

How do distributions of belowground grass-fungal symbioses
change over altitudinal gradients in the Colorado Rocky
Mountains?

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Title:

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Abstract:

Fungal symbionts are pervasive and crucial components of terrestrial ecosystems. They can often reduce the effects of stressors such as drought and heat and can increase nutrient and water uptake for plants. Because of these roles, fungal symbionts may be important in mitigating the stressors of climate change. Because plants will likely move up in elevation in search of more favorable environments as climate change occurs, altitudinal gradients can be used to model the effects of climate change. We aimed to gain an understanding of the distribution of fungal symbionts over altitudinal gradients in the Colorado Rocky Mountains. We collected roots from 14 different grass species over 6 altitudinal gradients and quantified colonization by arbuscular mycorrhizal fungi, fine arbuscular mycorrhizal fungi, dark septate endophytes, and saprotrophs. The distribution of fungal symbionts over altitudinal gradients is not well understood and many studies focus on only one host species, one fungal group, or use only one gradient. We looked at multiple host species, fungal groups, gradients, and variables in an attempt to isolate the most important factors in determining distributions of fungal symbionts. We found that colonization patterns with altitude vary by host and many other factors may play important roles in determining the distribution of fungal symbionts.

Introduction:

Distributions of fungal symbionts over altitudinal gradients are not well understood and may be important in predicting future fungal distributions as well as terrestrial ecosystem distributions in response to climate change. As climate change progresses global surface temperatures are predicted to increase at a rate of 0.3°C per decade (Bernstein et al., 2007). This environmental shift will likely have serious implications for plant communities as plants will shift from newly hostile environments and distribute to more favorable areas, often at higher elevations (Kelly and Goulden, 2008). Symbioses between plant roots and fungi may play a critical role in mitigating this shift. These symbioses are widespread and come in various forms. The relationship between root colonizing fungi and their host plants can range from mutualistic to parasitic (Smith and Read, 2008). In many cases these partnerships can increase a plants stress-tolerance, growth rate, water, and nutrient uptake (Rodriguez, 2004; Fracchia et al., 2011). Mycorrhizal fungi may also be important for seedling establishment and plant survival, especially in arid and semi-arid environments (Fracchia et al., 2011). Fungal symbionts can even ameliorate abiotic stressors posed by climate change, such as drought and temperature increase (Kivlin et al., 2013).

Altitudinal gradients can impose a range of abiotic factors on plants and can model conditions under climate change. It has been found that as altitude increases generally temperature, soil nutrient levels, and pH decrease while precipitation increases (Laughlin and

Abella, 2007). Due to these altitudinal factors we may expect root symbionts to play different functional roles in the plant at different elevations. It has been shown that arbuscular mycorrhizal fungi (AMF) can help some plants acquire nutrients and water while dark septate endophytes (DSE) can increase drought and heat tolerance (Fracchia et al., 2011). AMF play an important role in helping plants to acquire nutrients (especially P) and water. AMF transfer inorganic P to the plant in exchange for organic C and it has been shown that AMF are less beneficial to plants where high levels of P are readily available such as at lower altitudes (Smith et al. 2010). DSE may also increase plant growth by accessing organic N, P, and soil moisture (Newsham 2011). DSE are also highly melanized which may allow them to withstand extreme temperatures and drought and therefore continue to benefit plants despite these stressors (Kivlin et al., 2013).

Other studies on the distributions of root fungi have largely focused on one species of fungus at a time. Some studies have shown AMF colonization levels and diversity to decline as elevation increases (Gai et al., 2012). Other studies on DSE have shown that DSE colonization can increase with elevation (Read and Haselwandter, 1981). However, studies have shown different trends and fungal distributions have often been more related to other geographical or abiotic factors rather than altitude (Ranelli et al., 2015)

I estimated root fungal colonization from grasses collected from sites along 6 different altitudinal gradients. These data add to a larger study and a plethora of data collected at these sites in 2014 (Table 2). The overall study is the largest altitudinal survey of fungal symbionts in the world that we know of. The fact that this study looks at multiple altitudinal gradients also gives us more robust data and lets us differentiate between the effects of altitude vs. those of geographic or other abiotic factors. While many studies focus on one fungal group and its distribution with one host species, this study looks at both above and below ground fungal groups and uses multiple host species which allows us to assess interactions between plant and fungal species across altitudinal gradients. We can pose several questions about these data: 1) How does colonization of different fungal groups change with elevation? 2) How sensitive are altitudinal patterns to host species identity? 3) What other factors besides elevation explain colonization rates?

Methods:

Study System:

I quantified the root colonization of regular AMF, fine AMF, DSE, and saprotrophic fungi in select host grass species (Table 1). Mycorrhizal fungi, in the phylum Glomeromycota, usually form mutualistic relationships with host plants (Smith and Read, 2008). AMF is the most common root-fungal symbiont for terrestrial plants and is found in approximately 80% of vascular plants (Smith et al., 2010). AMF live in the soil as well as plant roots and essentially extend the plant's root system. AMF exchange nutrients with plants in exchange for carbon at interchange sites called arbuscules. Plants and mycorrhizal fungi likely have a co-evolutionary history and mycorrhizae are believed to be responsible for the colonization of land by plants

(Smith and Read, 2008). DSE, in the phylum Ascomycota, may also be beneficial symbionts for plants and are believed to be more common in stressful environments such as with high heat or episodic precipitation (Zubek et al., 2009; Porras-Alfaro et al., 2011). Saprotrophs may be important in nutrient cycling and plant fitness, yet may also help to spread diseases among plants (Otten et al., 2004).

