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Biotic and abiotic predictors of fungal colonization in grasses of the Colorado Rockies

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ABSTRACT

Aim Fungal symbionts are ubiquitous in plants and can mitigate abiotic stressors associated with climate change. Predicting fungal symbiont distributions under future climates first requires knowledge of current distributions and their potential drivers.

Location We documented colonization by fungal symbionts in perennial, cool-season grasses along altitudinal gradients in the Rocky Mountains of Colorado, USA.

Methods Across seven replicate altitudinal gradients, spanning *c.* 1400 vertical meters, we scored fungal colonization for 46 grass species. We documented altitudinal clines in colonization by both above-ground and below-ground fungal symbionts for the first time, including localized foliar endophytes (LFE) and systemic endophytes (epichloae) in leaves and arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE) in roots. For a subset of 16 well-sampled grass species, we used model selection procedures to evaluate the relative importance of geography, edaphic factors and host plant identity. We also assessed the influence of host phylogenetic relatedness and colonization by co-infecting fungi.

Results Levels of fungal colonization varied strongly with host plant identity, but the effects of particular host species were not consistent across fungal groups. In addition to the influence of host identity, epichloae colonization declined with elevation and varied with geography (latitude/longitude) and edaphic factors. Geography and collection date were important predictors of LFE colonization, with higher colonization later in the growing season. Colonization estimates for the obligately plant-associated fungi (epichloae, AMF) were phylogenetically conserved across the grass supertree. Positive correlations between AMF and DSE, which remained even after accounting for host plant relatedness, suggested possible synergisms between these fungal groups.

Main conclusions Our survey showed greater host specificity in patterns of fungal colonization than prior reports and revealed that different fungal symbiont groups do not share similar drivers. Conserving plant–fungal symbioses under future climates may require unique strategies for different plant species and fungal symbiont types.

Keywords

Climate change, distribution model, endophyte, mycorrhizal fungi, plant–fungal interactions, symbiosis.

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INTRODUCTION

Little is known about the geographic distributions of most fungal symbionts of plants, with current studies focused on a single fungal group [e.g. arbuscular mycorrhizal fungi (AMF)], host taxon or environmental gradient (e.g. Herrera *et al.*, 2010; Giauque & Hawkes, 2013; but see, U'Ren *et al.*, 2012). Spatially or phylogenetically narrow studies may fail to identify drivers of distributions when fungi respond to multiple spatial, abiotic and biotic factors (Opik *et al.*, 2010; Kivlin *et al.*, 2011). Because fungal symbionts can benefit plants by ameliorating the abiotic stressors associated with climate change, such as heat and drought (Compant *et al.*, 2010; van der Putten, 2012; Kivlin *et al.*, 2013; Worchel *et al.*, 2013), the ability to predict shifts in fungal distributions under future climates could be useful for understanding changes in terrestrial communities. To forecast future patterns, it is first necessary to elucidate current drivers of fungal symbiont distributions.

Climate and soil resources have been common predictors of fungal symbiont distributions (e.g. Lewis *et al.*, 1997; Lekberg *et al.*, 2007; Dumbrell *et al.*, 2010; Kivlin *et al.*, 2011; Johnson & Graham, 2013; Koorem *et al.*, 2014). However, whether the distributions of different fungal symbiont groups share associations with similar environmental conditions remains an open question, because fungal groups have been investigated independently. For example, there can be strong clines in AMF over phosphorus gradients (Liu *et al.*, 2011) as high phosphorus availability to the plant reduces the benefits gained from AMF symbiosis (Kiers *et al.*, 2011; Smith & Smith, 2011). In contrast, drought has been positively correlated with the proportion of plants colonized by foliar endophytes (Lewis *et al.*, 1997), which can ameliorate osmotic stress (Malinowski & Belesky, 2000). Few studies have simultaneously evaluated the relative importance of edaphic factors, such as soil nutrients and pH, versus climate on fungal distributions across broad, spatial gradients (e.g. Vare *et al.*, 1997; Bahram *et al.*, 2012; Hu *et al.*, 2013; Vanesa *et al.*, 2013).

While abiotic factors are often invoked as primary drivers (e.g. Soudzilovskaia *et al.*, 2015), biotic factors such as host identity and the occurrence of co-infecting fungi may additionally influence fungal distributions. The importance of host identity or host evolutionary history may depend on whether fungi are obligate or facultative symbionts. Above ground, plants can associate with systemic endophytes (Class I, Clavicipitaceae, Ascomycota) as well as localized foliar endophytes (LFE) (Class II & III, mainly Ascomycota) (Rodriguez *et al.*, 2009). Epichloae are obligate associates of living plants (Schardl *et al.*, 2008) and therefore may exhibit high host fidelity in their distributions, whereas LFE can grow facultatively as saprotrophs in litter and soil (e.g. Osono, 2006; Unterseher *et al.*, 2013a). Below ground, AMF and dark septate endophytes (DSE) colonize plant roots, often improving nutrient uptake or stress tolerance (Smith & Read, 2008; Porras-Alfaro & Bayman, 2011). Like the epichloae, AMF are obligate symbionts of living plants (Smith & Read, 2008). In contrast, DSE have been reported from other environments, such as organic debris (Menkis *et al.*, 2004; Day & Currah, 2011) and biological soil crusts (Green *et al.*, 2008). The abundance of co-infecting fungal groups adds additional complexity to biotic drivers of fungal distributions (Larimer *et al.*, 2010; Afkhami *et al.*, 2014). For instance, epichloae in leaves reduced AMF colonization in roots (Mack & Rudgers, 2008). A first step towards evaluating the effects of fungal groups on each other is observing epichloae, LFE, DSE and AMF together in the same plants.

