

belowground arbuscular mycorrhizal colonization as related to soil moisture (Fig 4). It seems likely that decreasing frequency of AMF infection with increased water availability could indicate plants no longer require the AMF for water acquisition at higher soil moisture.

3. Impact of elevation on fungal frequency

Because of evidence indicating that under stressful conditions for the hosts selection strongly favors grass-fungus mutualisms (Clay & Schardl 2002), we predicted higher endophyte infection frequency at higher elevations. We observed contradictory trends in the four species in which we found endophytes. For both *Festuca thurberi* and *Festuca brachyphylla* we observed a negative correlation between elevation and endophyte infection frequency; the trend was only significant in *F. brachyphylla* (Fig 5). Perhaps high elevations are too cold for endophytes or the benefits from endophytes are not as needed or selected for at high elevations. For example, one study describes mean herbivory decreasing with elevation (Alonso 1999) so protection from herbivory by endophytes at higher elevations may not be an advantage. There is at best equivocal evidence for trends of decreasing herbivory with increasing elevation, however, with numerous studies stating opposing results (e.g. Hodkinson 2005, Scheidel et. al. 2003).

Looking across all grass species we collected, we found a significant negative correlation between elevation and infection frequency of non-systemic foliar endophytes (Fig. 6). There were no significant patterns with root colonization (both AMF and non-AMF) and elevation, but when our sample size increases this may change. The lack of observed pattern, however, is in contrast to previous findings that AMF colonization decreased and root endophytes (a subset of non-AMF) increased with elevation (Opik et al. 2010).

4. Fungal interactions

Assuming limited plant photosynthate available for allocation to fungal symbionts, we predicted there would be competitive exclusion between groups of fungi. We expected to see that as frequency of infection of one species of fungus increased in a host, frequencies of all other fungi would decrease. Instead we observed more complex patterns, perhaps indicative of facilitation and exclusion between groups of fungi. In fact, we only found significant negative correlations between groups of phyllospheric and rhizospheric fungi in one grass genus (Fig. 7). Within each of these spheres we were surprised to find positive correlations between frequencies of fungal taxa in some grass hosts (Fig. 8), suggesting facilitation. Because our study was exclusively observational, we cannot assess any causation for these relationships, but we believe that either there is competitive exclusion between rhizosphere and phyllosphere fungi as entire groups, or there are additional variables, potentially abiotic, that drive the negative correlation. It could be that soil moisture has a large impact on the interactions we observe between above and belowground fungi. As stated above, we observed increasing rates of infection by systemic and non-systemic foliar endophytes as soil moisture increased, while root fungi declined in frequency along the same abiotic gradient. The “opposite” responses of aboveground fungi and belowground fungi to water could be driving the negative correlation between the two fungal groups. However, direct interactions between fungal groups could also explain the trend. Other experimental studies showed that the presence of systemic endophytes reduced mycorrhizal colonization rates, but they did not show the reverse (Mack and Rudgers 2008, Omacini et al. 2006) nor did they examine whether symbiont diversity within a sphere of the host plant—either in the rhizosphere or in the phyllosphere—facilitated additional species diversity in that area.

CONCLUSIONS

Overall, the proportion of grass species with systemic endophytes (8.7%) is lower than the estimated 20-30% species in Poaceae that associate with systemic fungal endophytes (Leuchtman 1992). Perhaps the observed trend of decreasing fungal frequency with elevation continues to sea level so we missed some species on that continuum. Despite the low frequency of endophytes, we noticed a very interesting pattern of relationships between and within above ground and below ground fungi. It seems like our data is the first to suggest positive correlations between both phyllosphere and rhizosphere fungi separately, while simultaneously noticing negative relationships between the two groups. We would like to investigate these patterns further as they might indicate plant mechanisms for facilitating groups of internal symbionts rather than individuals, as well as potential antagonism between phyllospheric and rhizospheric fungal groups. Finally, the soil nutrient and carbon data has not been used in analysis yet to evaluate how they might influence rate of infection by fungal symbionts. These abiotic factors are likely important determinants of a fungal community and we look forward to incorporating that information.