Table 1. Host grass species sampled for root fungal symbionts along 6 separate altitudinal gradients in Gunnison County, Colorado, USA.

Tribe-subtribe	Broad altitudinal distribution	Restricted to high elevation	Restricted to low elevation
Poeae-Aveninae-Alopecurinae	<i>Trisetum spicatum</i>	<i>Phleum alpinum</i>	X
Poeae-Loliinae	<i>Festuca thurberi</i>	<i>F. brachyphylla</i>	<i>F. saximontana</i>
Poeae-Poinae	<i>Poa stenantha</i>	<i>P. alpina</i>	<i>P. pratensis</i>
Poeae-Poinae	<i>P. leptocoma</i>	X	X
Stipeae	<i>Achnatherum lettermanii</i>	X	<i>A. nelsonii</i>
Triticeae	<i>Elymus trachycaulus</i>	<i>E. scribneri</i>	<i>E. elymoides</i>

Site description:

Samples were collected from 6 different altitudinal gradients in the Upper Gunnison Basin within the Colorado Rocky Mountains. This region shows an increase in precipitation and a decrease in atmospheric pressure, air temperature, and soil nutrients as altitude increases (Kittel et al., 2002). Sites were spaced at 100m altitudinal increments for a total of 66 sites (Appendix A).

Table 2. Summary of datasets collected and to be collected along the altitudinal gradients.

RESPONSE VARIABLE	DATA COLLECTION METHOD AND SOURCE	COMPLETION
(1a) Goal: Gauge the strength and correlates of altitudinal gradients in fungal symbiont communities		
Geographic Data		
Elevation, Latitude, Longitude	Trimble GEOXT GPS unit and altimeter	completed
Gradient identity	Six independent gradients, 11 sites per gradient = 66 sites	completed
Climate Data		
Air temperature	PRISM climate group data, RMBL and NOAA weather stations	ongoing
Precipitation	PRISM climate group data, NOAA and RMBL Weather stations	ongoing
Other Abiotic Correlates		
Soil moisture, temperature	TDR soil moisture/ temperature probe (% volumetric water content)	completed
pH	Michigan State University Soil Testing Laboratory:	completed
Nutrients	Bray P1 ppm, K ppm, Ca ppm, Mg, ppm, NO ₃ ppm, NH ₄ ppm, % Organic matter	completed
Phenology Data		
Collection date	Date adjusted to annual snowmelt date for the region	completed
Host Plant Correlates		
Host distribution pattern	Broad, restricted to low, or restricted to high elevation	completed

Host clade	Eight genera/subfamilies and their phylogeny	completed
Host species identity	Three species per clade, 6 individuals pooled per species per site	completed
Fungal Colonization Rate		
VTE	Percentage of individuals colonized	completed
HTE, AMF, DSE	Percentage of tissue colonized	completed
Fungal Community Composition		
VTE, HTE, DSE	NGS amplicons ITS2 primers, Illumina MiSeq, and their phylogenies	proposed
AMF	NGS amplicons Krueger et al. (2012) primers, and their phylogenies	proposed
Pathogen attack on leaves	Visual estimate of percentage damage per plant	completed
Herbivory	Visual estimate of percentage damage per plant	completed
Plant diversity	Visual estimates of plant cover in 33 quadrats per site	completed
Abundance of grasses	Counts of number of individuals along transects	completed
Total plant cover	Visual estimates of plant cover in 33 quadrats per site	completed

Lab methods:

To estimate root fungal colonization levels I analyzed roots samples collected from the 6 altitudinal gradients in summer 2014. I cleared and stained roots, mounted roots on microscope slides, and scored them for fungal colonization.

Roots were first cleared so that root tissue was translucent and then stained so that fungal structures were visible. Roots were rinsed with tap water and cut into segments to fit into tissue cassettes. The roots in cassettes were then placed in 10% w:v KOH solution and soaked for 4-5 days. KOH solution was then poured off and cassettes were thoroughly rinsed with tap water to rid them of any KOH residue before acidification. Roots then soaked overnight in 0.1N HCl. The HCl was then poured off and the cassettes were covered with pre-heated 5% vinegar-ink staining solution and soaked for 15-20 minutes. The cassettes were then rinsed thoroughly with tap water and stored in clean tap water for a few days to allow non-AMF structures to destain. Cassettes were stored in a 50:50 solution of glycerol and DI water until they are mounted onto microscope slides (Vierheilig et al., 1998).

Cleared and stained roots were then mounted onto microscope slides to score for fungal colonization. About 10-20 root segments 1-2cm long were mounted on each microscope slide and a microscope coverslip with a thin layer of polyvinyl lactoglycerol (PVLG) was pressed over them to secure them in place (McGonigle et al., 1990).

Root segments were then scored for fungal colonization by the magnified intersections methods. Roots were examined at x 200 magnification on a compound microscope. The microscope field of view was shifted to pass perpendicular to the root segments. A crosshair was rotated so that it was perpendicular to the root segment and a fungal structure was counted if it intersected with the crosshair. Fine and regular AMF structures were identified and categorized by the presence of arbuscules, vesicles, and/or hyphae (McGonigle et al., 1990). DSE were identified by hyphae that were septate, darker in color, and lacked arbuscules and vesicles. Saprotrophic fungi were identified by their dark blue to black, septate, and branched hyphae.

Results:

We found that relationships between elevation and fungal colonization varied among host species. Trends in AMF colonization by elevation were not consistent among species. We found DSE to decrease and fine AMF to increase with elevation. We also found vesicles and arbuscules to consistently increase with elevation among species.