Climatic gradients provide natural variation over which to investigate the relative importance of abiotic and biotic drivers of fungal distributions (Sundqvist *et al.*, 2013). In particular, mountain ecosystems provide sharp natural gradients of soil nutrients, temperature, precipitation and plant communities over relatively short distances (*c.* 2–3 km) that fall within the potential dispersal ranges of many fungi (Lekberg *et al.*, 2007; Wolfe *et al.*, 2010). The use of multiple gradients across a regional scale can disentangle the relative effects of elevation, geographic location, abiotic factors and host identity. While altitudinal gradients in fungal colonization have been documented in various ecosystems and for several individual host species (e.g. Fisher *et al.*, 1995; Vare *et al.*, 1997), prior work has been restricted to the same host tissues (e.g. either roots or leaves, but not both), limiting detection of general patterns. In roots, AMF colonization and diversity have been shown to decline with elevation (e.g. Gardes & Dahlberg, 1996; Wu *et al.*, 2007; Gai *et al.*, 2012). In contrast, DSE colonization can increase with elevation (e.g. Read & Haselwandter, 1981; Schmidt *et al.*, 2008). Too few data are available to draw generalizations for the LFE (Fisher *et al.*, 1995; Hashizume *et al.*, 2008; Cordier *et al.*, 2012) or epichloae (Granath *et al.*, 2007; Gonzalo-Turpin *et al.*, 2010; Kirkby *et al.*, 2011). Despite clear evidence for host specificity of fungal symbionts (Becklin *et al.*, 2012; Unterseher *et al.*, 2013b), few studies have evaluated the degree to which fungal symbiont distributions vary amongst host species (but see, Granath *et al.*, 2007).

To improve understanding of the factors that influence fungal distributions, we documented levels of colonization by fungal symbionts in roots and leaves of perennial, cool-season grasses along replicated, altitudinal gradients. We addressed the following questions. (1) What factors (elevation, geographic location, edaphic factors or host identity) best predict the percentage of host tissue colonized by fungal symbionts? (2) To what degree are altitudinal patterns specific to host taxon? (3) Is there evidence for host plant phylogenetic signal in levels of fungal colonization? (4) Are associations between different fungal groups suggestive of synergisms or antagonisms among symbiont groups inhabiting the same plants? Based on known costs and benefits of symbiosis, we posed specific hypotheses for each fungal group (Table 1).

Table 1 Hypotheses on the responses of fungal colonization to abiotic and biotic predictors for four fungal symbiont groups: Epichloae, LFE = localized foliar endophytes, AMF = arbuscular mycorrhizal fungi, and DSE = dark septate endophytes. Hypotheses were based on previously documented patterns and known costs and benefits of each symbiont group. Where predicted trends are shown with arrows, question marks indicate that to our knowledge, the relationship has not yet been characterized

Fungal group	Location	Association	Hypotheses
What factors best predict the percentage of host tissue colonized by fungal symbionts?			
			Relative importance of biotic versus abiotic predictors
Epichloae	Leaves	Obligate	Host identity outweighs environmental factors due to obligate host association and vertical mode of transmission
LFE	Leaves	Facultative	Environmental factors outweigh host identity due to facultative associations with host and horizontal mode of transmission
AMF	Roots	Obligate	Host identity outweighs environmental factors due to obligate host association
DSE	Roots	Facultative	Environmental factors outweigh host identity due to facultative associations with host and horizontal mode of transmission

			Direction of abiotic predictors				
			Elevation	Nitrogen	Phosphorus	Soil moisture	References
Epichloae	Leaves	Obligate	↓	↑	↓	↓	Granath *et al.* (2007) and Malinowski & Belesky (2000)
LFE	Leaves	Facultative	↓	↑	?	↓	Giauque & Hawkes (2013)
AMF	Roots	Obligate	↓	↓	↓	↓	Auge (2004), Smith & Read (2008) and Camenzind *et al.* (2014)
DSE	Roots	Facultative	↑	↑	?	↓	Newsham (2011) and Kivlin *et al.* (2013)

To what degree are altitudinal patterns specific to host taxon?			
Epichloae	Leaves	Obligate	Altitudinal patterns are specific to host taxa because fungi are obligately plant associated
LFE	Leaves	Facultative	Altitudinal patterns are similar across host taxa because fungi are facultative associates
AMF	Roots	Obligate	Altitudinal patterns are specific to host taxa because fungi are obligately plant associated
DSE	Roots	Facultative	Altitudinal patterns are similar across host taxa because fungi are facultative associates

Is there evidence for host plant phylogenetic signal in levels of fungal symbiont colonization?			
Epichloae	Leaves	Obligate	Strong host phylogenetic signal occurs because fungi are obligately plant associated
LFE	Leaves	Facultative	There is weak to no host phylogenetic signal due to facultative host association
AMF	Roots	Obligate	Strong host phylogenetic signal occurs because fungi are obligately plant associated
DSE	Roots	Facultative	There is weak to no host phylogenetic signal due to facultative host association

Are associations between different fungal groups suggestive of synergisms or antagonisms among symbionts inhabiting the same plants?			
Epichloae	Leaves	Obligate	Colonization is negatively correlated with LFE due to competition for similar host tissue (leaves) and uncorrelated or positively correlated with AMF and DSE (roots)
LFE	Leaves	Facultative	Colonization is negatively correlated with Epichloae due to competition for similar host tissue (leaves) and uncorrelated or positively correlated with AMF and DSE (roots)
AMF	Roots	Obligate	Colonization is negatively correlated with DSE due to competition for similar host tissue (roots) and uncorrelated or positively correlated with symbionts in leaves
DSE	Roots	Facultative	Colonization is negatively correlated with AMF due to competition for similar host tissue (roots) and uncorrelated or positively correlated with symbionts in leaves

METHODS

Study sites

We surveyed fungal symbiont colonization of plant tissues in the Colorado Rocky Mountains (Appendix S1 in Supporting Information), with a focus on the Upper Gunnison Basin. The region shows altitudinal declines in air temperature (*c.* 0.8 °C per 100 m), atmospheric pressure and soil nutrients, but increases in precipitation (Kittel *et al.*, 2002; Dunne *et al.*, 2003). Warming trends (0.5–1 °C per decade) include higher air temperatures and steeper surface temperature lapse rates in more recent years (Pepin & Losleben, 2002; McGuire *et al.*, 2012; Rangwala & Miller, 2012).