Our project, though time consuming, was worthwhile in many regards, and we would like to encourage similar projects worldwide. Such surveys offer insight on numerous valuable concepts to the scientific community. For example, it is stunning that there is no consensus on how herbivory, soil nutrients, and soil moisture changes with elevation. In order to properly execute experiments, scientists need to know what variables to manipulate and why. If more surveys describe the environment that certain plants and their fungal symbionts are in, future experiments could determine more definite causal relationships. Furthermore, our survey is powerful because it couples the abiotic measurements with frequencies of groups of organisms—

the host grasses, systemic foliar endophytes, localized foliar endophytes, AM fungi, other root fungi. It not only informs how organisms thrive and under what circumstances, but also sheds light on how interactions between organisms—already shown to structure entire communities—are affected by the environment.

LITERATURE CITED

- Allen, M. F., W. Swenson, J. I. Querejeta, L. M. Egerton-Warburton, and K. K. Treseder. 2003. Ecology of mycorrhizae: a conceptual framework for complex interactions among plants and fungi. *Annual Review of Phytopathology* 41:271-303.
- Alonso, C. 1999. Variation in herbivory by *Yponomeuta mahalebella* on its only host plant *Prunus mahaleb* along an elevation gradient. *Ecological Entomology* 24:317-379.
- Bacon C. W. 1993. Abiotic stress tolerances (moisture, nutrients) and photosynthesis in endophyte-infected tall fescue. *Agriculture, Ecosystems & Environment* 44:123-141.
- Bacon, C. W., and J. F. White, Jr. 1994. Stains, media, and procedures for analyzing endophytes. *Biotechnology of endophytic fungi of grasses*, ed. C. W. Bacon and J. F. White, Jr., pp 47-56. Boca Raton, Florida: CRC Press.
- Barkworth, M. E., K. M. Capels, S. Long, L. K. Anderton, and M. B. Piep. 2007. *Flora of North America Volume 24: North of Mexico: Magnoliophyta: Commelinidae (in part): Poaceae, part 1*. New York, Oxford University Press.
- Bryant, J. A., C. Lamanna, H. Morlon, A. J. Kerkhoff, B. J. Enquist, and J. L. Green. 2008. Microbes on mountainsides: contrasting elevational patterns of bacterial and plant diversity. *Proceedings of the National Academy of Sciences of the United States of America* 105:11505-11511.
- Burnham, K. P. and D. Anderson. 2002. *Model selection and multimodel inference: a practical information-theoretic approach*. 2nd ed. New York: Springer.
- Cheplick G. and K. Clay. 1988. Acquired chemical defenses in grasses - the role of fungal endophytes. *Oikos* 52:309-318.
- Cheplick, G.P. and S.H. Faeth. 2009. *Ecology and evolution of the grass-endophyte symbioses*. New York: Oxford University Press.
- Clay, K. and C. Shardl. 2002. Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. *The American Naturalist* 160:S99-S127.
- Dunne, J. A., J. Harte, and K. J. Taylor. 2003. Subalpine meadow flowering phenology responses to climate change: integrating experimental and gradient methods. *Ecological Monographs* 73:69-86.
- Fierer, N. and R. B. Jackson. 2006. The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences of the United States of America* 103:626-631.
- Fierer, N., C. M. McCain, P. Meir, M. Zimmermann, J. M. Rapp, M. R. Silman, and R. Knight. 2010. Microbes do not follow the elevational diversity patterns of plants and animals. *Ecology* 92:797-804.