Table 3. Summary statistics for fungal colonization by elevation for each grass species

Species Code	Response	Predictor	<i>r</i> ²	slope	<i>P</i>	<i>n</i>
ACLE	AMFhyphae	Elevation	0.06	-0.00799	0.3788	15
ACNE	AMFhyphae	Elevation	0.09	-0.02771	0.3342	13
ELEL	AMFhyphae	Elevation	0.29	0.12188	0.3478	12
ELSC	AMFhyphae	Elevation	0.11	-0.06907	0.3492	11
ELTR	AMFhyphae	Elevation	0.02	-0.00611	0.4068	39
FEBR	AMFhyphae	Elevation	0.14	0.03021	0.1287	20
FESA	AMFhyphae	Elevation	0.00	0.00158	0.9141	21
FETH	AMFhyphae	Elevation	0.01	0.00373	0.6109	35
PHAL	AMFhyphae	Elevation	0.03	-0.02911	0.8302	4
POAL	AMFhyphae	Elevation	0.09	-0.03738	0.3532	13
POLE	AMFhyphae	Elevation	0.37	0.05361	0.5812	4
POPR	AMFhyphae	Elevation	0.30	0.04909	0.008	22
POST	AMFhyphae	Elevation	0.15	-0.03266	0.0411	29
TRSP	AMFhyphae	Elevation	0.22	-0.02659	0.0055	33
ACLE	FineAMFhyphae	Elevation	0.02	0.00176	0.58	15
ACNE	FineAMFhyphae	Elevation	0.02	-0.00292	0.6702	13
ELEL	FineAMFhyphae	Elevation	0.22	0.01575	0.4355	12
ELSC	FineAMFhyphae	Elevation	0.02	-0.01071	0.6688	11
ELTR	FineAMFhyphae	Elevation	0.13	0.00587	0.0344	39
FEBR	FineAMFhyphae	Elevation	0.06	-0.00997	0.338	20
FESA	FineAMFhyphae	Elevation	0.04	0.00531	0.3764	21
FETH	FineAMFhyphae	Elevation	0.03	-0.00336	0.3657	35
PHAL	FineAMFhyphae	Elevation	0.48	-0.02806	0.3105	4
POAL	FineAMFhyphae	Elevation	0.05	-0.02175	0.4743	13
POLE	FineAMFhyphae	Elevation	0.22	0.0376	0.6856	4
POPR	FineAMFhyphae	Elevation	0.01	0.00143	0.6264	22
POST	FineAMFhyphae	Elevation	0.06	0.01294	0.1911	29
TRSP	FineAMFhyphae	Elevation	0.04	0.0071	0.2618	33
ACLE	DSE	Elevation	0.01	0.00742	0.6778	15
ACNE	DSE	Elevation	0.02	-0.0148	0.6758	13
ELEL	DSE	Elevation	0.01	-0.0278	0.8709	12
ELSC	DSE	Elevation	0.01	-0.00871	0.828	11
ELTR	DSE	Elevation	0.02	0.00703	0.3737	39
FEBR	DSE	Elevation	0.00	-0.00181	0.9382	20
FESA	DSE	Elevation	0.00	0.00389	0.7908	21
FETH	DSE	Elevation	0.02	-0.0075	0.4313	35
PHAL	DSE	Elevation	0.05	0.01408	0.7791	4

POAL	DSE	Elevation	0.27	-0.05888	0.0843	13
POLE	DSE	Elevation	0.13	0.0192	0.7613	4
POPR	DSE	Elevation	0.00	-0.00378	0.7967	22
POST	DSE	Elevation	0.03	-0.01215	0.3876	29
TRSP	DSE	Elevation	0.23	-0.02214	0.0048	33
ACLE	Saprotrophic	Elevation	-	0	-	15
ACNE	Saprotrophic	Elevation	0.04	-0.00111	0.5458	13
ELEL	Saprotrophic	Elevation	0.56	0.0667	0.146	12
ELSC	Saprotrophic	Elevation	0.10	-0.01167	0.3708	11
ELTR	Saprotrophic	Elevation	0.00	-0.00022	0.854	39
FEBR	Saprotrophic	Elevation	0.01	-0.00083	0.7744	20
FESA	Saprotrophic	Elevation	0.10	-0.00485	0.1664	21
FETH	Saprotrophic	Elevation	0.05	-0.00096	0.1945	35
PHAL	Saprotrophic	Elevation	0.21	-0.00812	0.5438	4
POAL	Saprotrophic	Elevation	0.09	-0.00281	0.3539	13
POLE	Saprotrophic	Elevation	0.54	0.00347	0.4726	4
POPR	Saprotrophic	Elevation	0.01	0.00131	0.5976	22
POST	Saprotrophic	Elevation	0.00	-2.4E-05	0.9919	29
TRSP	Saprotrophic	Elevation	0.00	0.000455	0.8287	33
ACLE	Vesicles	Elevation	0.19	0.00653	0.1017	15
ACNE	Vesicles	Elevation	0.03	-0.00363	0.5615	13
ELEL	Vesicles	Elevation	0.01	0.00491	0.8662	12
ELSC	Vesicles	Elevation	0.07	0.00994	0.4446	11
ELTR	Vesicles	Elevation	0.00	0.000806	0.7158	39
FEBR	Vesicles	Elevation	0.03	0.00691	0.5212	20
FESA	Vesicles	Elevation	0.03	0.00474	0.4334	21
FETH	Vesicles	Elevation	0.13	0.00844	0.0334	35
PHAL	Vesicles	Elevation	0.36	-0.01004	0.4042	4
POAL	Vesicles	Elevation	0.06	0.00968	0.4412	13
POLE	Vesicles	Elevation	0.28	0.00467	0.6485	4
POPR	Vesicles	Elevation	0.36	0.01634	0.0029	22
POST	Vesicles	Elevation	0.01	0.00206	0.6667	29
TRSP	Vesicles	Elevation	0.11	-0.00484	0.0594	33
ACLE	Arbuscules	Elevation	0.09	0.000306	0.2754	15
ACNE	Arbuscules	Elevation	0.02	0.00141	0.7034	13
ELEL	Arbuscules	Elevation	0.96	0.02325	0.0033	12
ELSC	Arbuscules	Elevation	0.08	-0.00842	0.4388	11
ELTR	Arbuscules	Elevation	0.03	-0.00066	0.3254	39
FEBR	Arbuscules	Elevation	0.01	0.00122	0.6442	20
FESA	Arbuscules	Elevation	0.11	0.00204	0.1628	21
FETH	Arbuscules	Elevation	0.07	0.00131	0.1295	35
PHAL	Arbuscules	Elevation	0.65	-0.02138	0.1957	4
POAL	Arbuscules	Elevation	0.06	-0.00254	0.4253	13
POLE	Arbuscules	Elevation	0.37	0.04529	0.5823	4
POPR	Arbuscules	Elevation	0.20	0.00236	0.0378	22
POST	Arbuscules	Elevation	0.03	-0.00033	0.3572	29
TRSP	Arbuscules	Elevation	0.01	0.000565	0.6584	33