Host plant species

We used grasses (Poaceae, subfamily Pooideae) as a model host clade. Grasses are particularly tractable owing to their diversity [*c.* 10,000 species (Barker *et al.*, 2001)] and ecological dominance [cover *c.* 20% of land area (Shantz, 1954)]. Grasses dominate montane to alpine meadows and, unlike trees, span the full altitudinal range (Shaw, 2008). By constraining hosts to a single functional group, we reduced confounding effects of hosts' ecological roles. Prior altitudinal surveys of grass-fungal symbioses have focused on a single fungal group and a single or small number of host species (Fisher & Fule, 2004; Granath *et al.*, 2007; Gonzalo-Turpin *et al.*, 2010; Kirkby *et al.*, 2011; Casper *et al.*, 2012; Lugo *et al.*, 2012).

Sampling methods

We focused on four fungal groups: systemic endophytes (Epichloae, Leuchtmann *et al.*, 2014), LFE, AMF and DSE. Our survey spanned seven replicate altitudinal gradients, 46 grass species and 5 years (2008, 2009, 2011–2013) (Appendix S1).

Between July and September, grasses were collected according to a nested sampling design. Gradients spanned the peak of a mountain to its base and were selected to avoid overlap among low elevation sites and to follow separate watersheds (Appendix S2). Gradients were split into sites positioned at *c.* 200 vertical meter intervals, descending from the highest vegetated site, with an average of seven sites per gradient. At each site, we sampled the six to seven dominant grass species (Appendix S1). For each grass species × site combination, we randomly selected a minimum of six individual plants at the seed production stage, located at 5-m intervals along a 50-m transect placed horizontally with respect to the altitudinal gradient. For each individual plant, we sampled a minimum of two asymptomatic tillers and *c.* 5 g of fine roots. Plant material was refrigerated the same day it was collected and processed within *c.* 1 week. For each grass species × site combination, we used the six individual plants to obtain a single estimate of fungal colonization.

Fungal symbiont colonization estimates

Leaves

For each individual plant, approximately two thin sections from the inner leaf sheaths of each of two separate culms were mounted on a microscope slide and stained with aniline blue lactic acid (Bacon & White, 1994). Slides were scored via light microscopy at 200–400× (Appendix S3). Epichloae presence was indicated by multiple views showing the characteristically long, rarely branching hyphae in the plant apoplast (N = 2748 slides for 458 populations). LFE presence or absence (across all tissue) was also scored for each individual plant (N = 1830 slides, 305 populations). We calculated the percentage of symbiotic individuals for each grass species × site combination (i.e. one estimate per population). We did not correct for differences among individuals in the number of leaf cells or the width of leaf sheaths; thus, this was a coarse estimate of colonization.

Roots

Root tissue samples were first pooled by volume across individuals within each grass species × site combination (N = 283 populations). Samples were placed into plastic tissue cassettes, soaked in 10% w:v KOH for 10 h, heated to boiling, then cooled to 25 °C. To stain, samples were removed from KOH and rinsed in water (5 min, four times), soaked in 1% HCl for 20 min, soaked in preheated 5% Sheaffer black ink and vinegar stain for 20 min, and finally rinsed in water (5 min, four times) (McGonigle *et al.*, 1990; Vierheilig *et al.*, 1998). We scored samples via light microscopy with the gridline intercept method at 200× (McGonigle *et al.*, 1990). Root colonization of AMF (aseptate hyphae with vesicles and/or arbuscules, including 'fine endophytes,' Thippayarugs *et al.*, 1999) and DSE (dark, melanized, septate hyphae, Appendix S3) was calculated as the percentage colonization out of 100 views per sample. In 2011, roots were stained with the same procedure, except for the substitution of acid fuchsin for ink (Koske & Gemma, 1989).

Edaphic factors

In 2012 only, we characterized abiotic factors at each collection site (N = 44 sites, Appendix S2, Table S2). At each site, we measured soil volumetric water content (%) (FieldScout TDR probe, 10 cm probes; Spectrum Technologies, Aurora, IL, USA) and collected *c.* 50-mL soil (at 10–15 cm depth) at each 5-m interval along the 50-m transect. Soil samples were pooled by volume within each site and analysed at the Michigan State Soil Testing Laboratory (East Lansing, MI, USA) for available phosphorus [Bray P1 by ascorbic acid, spectrophotometer (U.S. Environmental Protection Agency, 1993)], nitrate [NO_3^-, cadmium reduction, flow injection analyser (Huffman & Barbarick, 1981)] and ammonium [NH_4^+, KCl extract, flow injection analyser (Nelson, 1983)].

Statistical analyses

Multivariate predictors of fungal colonization

We used model selection procedures and multimodel inference (Burnham & Anderson, 2002) on data from year 2012 for 16 well-represented grass species (Appendices S4 and S5). The models evaluated *a priori* hypotheses on the relative importance of potential drivers of colonization (Table 1). Our global model included elevation, soil moisture, available soil phosphorus, available soil nitrogen (NO_3^-, NH_4^+), geographic location (latitude, longitude) and host species identity as predictors. Julian day of sample collection was included as a covariate. Site was included as a random effect to account for the non-independence of species that co-occurred at the same site (Proc Mixed with restricted maximum likelihood, SAS Institute Inc., 2012). Eighteen nested models tested two- and three-way combinations of geographic factors, edaphic factors, host identity and date of collection, as well as each predictor set alone, ensuring that predictors appeared in the same total number of models (Appendix S4). Continuous predictors were natural log-transformed to improve normality (except for elevation, latitude, longitude, Julian day) then standardized to mean = 0, standard deviation = 1. Response variables (proportion of tissue or individuals colonized) were logit-transformed (Warton & Hui, 2011). For epichloae models, we included only the grass taxa documented as epichloae hosts (four species, Appendix S5) to avoid zero-inflated data and used gradient identity rather than site as the random factor because most sites had a single epichloae host. Multimodel inference used the full model set to calculate model-averaged parameter estimates from the Akaike's information criterion (AICc) weights (w_i), unconditional confidence intervals and relative parameter importance (the sum of w_i across all models in which the parameter appeared) (Anderson, 2008). We obtained likelihood r^2 values by comparing each model against the null, which included only the random effect (Anderson, 2008). Edaphic variables were tested for spatial autocorrelation using the *Moran's.I* function in the ape package (Paradis *et al.*, 2004) in R v.2.15.2 (R Core Team, 2013).