- Fisher, M. A. and P. Z. Fulé. 2004. Changes in forest vegetation and arbuscular mycorrhizae along a steep elevation gradient in Arizona. *Forest Ecology and Management* 200:293-311.
- Gardes, M. and A. Dahlberg. 1996. Mycorrhizal diversity in arctic and alpine tundra: an open question. *New Phytologist* 133:147-157.
- Global Observation Research Initiative in Alpine Environments (G.L.O.R.I.A.). 14 May 2008. GLORIA at Rocky Mountain Biological Laboratory. Available from http://faculty.ucmerced.edu/lkueppers/GLORIA/GLORIA_page.html.
- Granath, G., M. Vicari, D. R. Bazely, J. P. Ball, A. Puentes, and T. Rakocovic. 2007. Variation in the abundance of fungal endophytes in fescue grasses along altitudinal and grazing gradients. *Ecography* 30:422-430.
- Groppe K., T. Steinger, I. Sanders, B. Schmid, A. Wiemken, and T. Boller. 1999. Interaction between the endophytic fungus *Epichloë bromicola* and the grass *Bromus erectus*: effects of endophyte infection, fungal concentration and environment on grass growth and flowering. *Molecular ecology* 8:1827-1835.
- Hodkinson, I.D. 2005. Terrestrial insects along elevation gradients: species and community responses to altitude. *Biological Review* 80:489-513.
- Krings M., T. N. Taylor, H. Hass, H. Kerp, N. Dotzler, and E. J. Hermesen. 2007. Fungal endophytes in a 400-million-yr-old land plant: infection pathways, spatial distribution, and host responses. *New Phytologist* 174:648-657.
- Laughlin, D. C. and S. R. Abella. 2007. Abiotic and biotic factors explain independent gradients of plant community composition in ponderosa pine forests. *Ecological Modeling* 205:231-240.
- Leuchtmann, A. 1992. Systematics, distribution, and host specificity of grass endophytes. *Natural Toxins* 1:150-162.
- Lugo, M. A., M. Ferrero, E. Menoyo, M. C. Estévez, F. Siñeriz, and A. Anton. 2008. Arbuscular mycorrhizal fungi and rhizospheric bacteria diversity along an altitudinal gradient in South American Puna Grassland. *Microbial Ecology* 55:705-713.
- Mack, K. M. L., and J. A. Rudgers. 2008. Balancing multiple mutualists: asymmetric interactions among plants, arbuscular mycorrhizal fungi, and fungal endophytes. *Oikos* 117:310-320.
- McGonigle, T.P., M.H. Miller, D.G. Evans, G.L. Fairchild, and J.A. Swan. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist* 115: 495-501.
- Omacini, M., T. Eggers, M. Bonkowski, A. C. Gange, and T. H. Jones. 2006. Leaf endophytes affect mycorrhizal status and growth of co-infected and neighbouring plants. *Functional Ecology* 20:226-232.
- Öpik, M., A. Vanatoa, E. Vanatoa, M. Moora, J. Davison, J.M. Kalwij, Ü. Reier, and M. Zobel. 2010. The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). *New Phytologist* 188:223-241.
- Porrás-Alfara, A., and P. Bay. 2011. Hidden fungi, emergent properties: endophytes and microbiomes. *Annual Review of Phytopathology* 49:291-315.
- Rudgers J. A., M.E. Afkhami, M.A. Rúa, A.J. Davitt, S. Hammer, and V.M. Huguet. 2009. A fungus among us: broad patterns of endophyte distribution in the grasses. *Ecology* 90:1531-1539.
- Rudgers, J. A. and K. Clay. 2008. An invasive plant–fungal mutualism reduces arthropod diversity. *Ecology Letters* 11:831-840.