In *Poa pratensis*, the proportion of roots with arbuscular mycorrhizal fungal (AMF) hyphae present was significantly correlated with elevation ($p = 0.008$). AMF hyphae increased with elevation (Figure 1).

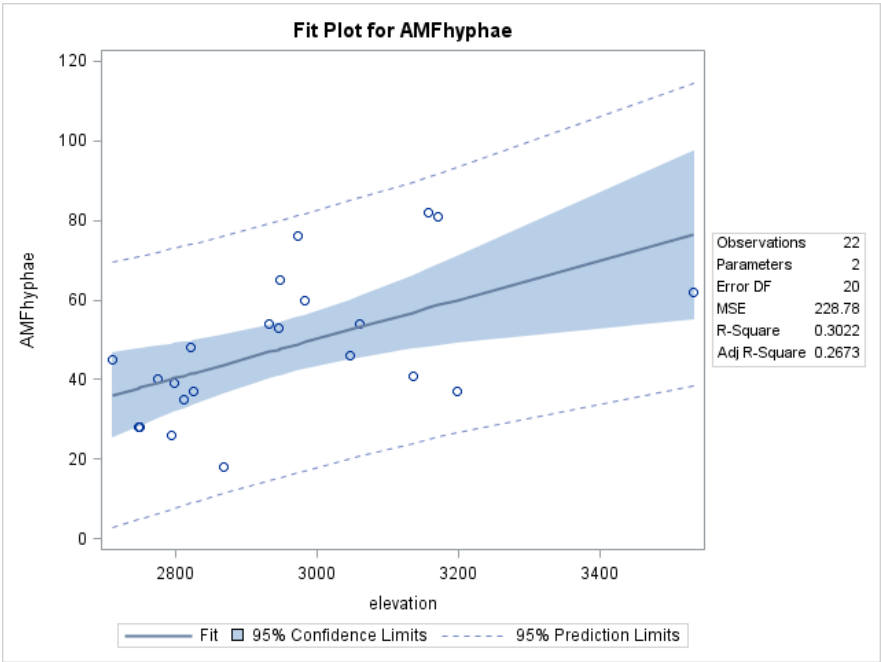


Figure 1. AMF hyphal colonization (number colonized out of 100) against elevation (meters) of *Poa pratensis*.

In *Poa stenantha*, AMF hyphae colonization was significantly correlated with elevation ($p = 0.0411$). AMF hyphae decreased as elevation increased (Figure 2).

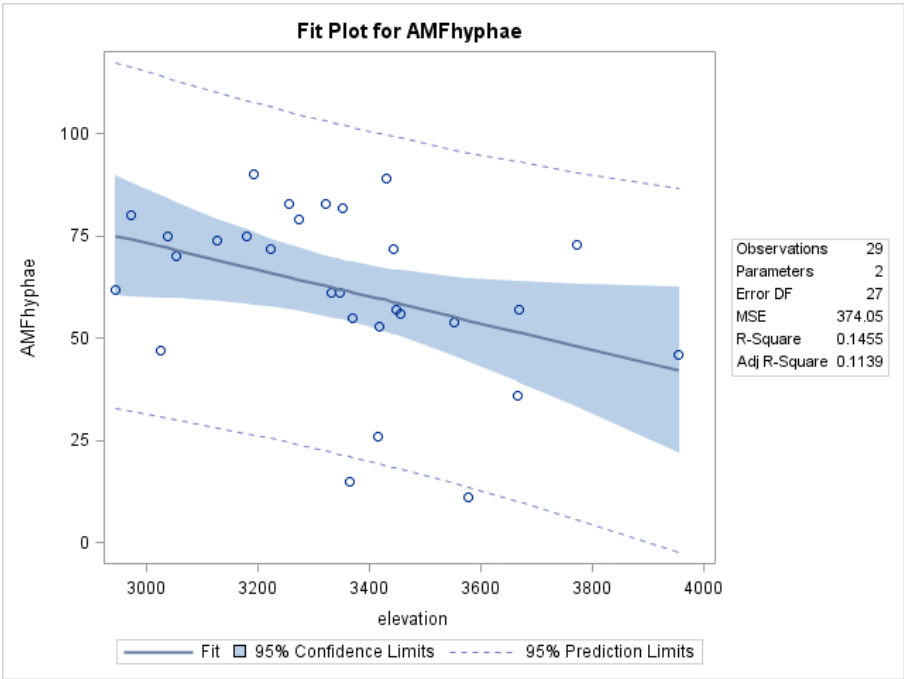


Figure 2. AMF hyphal colonization (number colonized out of 100) against elevation (meters) of *Poa stenantha*.