Altitudinal distributions

We had larger datasets for examining altitudinal patterns because elevation was recorded in every year (Epichloae $N = 148$ host populations; LFE $N = 305$; AMF/DSE $N = 283$, Appendix S5). A small number of grass populations were sampled in > 1 year: Epichloae (14.0%), LFE (3.9%), AMF/DSE (11.9%). The degree to which altitudinal clines in colonization varied amongst host species was evaluated with model selection procedures comparing a model that included the interaction between elevation and host species identity to a model lacking this interaction. Both models had the random effect of gradient identity. We also examined rank correlations of fungal colonization against elevation for each grass taxon × fungal group with sufficient replication ($N \geq 6$, Appendix S4), followed by Benjamini–Hochberg FDR corrections. To examine interannual variability, we performed Spearman rank correlations on colonization estimates between years for the subset of repeat samples.

Host plant phylogenetic signal

We determined the mean colonization percentage for each fungal group × plant species combination (logit-transformed). We then calculated Blomberg's K (Blomberg & Garland, 2002) along a grass supertree using PICANTE v.1.5-2 (Kembel *et al.*, 2010) in R v.2.15.1 (R Core Team, 2013). To create the grass supertree, we first generated individual phylogenies for the ITS, matK, rps16 and trnL genes using SATÉ v.2.1 (Liu *et al.*, 2012) with default settings. These genes represent a range of chloroplast (matK, rps16, trnL) and nuclear plant (ITS) genes and allowed us to encompass the greatest number of plant species in our dataset (Appendices S6 and S7). Plant DNA sequences were obtained from NCBI GenBank on 16 December 2013. Each plant species was represented by at least two genes. Individual phylogenies were then combined into a plant supertree using matrix representation parsimony in the PHYTOOLS package v.0.2-1 (Revell, 2012) in R v.2.15.1 (R Core Team, 2013). We ran ten iterations and retained the supertree with the lowest parsimony score, resulting in a supertree largely congruent with published phylogenies (e.g. Salamin *et al.*, 2002; Edwards *et al.*, 2010), with the exception that *Poa alpina* was paraphyletic to the rest of the *Poa* clade (Appendix S6).

Correlations among symbiont groups

We examined associations between pairs of fungal groups using Spearman rank correlations. Because host evolutionary history could drive associations between fungal groups, we additionally performed correlations on phylogenetically independent contrasts (PICs) (Garland *et al.*, 1992). PICs were calculated in PICANTE v.1.5-2 (Kembel *et al.*, 2010) in R v.2.15.1 (R Core Team, 2013) from the average logit-transformed colonization estimates for each fungal group × plant species in the grass supertree.

RESULTS

Altitudinal clines in edaphic factors

For the Upper Gunnison Basin, surveys of 44 sites spanning seven altitudinal gradients showed that for every 1 km increase in elevation, soil available phosphorous declined 28 p.p.m. and nitrate declined 8 p.p.m., whereas volumetric soil moisture increased *c.* 6% (no strong trend for ammonium) (Appendices S2 and S8). Elevation, phosphorus, ammonium and soil moisture were positively spatially autocorrelated ($P < 0.05$; Appendix S9).

What factors best predict the percentage of host tissue colonized by fungal symbionts?

Fungal colonization varied strongly with host identity, which was the most important predictor for all four fungal groups (Table 2). However, specific differences among host species were not consistent across fungal groups (Fig. 1). Epichloae colonization varied 14-fold across host species, from the lowest level of colonization in *P. alpina* (2.8 ± 2.8% SE) to the highest in *Festuca brachyphylla* (41.8 ± 9.2% SE) (Fig. 1a). LFE colonization varied 5.5-fold across host species, from the lowest level of colonization in *P. alpina* (6.1 ± 3.9% SE) to the highest in *Phleum alpinum* (40.0 ± 7.9% SE) (Fig. 1b). AMF colonization varied 6.8-fold across hosts, from the lowest level of colonization in *Bromelica spectabilis* (2.9 ± 0.6% SE) to the highest in *Elymus scribneri* (23.0 ± 4.3% SE) (Fig. 1c). Of the fungal groups, DSE showed the weakest variation among host species (1.6-fold), from the lowest level of colonization in *Poa pratensis* (12.6 ± 1.7% SE) to the highest in *E. scribneri* (32.6 ± 4.7% SE) (Fig. 1d).

The four fungal groups also differed in the relative importance of other factors that predicted their colonization of host tissues (Table 2). For the epichloae, the best models explained 52–56% of the variation in the data relative to the null model (Appendix S4) and included geography, elevation and edaphic factors, in addition to host identity (Table 2). Consistent with our hypotheses (Table 1), epichloae colonization declined with increasing elevation, soil moisture and soil available phosphorus, but increased with greater soil nitrogen (Table 2). In contrast, the strongest effect on LFE

Table 2 Model-averaged parameter estimates for each fungal group resulting from multimodel inference