- Rudgers, J. A., J. Holah, S. P. Orr, and K. Clay. 2007. Forest succession suppressed by an introduced plant-fungal symbiosis. *Ecology* 88:18-25.
- The Samuel Roberts Noble Foundation. 2012. Carolyn Young, Ph.D. Available from <http://www.noble.org/staff/young-carolyn/>.
- Scheidel, U., S. Röhl, and H. Bruelheide. 2003. Altitudinal gradients of generalist and specialist herbivory on three montane Asteraceae. *Acta Oecologica* 24:275-283.
- Shardl, C. L. 2010. The Epichloae, symbionts of the grass subfamily Pooideae. *Annals of the Missouri Botanical Garden* 97:646-665.
- Shaw, R. B. 2008. Grasses of Colorado. University Press of Colorado.
- Smith, S. E. and F. A. Smith. 2011. Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales. *Annual Review of Plant Biology* 62:227-250.
- Tadych, M., M. Bergen, F.M. Dugan, J.F. White Jr.. 2007. Evaluation of the potential role of water in spread of conidia of the *Neotyphodium* endophyte of *Poa ampla*. *Mycological Research* 111:466-472.
- Texas AgriLife Extension Service, Texas A&M System. 2012. Soil, Water, and Forage Testing Laboratory. Available from <http://soiltesting.tamu.edu/>.
- van der Heijden, M. G. A., J. N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken, and I. R. Sanders. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 396:69-72.
- Vierheilig, H., A.P. Coughlan, U. Wyss, and Y. Piché. 1998. Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. *Applied and Environmental Microbiology*: 5004-5007.
- White J. F. Jr. 1988. Endophyte-host associations in forage grasses. XI. A proposal concerning origin and evolution. *Mycologia* 80:442.

Appendix A. Figures cited in our paper.

