

DRIVERS OF ENDOPHYTE COMMUNITIES IN PACIFIC NORTHWEST PRAIRIES

By  
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## THESIS ABSTRACT

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Prairies of the Pacific Northwest are threatened systems, with only ~2% of historic land remaining. The combined risk of global climate change and land use change make these systems a high conservation priority. However, little attention has been paid to the microbiota. Fungal endophytes are ubiquitous in plants and are important in ecosystem functioning and host dynamics. To understand fungal community assembly, we used high-throughput sequencing to investigate the composition of fungal foliar endophyte communities in two native, cool-season (C3) bunchgrasses along a natural latitudinal gradient. We quantified the importance of host, host traits, climate, edaphic factors, and spatial distance in microbial community composition. We found that spatial distance was the strongest predictors of endophyte community, while host traits (e.g., plant size, density) and abiotic environment were less important for community structure. These findings underline the importance of dispersal in shaping microbial communities.

This thesis includes previously unpublished, co-authored material.

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# CHAPTER I

## INTRODUCTION

Due to their close co-evolutionary relationships with plants, fungi often have large consequences for plant communities (Hyde and Soyong 2008). For example, plant-fungal relationships, such as those between mycorrhizal fungi and plants are often beneficial, frequently buffering stress responses (Barea and Pozo 2005). However, fungal endophytes, which have been found in large numbers in every plant species examined (Rodriguez et al. 2009) represent a cryptic yet large component of plant-associated microbes (Hyde and Soyong, 2008, U'Ren *et al.*, 2012). Fungal endophytes are fungi that, for at least a part of their life cycle, colonize internal plant tissue, causing no apparent harm to the host (Hyde and Soyong 2008). Endophytes encompasses large variation in both taxonomy and ecology, and although their functions vary, endophytes are thought to be important drivers of plant communities (Rodriguez et al. 2010). As mutualists, they can increase plant recruitment, buffer environmental stresses, and mediate damage from parasites, pathogens, and herbivores (Porrás-Alfaro and Bayman 2011).

Endophytes can also affect pathogens via both direct and indirect effects. Directly, they have been observed to produce antibiotics and lytic enzymes, although there is debate as to the frequency and specificity of these effects (Gao et al. 2010). However, there is a large body of literature on how endophytes can indirectly affect pathogens. One mechanism is the activation of plant defenses. Many of the plant mechanisms involved in pathogen resistance involve signal cross-talk between plant and pathogen, and it is thought that endophytic fungi are capable of hijacking that system (Gao et al. 2010). On a simpler level, endophytes can occupy niche space to exclude other fungi. This can be in the form of space occupation, or competitive exclusion (Gao et al. 2010). As latent saprotrophs, these fungi are involved in ecosystem processes essential to plant growth and survival (Saikkonen et al. 2015). However, endophytes can also act as latent pathogens, with adverse effects upon both individuals and plant

communities. Infection could change competitive dynamics among plant species, and lead to widespread loss in fitness within communities (Porrás-Alfaro and Bayman 2011).

To add complication to the matter, the endophytic life strategy seems to be a plastic one, and is not evolutionarily stable. It is thought that endophytic fungi likely evolved from pathogenic fungi, and thus often have very similar strategies in plant colonization and transfer of nutrients (Wani et al. 2015). Factors such as changes in environment, host fitness, and host species have all been described as triggers that change life histories between mutualistic and pathogenic (Wani et al. 2015). Whatever the trophic mode displayed, fungi must also gain benefits from their plant hosts, as there are costs involved with circumventing host defenses. These benefits are almost completely unknown, but likely manifest in the form of shelter from external environment in favor of a stable one within the host, a stable source of carbon, and potentially an alternate mode of dispersal (Thomas et al. 2016).

Fungal endophytes are divided broadly into two classes based upon life history traits: Clavicipitaceous (C-endophyte), and non-Clavicipitaceous (NC-endophyte)(Rodríguez et al. 2009). C-endophytes belong to the family *Clavicipitaceae* (Ascomycota, Sordariomycetes, Hypocreales; O. E Erikss. 1982), and are believed to be limited to graminoid hosts (that is, species of grasses, rushes, and sedges). C-endophytes are primarily transmitted vertically between hosts, passing from parent to offspring through seed infection, and thus often form systemic infections (Rodríguez et al. 2009). Although the rate of colonization often is high, diversity within the plant tissue is often low, sometimes with only a single species present (Rodríguez et al. 2009). Therefore, C-endophytes are thought to be more tightly linked with plant species, as opposed to environmental or spatial constraints (Giauque and Hawkes 2013). They sometimes confer benefits to host plants, including a reduction in herbivory through secondary metabolites, drought tolerance, and increased growth. although they have also been observed as latent pathogens and saprotrophs (Rodríguez et al. 2009). Indeed, even when considered mutualistic, these fungal infections often result in decreased host fitness, gaining them the nickname of balanced antagonists (Schulz and Boyle 2005).