Fungal group	Factor	β	95% CI		Relative importance
Epichloae	**Species** (range)	−6.31 to 11.69			**1.0000**
Epichloae	**Latitude**	0.761	−0.528	2.050	**0.9920**
Epichloae	**Longitude**	0.715	−0.558	1.988	**0.9920**
Epichloae	**Elevation**	−0.444	−1.966	1.078	**0.9920**
Epichloae	**Phosphorus**	−1.651	−3.348	0.045	**0.9916**
Epichloae	**NO_3**	0.899	−0.442	2.241	**0.9916**
Epichloae	**VWC**	−0.714	−2.202	0.774	**0.9916**
Epichloae	**NH4**	−0.005	−1.261	1.250	**0.9916**
Epichloae	**Julian day**	0.306	−0.981	1.593	**0.7605**
LFE	**Species** (range)	0.22 to 3.65			**1.0000**
LFE	**Julian day**	0.753	0.301	1.205	**0.9922**
LFE	**Latitude**	−0.191	−0.579	0.196	**0.6699**
LFE	**Elevation**	−0.138	−0.564	0.288	**0.6699**
LFE	**Longitude**	−0.004	−0.317	0.309	**0.6699**
LFE	NO_3	0.143	−0.194	0.479	0.4601
LFE	Phosphorus	−0.120	−0.525	0.284	0.4601
LFE	NH4	0.074	−0.213	0.360	0.4601
LFE	VWC	0.001	−0.314	0.316	0.4601
AMF	**Species** (range)	−0.56 to 1.50			**1.0000**
AMF	Julian day	0.008	−0.092	0.105	0.1836
AMF	Longitude	0.005	−0.082	0.075	0.0350
AMF	Elevation	−0.002	−0.061	0.058	0.0350
AMF	Latitude	0.000	−0.259	0.338	0.0350
AMF	Phosphorus	0.000	−0.026	0.025	0.0034
AMF	VWC	0.000	−0.024	0.022	0.0034
AMF	NH4	0.000	−0.018	0.018	0.0034
AMF	NO_3	0.000	−0.015	0.015	0.0034
DSE	**Species** (range)	0.51 to 1.63			**1.0000**
DSE	Julian day	0.000	−0.026	0.027	0.1503
DSE	Longitude	−0.005	−0.059	0.049	0.0416
DSE	Latitude	0.003	−0.038	0.044	0.0416
DSE	Elevation	0.001	−0.036	0.038	0.0416
DSE	VWC	−0.002	−0.040	0.036	0.0130
DSE	Phosphorus	−0.002	−0.045	0.040	0.0129
DSE	NH4	−0.001	−0.026	0.024	0.0129
DSE	NO_3	0.000	−0.017	0.017	0.0129

AMF, arbuscular mycorrhizal fungi; DSE, dark septate endophytes; LFE, localized foliar endophytes.

Factors shown in bold have importance values > 0.50. Unconditional 95% confidence intervals incorporate the model-averaged variance estimate as well as the variance among models. Factors are ranked in order of relative importance.

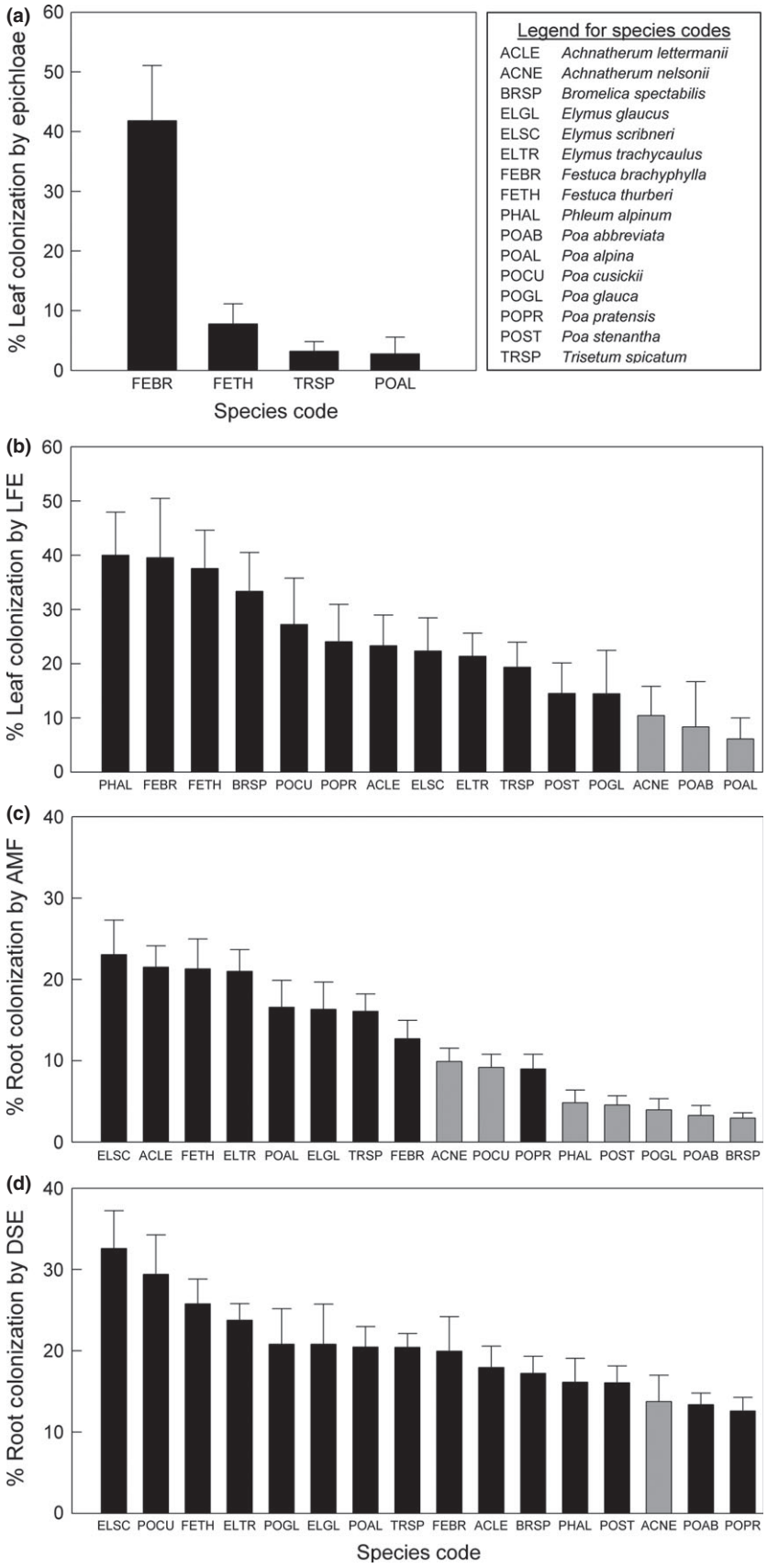

Figure 1 Fungal colonization estimates (mean % ± SE) by host grass species identity. Variation in colonization was not uniform across the four fungal symbiont types: (a) Foliar epichloae, (b) Localized foliar endophytes (LFE), (c) Arbuscular mycorrhizal fungi (AMF), (d) Dark septate endophytes (DSE). Only grass species that were hosts to a fungal symbiont type are included. Bars shaded black have unconditional confidence intervals (not shown) for model-averaged parameter estimates that do not include zero.

colonization besides host identity was increased colonization on later collection dates (Julian day, Table 2), perhaps due to a longer window of leaf exposure to dispersing spores. LFE colonization also showed geographic variation, declining with higher elevation and higher latitude (Table 2). Species identity was the only important predictor of AMF and DSE colonization of roots (Table 2). The best models explained more variation in colonization of AMF (*c.* 42%) than DSE (7–9%) (Appendix S4). Edaphic factors were not important predictors of LFE, AMF or DSE colonization (Table 2).