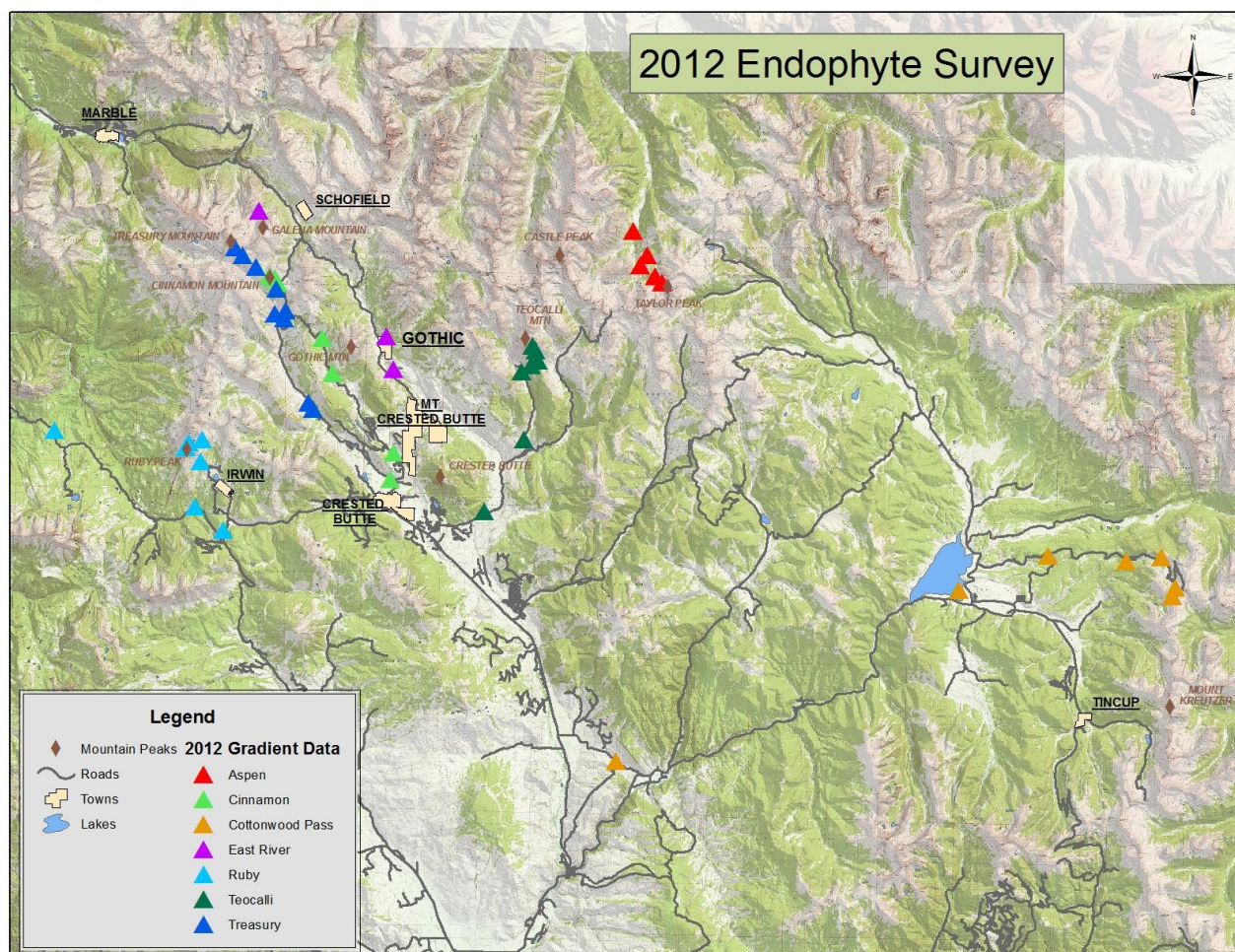


Figure 1. Map of research sites in Colorado (Gunnison County). All sites within an elevational gradient are the same color, and each site was separated by 200 elevational m.

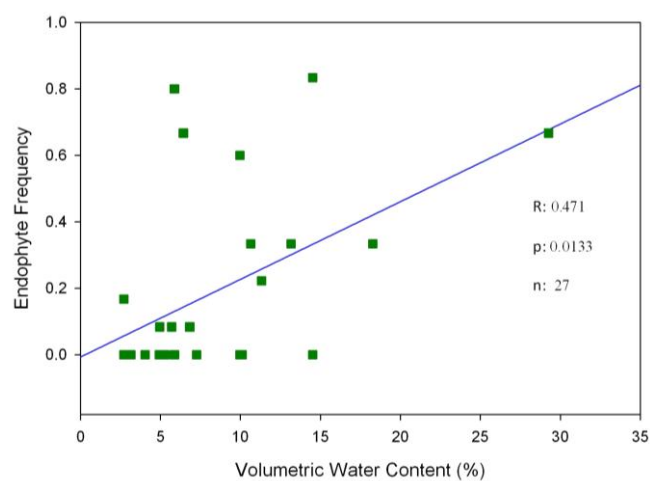


Figure 2. Regression analysis and spearman rank correlations relating soil moisture and systemic endophyte colonization frequency in *Festuca*.

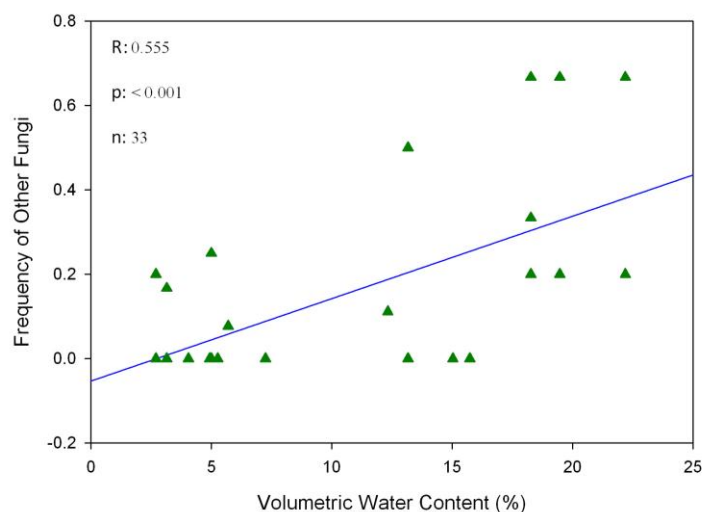


Figure 3. Regression analysis and spearman rank correlations relating soil moisture and colonization frequency of localized foliar endophytes (other fungi) in *Poa*.