In *Trisetum spicatum*, AMF hyphae colonization was significantly correlated with elevation ($p = 0.0055$). AMF hyphae decreased as elevation increased (Figure 3).

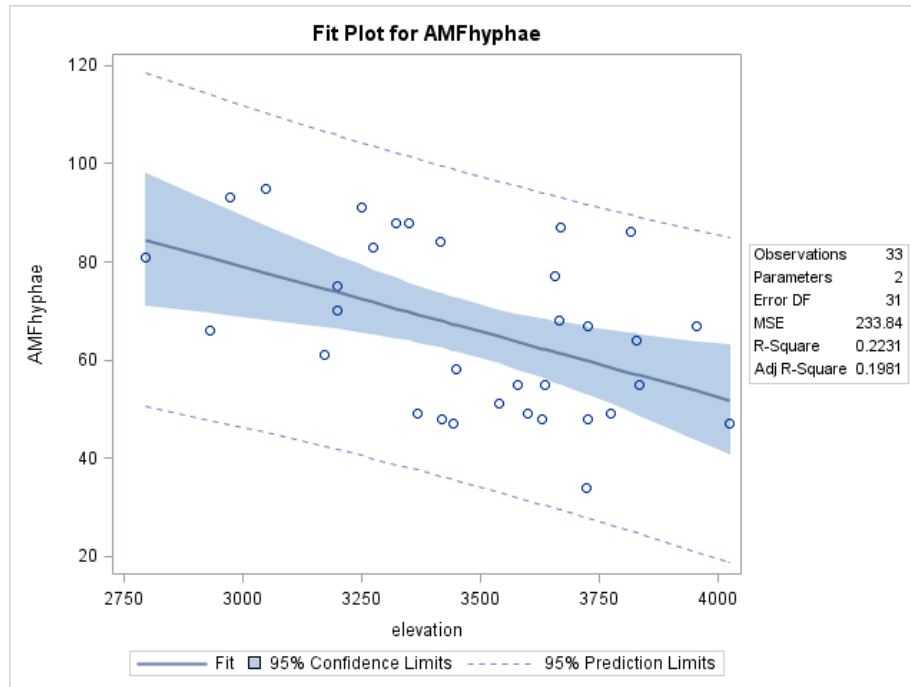


Figure 3. AMF hyphal colonization (number colonized out of 100) against elevation (meters) of *Trisetum spicatum*.

In *Elymus trachycaulus*, fine AMF hyphae colonization was significantly correlated with elevation ($p = 0.0344$). Fine AMF hyphae increased as elevation increased (Figure 4).

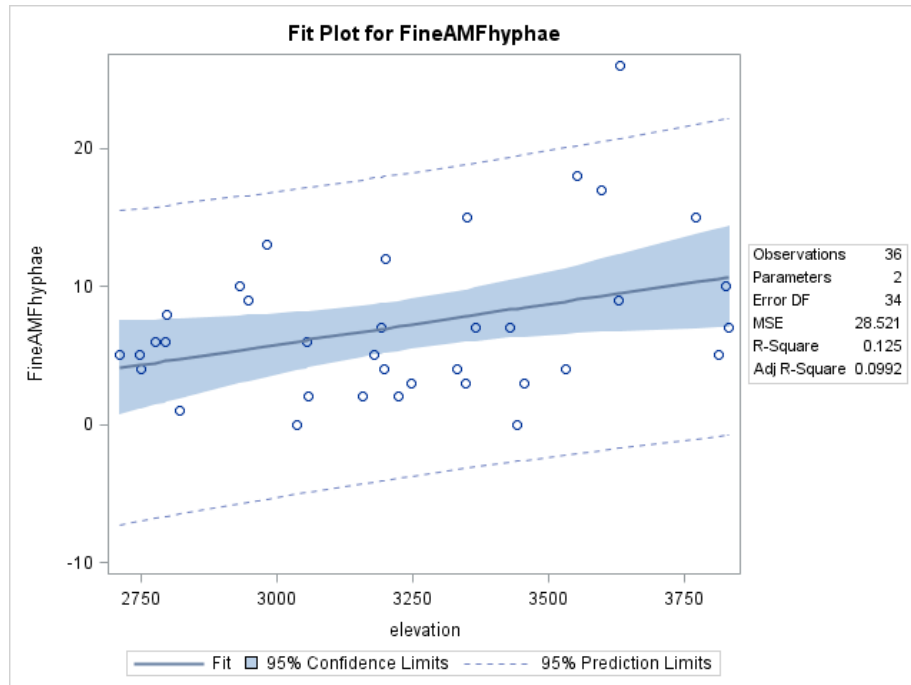


Figure 4. Fine AMF hyphal colonization (number colonized out of 100) against elevation (meters) of *Elymus trachycaulus*.

In *Trisetum spicatum*, DSE colonization was significantly correlated with elevation ($p = 0.0048$). DSE colonization decreased as elevation increased (Figure 5).