To what degree are altitudinal patterns specific to host taxon?

Foliar epichloae

The epichloae were the only group not present in all grass species, showing *c.* 7% colonization over all grasses studied (Appendix S5). Of the seven grass taxa with epichloae present, three showed declines in colonization with increasing elevation (Fig. 2a, Appendix S5). Model selection did not support a model including an elevation × host species interaction term (AICc = 831.9) over a model lacking this interaction (AICc = 812.1). Consistent with the long generation times and vertical mode of transmission of epichloae, colonization was highly correlated across years ($r = 0.75$, $P < 0.0001$, $N = 64$ populations).

Localized foliar endophytes

In individual analyses, 21 of 24 correlations with elevation were negative (Appendix S5). Altitudinal declines were strongest for the alpine grass *Poa alpina* and the broadly distributed *Achnatherum lettermanii* (Fig. 2b). Model selection did not support a model including an elevation × host species interaction term (AICc = 1251.8) over a model lacking this interaction (AICc = 1105.4). LFE colonization was not consistent between years ($r = 0.13$, $P = 0.697$), but the number of populations resampled was small ($N = 12$).

Arbuscular mycorrhizal fungi

Altitudinal patterns in AMF colonization varied among hosts. *Achnatherum nelsonii* and *Elymus elymoides* (both restricted to low elevations) showed the strongest altitudinal declines in AMF colonization, and no taxa showed a strong positive correlation with elevation (Appendix S5). Model selection did not support an interaction between host identity and elevation (interaction AICc = 753.4, no interaction AICc = 591.6). Within the same host population, AMF colonization was similar across years ($r = 0.38$, $P = 0.030$, $N = 33$).

Dark septate endophytes

Elevation was generally not an important predictor of DSE colonization (Table 2, Appendix S5). DSE colonization increased with higher elevation in the *Elymus* genus and in the broadly distributed species, *Elymus glaucus* (Fig. 2), but declined with higher elevation in *E. scribneri*, a high-elevation species. A model allowing grass species to vary in the slope of colonization on elevation (AICc = 682.6) was not a better fit to the data than a model lacking this interaction (AICc = 519.8). Within the same host population, DSE colonization was inconsistent between years ($r = 0.09$, $P = 0.625$, $N = 33$).

Is there evidence for host plant phylogenetic signal in levels of fungal symbiont colonization?

Colonization estimates for facultatively symbiotic fungi (LFE, DSE) were not phylogenetically conserved across the grass phylogeny (LFE Blomberg's $K = 0.484$, $P = 0.113$; DSE Blomberg's $K = 0.281$, $P = 0.835$). In contrast, colonization estimates for the obligately plant-associated epichloae ($K = 0.497$, $P = 0.014$) and AMF ($K = 0.526$, $P = 0.026$) were phylogenetically conserved.

Are associations between different fungal groups suggestive of synergisms or antagonisms among symbiont groups inhabiting the same plants?

The strongest associations were positive relationships between AMF and DSE (Spearman $r = 0.46$, $P < 0.001$, $N = 283$). Also, LFE colonization was positively correlated with colonization estimates for both epichloae ($r = 0.15$, $P = 0.007$, $N = 305$) and DSE ($r = 0.15$, $P = 0.022$, $N = 228$). After accounting for the evolutionary history of hosts, only the positive correlation between AMF and DSE remained strong (PICs, $r = 0.50$, $P = 0.033$, $N = 18$ species).

DISCUSSION

This work represents one of the largest altitudinal surveys of fungal symbionts to date, and the first, to our knowledge, to examine colonization of both leaves and roots. Our results suggest that edaphic, biotic and spatial drivers influencing fungal symbiont distributions are more complex than previously hypothesized. Functionally different groups of fungal symbionts did not share common responses to host identity or to environmental gradients. Different symbiont groups were positively correlated, indicating host-related hotspots of fungal abundance, particularly for AMF and DSE. We suspect that as more data accumulate, patterns will show greater host and fungal specificity than reported by more limited surveys (e.g. Haselwandter & Read, 1980; Newman & Reddell, 1988; Ruotsalainen *et al.*, 2004; Bahram *et al.*, 2012; Gai *et al.*, 2012).

The importance of host plant phylogeny varied among fungal groups

While colonization levels were most strongly influenced by host identity for all four fungal groups, only the obligately