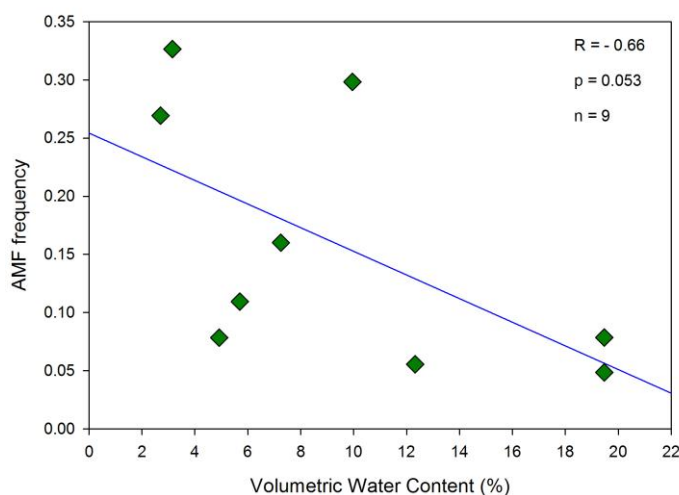


Figure 4. Regression analysis and spearman rank correlations showed an almost significant relationship between soil moisture and arbuscular mycorrhizal fungi colonization frequency in *Elymus*.

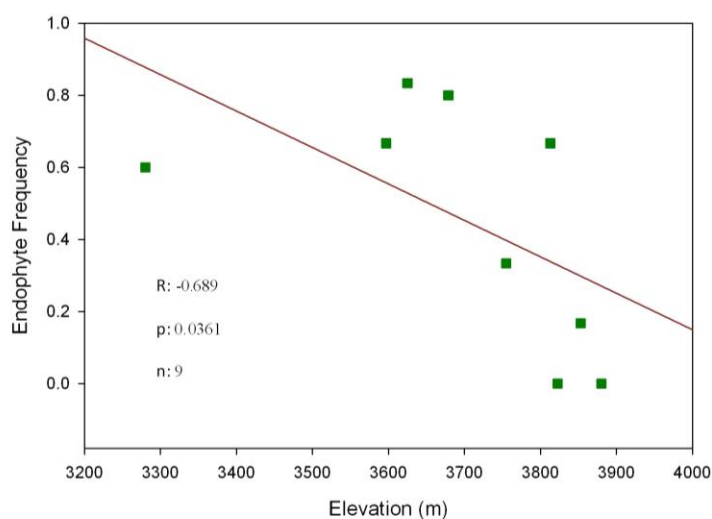


Figure 5. Regression analysis and spearman rank correlations relating elevation and endophyte colonization frequency in *Festuca brachyphylla*. The grass species *Festuca thurberi* also exhibited a negative correlation between elevation and systemic endophyte frequency, however it was not significant ($R = -0.23354$, $p = 0.0701$).

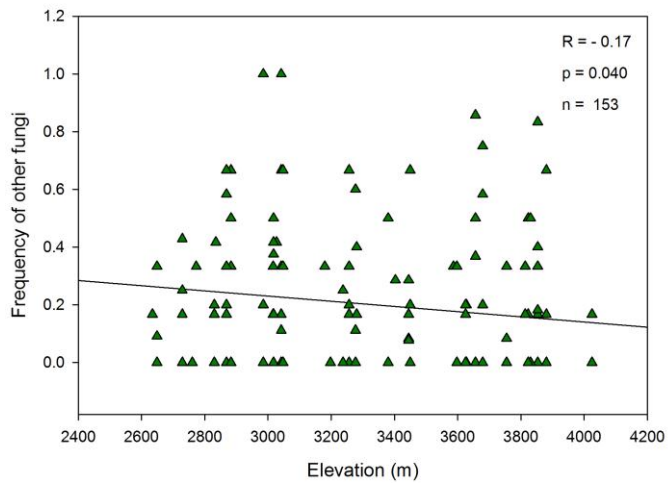


Figure 6. Regression analysis and spearman rank correlations relating increasing elevation to the frequency of localized foliar endophytes (other fungi) in all grasses sampled.