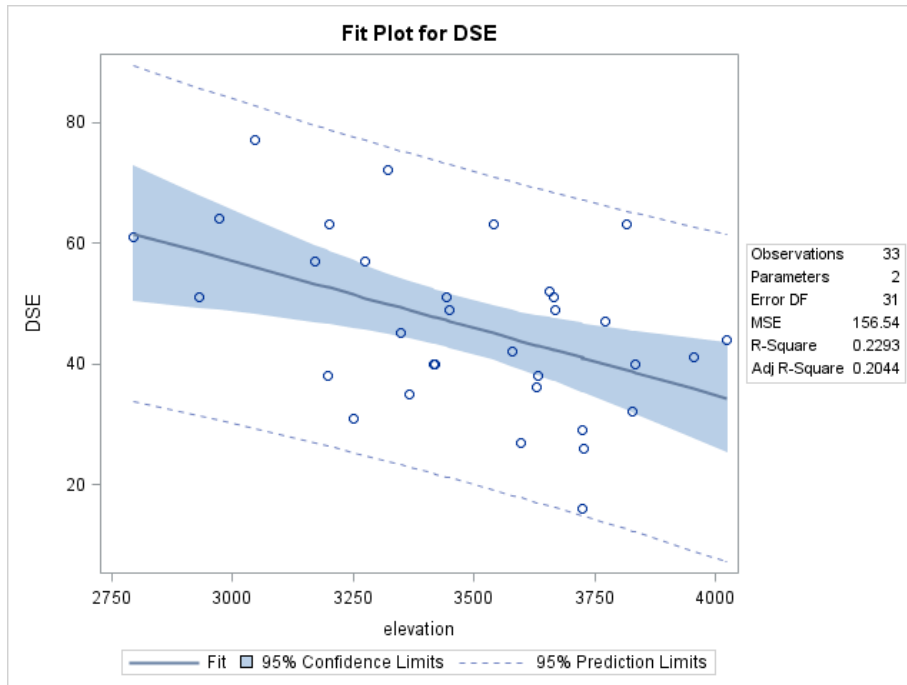


Figure 5. DSE hyphal colonization (number colonized out of 100) against elevation (meters) of *Trisetum spicatum*.

In *Festuca thurberi*, AMF vesicle colonization was significantly correlated with elevation ($p = 0.0334$). AMF vesicle colonization increased as elevation increased (Figure 6).

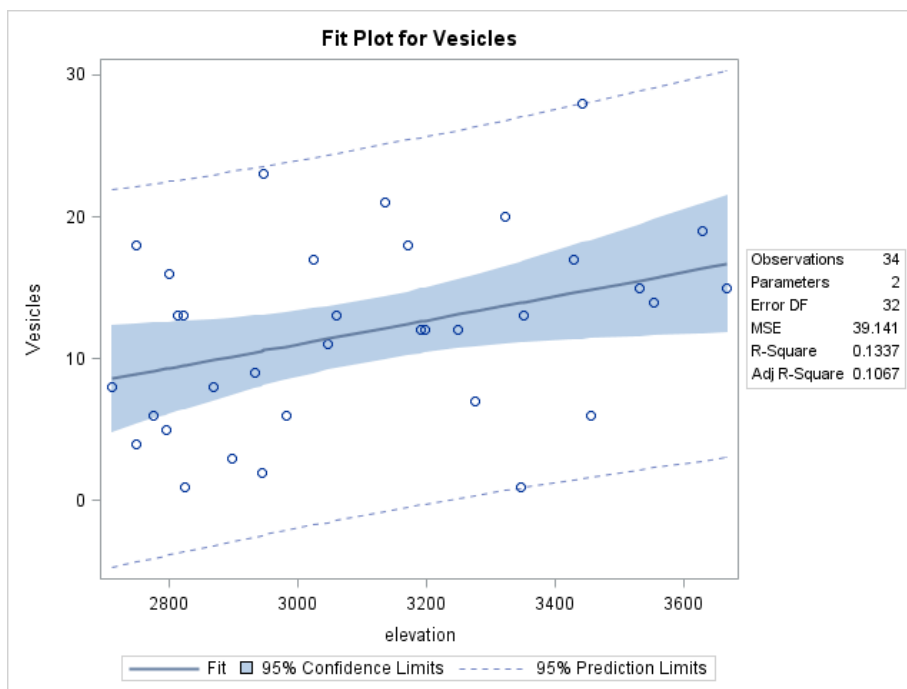


Figure 6. AMF vesicle colonization (number colonized out of 100) against elevation (meters) of *Festuca thurberi*.

In *Poa pratensis*, AMF vesicles were significantly correlated with elevation ($p = 0.0029$). AMF vesicle colonization increased as elevation increased (Figure 7).