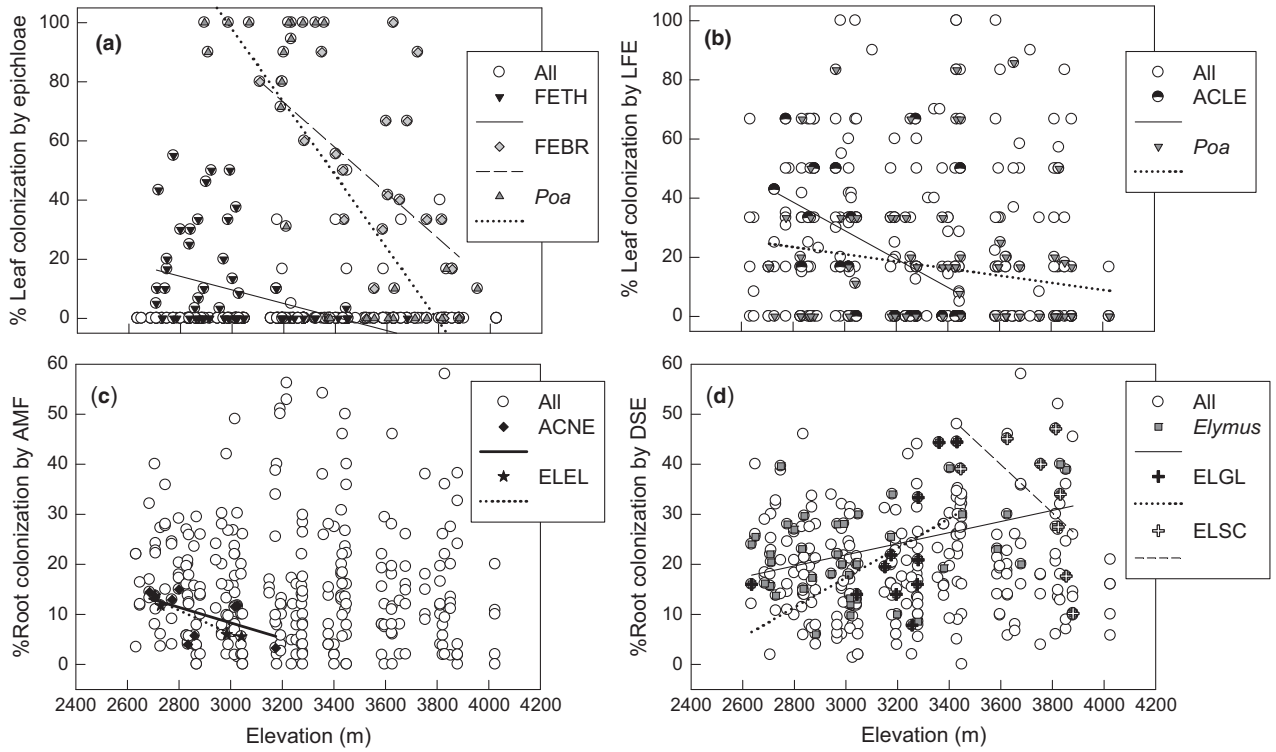

Figure 2 Relationships between fungal colonization and elevation for four fungal symbiont groups. (a) Foliar epichloae, (b) Localized foliar endophytes (LFE), (c) Arbuscular mycorrhizal fungi (AMF), (d) Dark septate endophytes (DSE). Open circles show all populations (grass species × site combinations) sampled (All) and filled symbols show grass taxa with significant ($P < 0.05$) correlations (see Appendix S5 for statistical results). Grass host codes: ACLE, *Achnatherum lettermanii*; ACNE, *Achnatherum nelsonii*; ELEL, *Elymus elymoides*; ELGL, *Elymus glaucus*; ELSC, *Elymus scribneri*; *Elymus*, *Elymus* genus; FETH, *Festuca thurberi*; FEBR, *Festuca brachyphylla*; *Poa, Poa* genus.

plant-associated symbionts (epichloae, AMF) showed an influence of host plant evolutionary history. For the epichloae, the observation of strong host phylogenetic signal was consistent with patterns of co-cladogenesis for other grass–epichloae species pairs (Schardl *et al.*, 2008; Schardl, 2010). For the AMF, our results conflict with prior studies showing that host identity and evolutionary history have little effect on colonization or fungal composition (e.g. Dumbrell *et al.*, 2010; Öpik *et al.*, 2013). However, some evidence points to AMF specificity at the larger scales of plant functional group (Yang *et al.*, 2012) and superorder (Opik *et al.*, 2010). For the obligately plant-associated fungi, the detection of host phylogenetic signal was consistent with the observation that colonization levels were similar across years for the populations that were resampled. The lack of plant phylogenetic signal for the facultative symbionts (LFE, DSE) and their inconsistency across years suggests that geographic and edaphic factors as well as context-dependent interactions with host plants may be more important drivers of their colonization levels.

The relative importance of elevation, geography and edaphic factors for fungal colonization

Our analyses focused on subsets of possible abiotic and biotic variables that may affect colonization of plants by fungi. Overall, host species identity was the most important predictor of colonization, regardless of the fungal group. This result supports an overriding influence of host identity, even within a host clade that was constrained in its ecological function (cool-season grasses). However, host species that had high colonization by one fungal group were not universally highly colonized by all fungi. We also found stronger support for edaphic and geographic predictors of above-ground fungal colonization (epichloae, LFE) than for colonization by root fungi (AMF, DSE). It is possible that the root fungi show strong within-site (e.g. microsite) variation, which was not captured by our sampling design. In addition to microsite variation, other factors not included here (e.g. animal dispersal of AMF spores) could be useful to consider in future work.

Foliar epichloae

Results supported our hypotheses that epichloae colonization would decline with higher elevation, increase with greater soil nitrogen and decrease with higher soil available phosphorus and soil moisture (Table 1). The decline in epichloae colonization with elevation may reflect reduced benefits through endophyte-mediated herbivore deterrence (Schardl, 2010), given that higher elevations have a shorter window of herbivore exposure as a function of the abbreviated growing season. Our results also suggest more attention should be

directed to possible roles of epichloae in phosphorus dynamics. In the best studied grass–epichloae interaction (*Schedonorus arundinaceus* with *Epichloë coenophiala*), plants grown in phosphorus-deficient soils accumulated more phosphorus in leaf and root tissues than endophyte-free isogenic lines and showed higher endophyte-mediated increases in biomass than in phosphorus-rich soils (Malinowski *et al.*, 1998, 2000; Malinowski & Belesky, 1999). In contrast to the decline in epichloae colonization with phosphorus, there was an increase with soil nitrates. It is possible that the epichloae symbiosis directly benefits from soil nitrates because epichloae produce nitrogen-rich alkaloids (Schardl, 2010) and can become costly to plants under nutrient limitation (Cheplick, 2007). The negative association between epichloae colonization and soil moisture was consistent with prior studies, which showed increased benefits of epichloae under drought (e.g. Elmi & West, 1995; Malinowski & Belesky, 2000; Kannadan & Rudgers, 2008; Davitt *et al.*, 2011). However, climatic patterns are highly variable among mountains and seasons (Rangwala & Miller, 2012), and we used only a one-time measure of soil moisture. While temperature and rainfall data loggers were beyond our budget, installation of such infrastructure could be valuable for future work. Also valuable would be direct manipulations of temperature (e.g. Rudgers *et al.*, 2014).