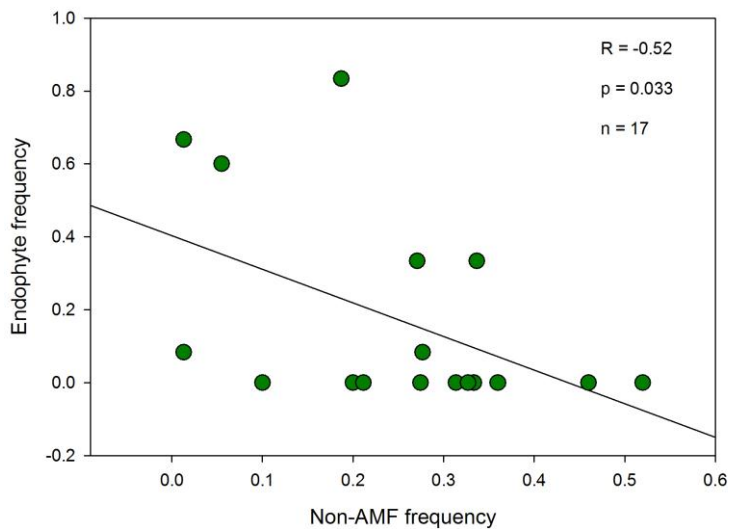


Figure 7. Regression analysis and spearman rank correlations relating frequency of non-AMF colonization with systemic foliar endophytes in the genus *Festuca*.

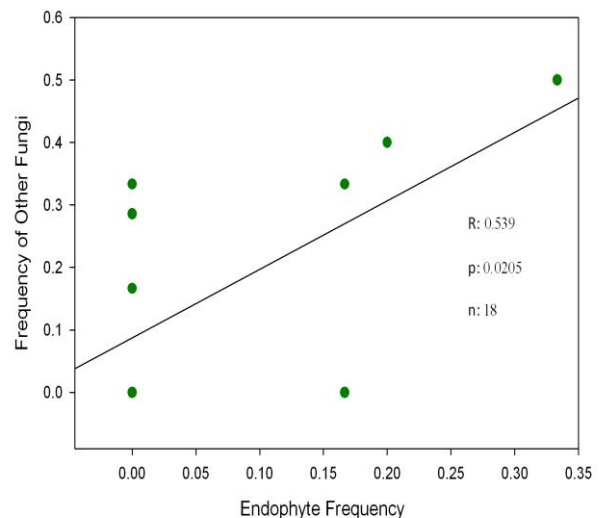
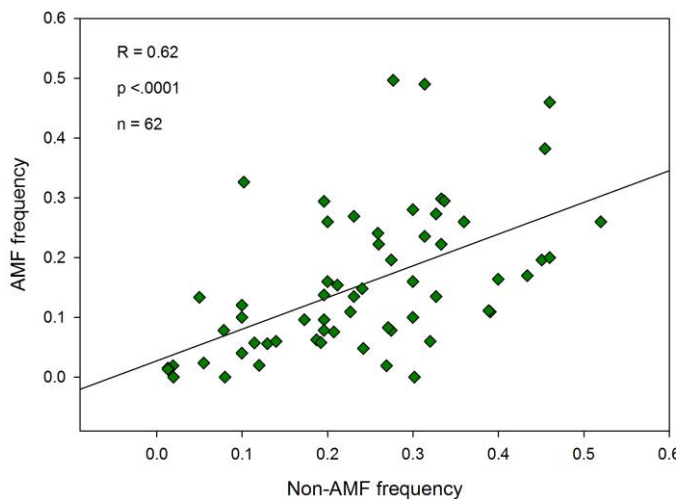


Figure 8. Regression analyses and spearman rank correlations showing positive correlations between colonization frequency of rhizospheric fungi in all grasses (left) and colonization frequency of phyllospheric fungi in *Trisetum spicatum* (right).