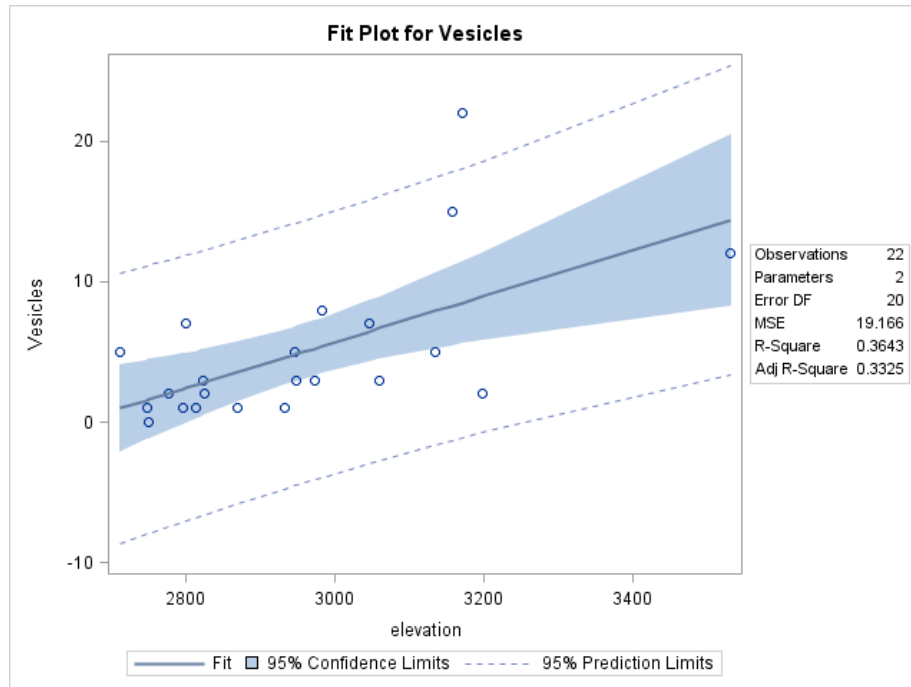


Figure 7. AMF vesicle colonization (number colonized out of 100) against elevation (meters) of *Poa pratensis*.

In *Elymus elymoides*, AMF arbuscule colonization was significantly correlated with elevation ($p = 0.0033$). AMF arbuscule colonization increased as elevation increased (Figure 8).

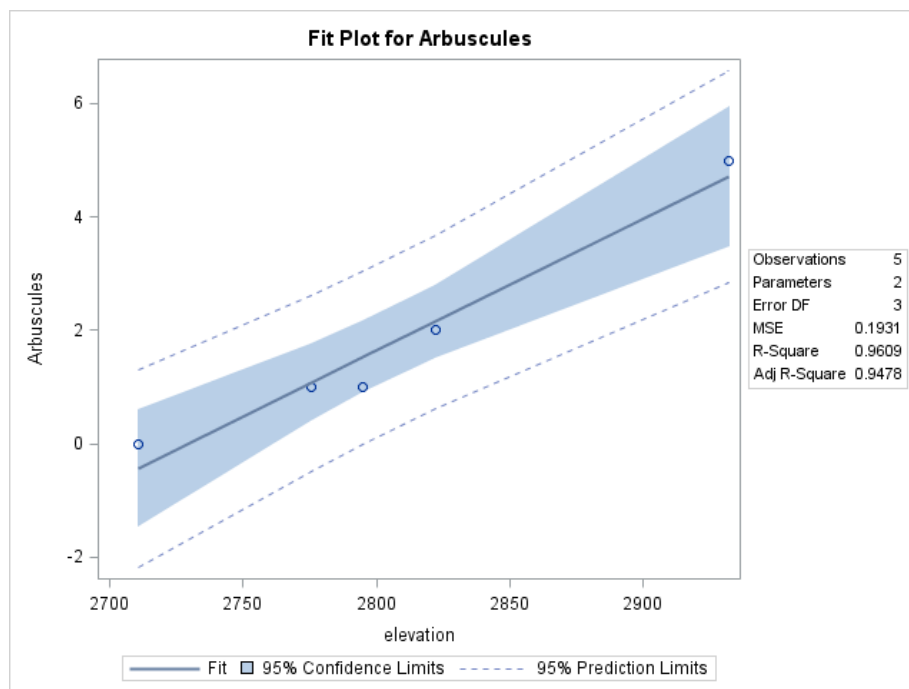


Figure 8. AMF arbuscule colonization (number colonized out of 100) against elevation (meters) of *Elymus elymoides*.

In *Poa pratensis*, AMF arbuscule colonization was significantly correlated with elevation ($p = 0.0378$). AMF arbuscule colonization increased as elevation increased (Figure 9).

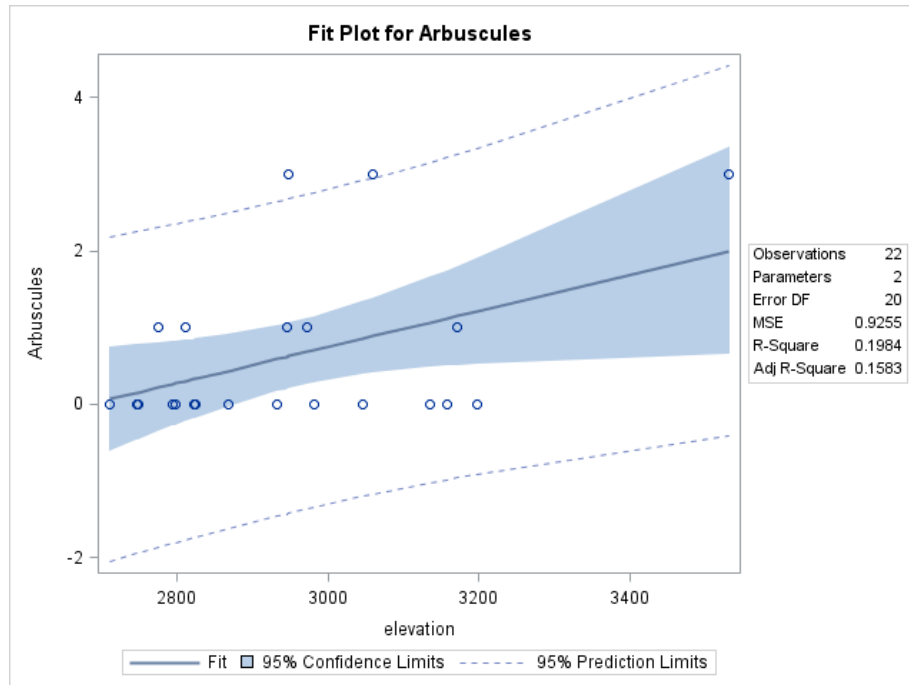


Figure 9. AMF arbuscule colonization (number colonized out of 100) against elevation (meters) of *Poa pratensis*.