Localized foliar endophytes

Few prior studies have explored factors associated with colonization by the horizontally transmitted LFE (e.g. U'Ren *et al.*, 2012). Here, the timing of sample collection, elevation and geography were important correlates. In contrast, edaphic factors, including nutrient availability and soil moisture, were not strong predictors. Thus, our colonization data did not support a hypothesis of higher colonization in drier soils, associated with greater plant investment in LFE under drought (e.g. Giauque & Hawkes, 2013). The influence of date of collection highlights the importance of considering plant (and fungal) phenology in sampling designs, particularly if high and low elevation sites are sampled on the same calendar date. We suspect that effects of collection date are likely to be most important early in the growing season, when low sites lack snow, but high sites are just recovering from winter snow pack. Finally, it is important to note that drivers of colonization extent may differ from drivers of fungal composition. For example, Zimmerman & Vitousek (2012) determined that precipitation and temperature, but not soil nutrients, influenced LFE composition along an altitudinal gradient in Hawaii, and surveys of LFE composition in tropical trees showed a strong role of host plant identity (Kembel & Mueller, 2014).

Arbuscular mycorrhizal fungi

Prior surveys have documented declines in AMF with elevation (e.g. Gardes & Dahlberg, 1996; Wu *et al.*, 2007; Gai *et al.*, 2012). However, elevation was not a strong predictor of AMF colonization in our survey. This disparity may reflect the span of the altitudinal gradient we studied. Even our lowest sites (*c.* 2600 m) were high in elevation compared to past studies (e.g. Wu *et al.*, 2007). In addition, AMF colonization levels (mean: 15%, range: 2.9–53.8%) were on the low end reported for grasses, consistent with the hypothesis that our surveys began above where large declines in AMF had already occurred. Finally, the strongest negative relationships between AMF colonization and elevation occurred for grass species restricted to the lowest elevations (Fig. 2c). These patterns suggest that accounting for host elevation range (e.g. species distribution models) could be an important tool for refining predictions on fungal symbiont distributions. We also expected declines in AMF colonization with higher levels of soil phosphorus, nitrogen and moisture, because AMF can improve plant nutrient and water acquisition (Auge, 2004; Smith & Read, 2008). Surprisingly, none of these predictions were supported. Instead, colonization varied most with host identity and phylogenetic relatedness. The high degree of variability in colonization among host species may suggest additional variation in AMF species composition among grass hosts. DNA sequence data will be useful for evaluating such patterns.

Dark septate endophytes

While altitudinal increases in DSE colonization have been reported elsewhere (e.g. Read & Haselwandter, 1981; Schmidt *et al.*, 2008), such patterns should not be assumed to be general across host taxa. Elevation was not an important predictor of DSE colonization in our study; only two grass taxa showed strong increases in colonization with elevation (Fig. 2d). Edaphic conditions were also not important correlates of colonization of roots by DSE, despite their roles in prior studies (Herrera *et al.*, 2011; Newsham, 2011; Kivlin *et al.*, 2013). Of the four fungal groups we considered, DSE were the least likely to have predictable colonization patterns, leaving open the question of what factors influence their distributions.

Fungal symbiont groups showed primarily positive associations

Studies that ignore the complexity of multiple symbionts within shared hosts may underestimate host responses if multiple symbionts interact synergistically to promote host resilience to abiotic stress (Afkhami *et al.*, 2014) or overestimate effects, if antagonisms among symbionts reduce net benefits to hosts (e.g. Mack & Rudgers, 2008). We expected that fungi competing for similar host tissues (e.g. leaves) would show negative correlations, whereas fungi occupying leaves would be uncorrelated or positively associated with root fungi (Larimer *et al.*, 2010; Afkhami *et al.*, 2014). Instead, most fungal groups showed positive correlations, suggesting that host quality or microsite-level abiotic factors

simultaneously affect multiple symbiont groups. The strongest positive association occurred between AMF and DSE, both colonists of plant roots, and this relationship remained even after we accounted for host evolutionary history. Given the lack of strong abiotic correlates of root colonization in our survey, we suggest that AMF and DSE may provide complementary, rather than overlapping, benefits to hosts (reducing the possibility for competition) or rely on similar aspects of host quality.

CONCLUSION

Our survey of grasses along altitudinal gradients in the Rocky Mountains suggests that the responses of fungal symbionts to climate change are likely to be complex and mediated by host plant specificity, edaphic factors and interactions among fungal groups. Given the influence of fungal symbionts on host resilience to stress (Kivlin *et al.*, 2013), the potential for climate change to alter symbioses deserves greater attention.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 List of samples for fungal symbiont surveys in the Colorado Rocky Mountains.

Appendix S2 Map of study sites for fungal symbiont surveys in the Colorado Rocky Mountains with corresponding table of edaphic data for each site from 2012.

Appendix S3 Photographic images of each fungal symbiont group.

Appendix S4 Statistical results of model selection procedures.

Appendix S5 Means and spearman rank correlations for fungal colonization estimates by individual grass taxa.

Appendix S6 Grass supertree for the fungal symbiont survey.

Appendix S7 Genbank IDs for grass species included in the supertree.

Appendix S8 Statistical results for edaphic variables against elevation.

Appendix S9 Results of the Moran's *I* test for spatial autocorrelation.

BIOSKETCHES

This research team aims to understand the responses of plant–fungal symbioses to future climates, with a focus on mountain ecosystems.

Author contributions: S.K. and J.R. conceived the study; all authors collected and processed samples; J.R. primarily scored foliar endophytes, S.K. primarily scored root colonization; L.R., J.R., J.L. and S.K. analysed the data; L.R. and J.R. led the writing.

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