Appendix B. Collection and systemic endophyte frequency information for all grass species collected as of August 9, 2012. We list the genus, species, and subspecies (if available) of each grass and site information—number of populations sampled and the elevational range in which we found the species. The last two columns list the percent of populations (sites) in which an individual had the endophyte (% pop with endo) and the mean frequency of the endophyte in that grass species. Since the table and paper was compiled we also identified that we collected *Festuca saximontana* and that it has an endophyte.

Genus	species	subspecies	# Pop sampled	Max Elevation (m)	Min Elevation (m)	% Pop with endo	Mean Endo Freq
Achnatherum	lettermanii	(blank)	11	3450	2729	0	0
Achnatherum	nelsonii	(blank)	3	3016	2773	0	0
Achnatherum	pinetorum	(blank)	1	2649.16	2649.16	0	0
Agrostis	scabra	(blank)	2	2869	2830.08	0	0
Alopecurus	pratensis	(blank)	1	2729	2729	0	0
Bromelica	spectabilis	(blank)	8	3280	2860	0	0
Bromus	porteri	(blank)	1	3017.46	3017.46	0	0
Bromus	pubescens	(blank)	1	3198	3198	0	0
Calamagrostis	canadensis	(blank)	2	3586.56	3237.32	0	0
Calamagrostis	purpurescens	(blank)	1	3755	3755	0	0
Ceratochloa	cannate	(blank)	1	3402.38	3402.38	0	0
Ceratochloa	carinata	(blank)	3	3444.77	2984.9	0	0
Danthonia	intermedia	(blank)	3	3627.49	3380	0	0
Deschampsia	cespitosa	(blank)	7	4025.36	3449.62	0	0
Elymus	elymoides	(blank)	3	3042	2729	0	0
Elymus	glaucus	(blank)	5	3830.35	2634	0	0
Elymus	lanceolatus	(blank)	1	2649.16	2649.16	0	0
Elymus	multisetus	(blank)	1	3017.46	3017.46	0	0
Elymus	scribneri	(blank)	8	3880.23	3444.77	0	0
Elymus	trachycaulus	(blank)	21	3853	2634	0	0
Elymus	violaceus	(blank)	2	3586.56	3198	0	0
Festuca	brachyphylla	(blank)	11	4025.36	3280	0.636363636	0.451851852
Festuca	idahoensis	(blank)	2	2883.26	2830.08	0	0
Festuca	rubra	(blank)	3	3280	2649.16	0	0
Festuca	thurberi	(blank)	17	3679	2729	0.352941176	0.071180556
Koeleria	macrantha	(blank)	3	3017.46	2883.26	0	0
Phleum	alpinum	(blank)	7	3627.49	2634	0	0
Poa	abbreviata	(blank)	5	4025.36	3822.59	0	0
Poa	alpina	(blank)	5	3880.23	3625	0.2	0.083333333
Poa	arctica	(blank)	5	4025.36	3813	0	0
Poa	cusickii	(blank)	6	3679	3280	0	0
Poa	cusickii	epilis	4	3880.23	3237.32	0	0
Poa	fendleriana	fendleriana	1	3276.3	3276.3	0	0
Poa	glaucula	(blank)	5	3880.23	3402.38	0	0
Poa	interior	(blank)	1	2984.9	2984.9	0	0
Poa	KEY	(blank)	1	3430	3430	0	0
Poa	leptocoma	(blank)	4	3237.32	2634	0	0

Poa	palustris	(blank)	1	2773	2773	0	0
Poa	pratensis	(blank)	9	3280	2729	0	0
Poa	pratensis	agassizensis	1	2830.08	2830.08	0	0
Poa	pratensis	pratensis	1	2830.08	2830.08	0	0
Poa	reflexa	(blank)	2	3256.12	3237.32	0	0
Poa	secunda	(blank)	1	2830.08	2830.08	0	0
Poa	stenantha	(blank)	12	3880.23	2869	0	0
Poa	tracyi	(blank)	1	3047.37	3047.37	0	0
Trisetum	spicatum	(blank)	22	4025.36	2869	0.181818182	0.054385965