Discussion:

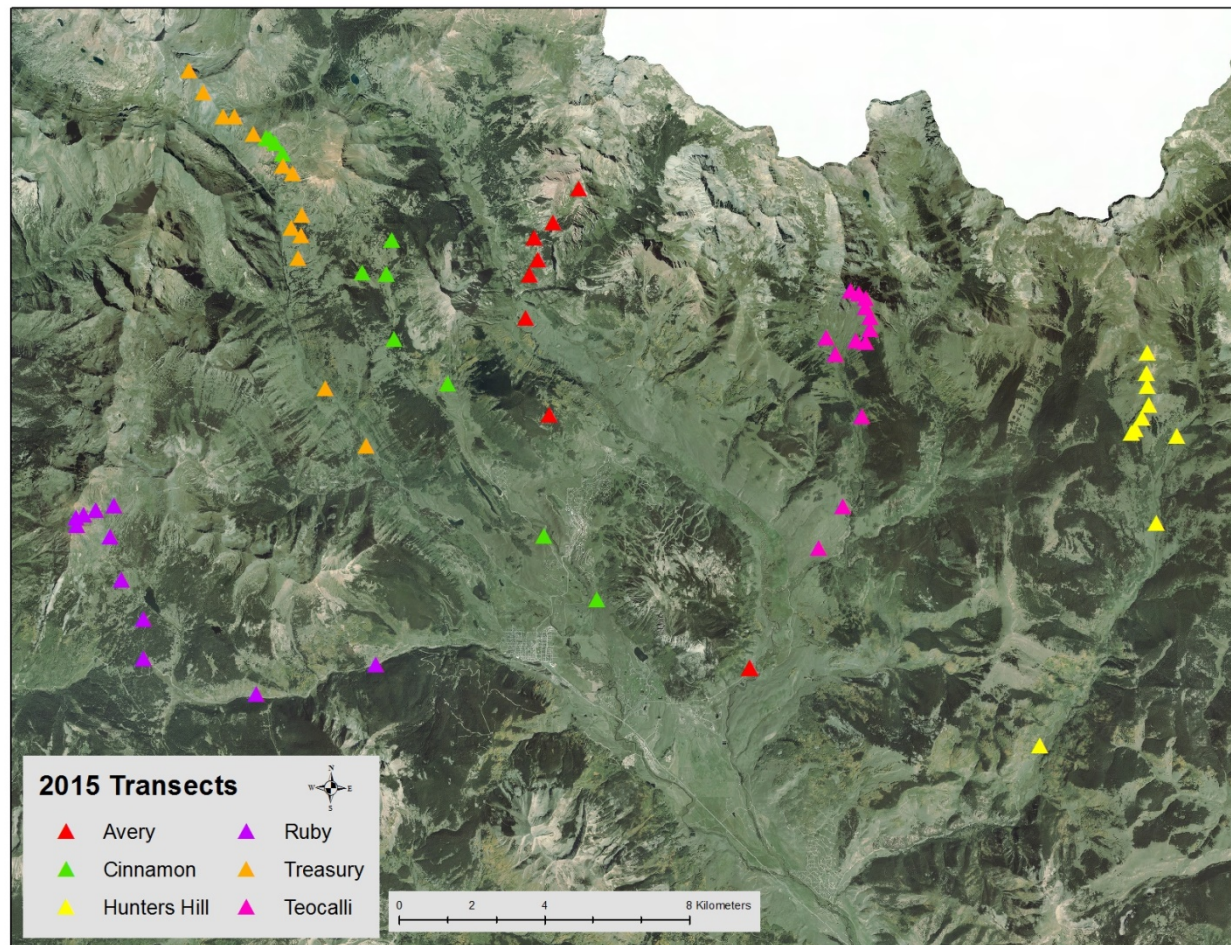
This study, as part of the largest altitudinal survey of fungal symbionts, looks at multiple host species and multiple altitudinal gradients. This has allowed us to look at larger trends in fungal symbiont distributions and host specificity. We found that colonization levels varied by host species as did colonization trends with elevation, a finding consistent with that of Ranelli et al (Ranelli et al, 2015).

Other studies have found AMF colonization to decrease with elevation (Gai et al., 2012). In two species, *Poa stenantha* and *Trisetum spicatum*, AMF decreased with elevation. However, in *Poa pratensis*, AMF colonization increased with elevation, a trend that is not consistent with what others have found. Also, other studies have found DSE to increase with elevation (Read and Haselwandter, 1981). However we found that in *Trisetum spicatum*, DSE colonization decreased with elevation. In addition we found that in *Elymus trachycaulus*, fine AMF colonization increased with elevation. The variance of these trends among host species and their departures from previous findings suggests that relationships between fungal symbionts and plants over altitudinal gradients may be more complex than previously hypothesized.

In *Festuca thurberi* and *Poa pratensis*, the concentration of vesicles increased with elevation, a trend that is to be expected. In *Elymus elymoides* and *Poa pratensis*, the concentration of arbuscules also increased with elevation, a trend that is not consistent with other findings. These

results show that altitudinal distributions of fungal symbionts are likely host specific and suggest that fungal symbionts may have different levels of importance for different grass species.

Appendix A. Samples were collected from each of 66 sites along the 6 altitudinal gradients in 2014.



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