In contrast, NC-endophytes represent a broad array of fungi, both taxonomically and ecologically. Species from both Ascomycota (Pezizomycotina) and Basidiomycota

(Agaricomycotina, Pucciniomycotina) have been reported as putative endophytes (Rodriguez et al. 2009). Colonization of host tissue is non-systemic, often limited to a specific tissue type, including leaves, shoots, and roots, although fungal diversity within these tissues is generally quite high. Moreover, they are thought to be less host specific, able to colonize a wide variety of plants (Rodriguez et al. 2009). General strategies including latent pathogenesis and saprotrophy have been described for NC-endophytes. Often, endophytes from this clade seem to switch from asymptomatic to pathogenic/saprobic only when conditions merit the change. This could be tissue senescence, attack by a more virulent pathogen, or host death. However, there is also strong evidence that some NC-endophytes confer mutualistic benefits such as stress tolerance and growth promotion on their host (Rodriguez et al. 2009).

To date, much of the current research has focused on the effects a single endophytic species has on plants. Of these, the majority focus on C-endophytes, namely *Epichloe* and *Neotyphodium* (Kuldau and Bacon 2008) that play roles in agriculturally significant plants. While these species are very important, there are often many more endophyte species to consider. Multiple species from both C and NC groups may be present, interacting both with the plant and with each other. This, in effect, creates an entire ecosystem within a single leaf of a plant. There is still little known about these communities.

In chapter two, I described a co-authored study in which we examine the communities of endophytes, both C and NC, present in the leaves of two native grass species along a latitudinal gradient stretching from southernmost Oregon to Northernmost Washington. The goal was to examine the potential roles of dispersal, the abiotic environment, and the biotic interactions in driving differences among communities.

## CHAPTER II

# DRIVERS OF ENDOPHYTE COMMUNITIES IN PACIFIC NORTHWEST PRAIRIES

This chapter includes co-authored material. The experimental design was developed through the collaboration of Dr. Bitty Roy and myself. I did most field work, lab work, data analysis and writing. Daniel Thomas contributed to the development of bioinformatics and statistical methodology used in this research. Dr. Scott Bridgham and Dr. Bitty Roy edited the document and contributed to idea development.

Fungal endophytes are ubiquitous among plant lineages, and comprise a cryptic, yet tremendously important aspect of plant and ecosystem functioning. To date, all plant species surveyed have been found to associate with fungal endophytes (Rodriguez et al. 2009). The term has been defined as any fungi living within the tissues of plants and causing no apparent harm for at least part of their life cycle (Rodriguez et al. 2009). While these fungi are commonly considered to be mutualistic, researchers have also described endophytic fungi as latently pathogenic or saprotrophic (Rodriguez et al. 2009). While traditionally pathogens have been excluded from the concept of endophytism (Hardoim et al. 2015), here we use the term endophyte to describe all fungi living within plant leaves. Recent evidence suggests that the role endophytes play within their host can change depending upon host species and environment (Hyde and Soyong 2008, Delaye et al. 2013, Hardoim et al. 2015). Moreover, research suggests that endophytic fungi often form complex communities, often with multiple species interacting between localized colonies within plant tissue (Saunders et al. 2010).

### *Community Assembly*

Here we examine the roles of dispersal, and the abiotic and biotic environments in driving endophyte community assembly. These drivers have colloquially become referred to as filters, acting to differentiate the regional species pool from the local (Kraft and Ackerly 2014). Dispersal limitation, while dependent on individual species' life history traits and dispersal capabilities, can be expressed in terms of spatial distance and

spatial structure. Abiotic factors, such as climate, edaphic factors, topography, etc. are thought to provide the framework defining a species fundamental niche, or all potential suitable habitats in which the species could survive. Finally, there is the influence of biotic interactions, largely thought of in terms of competition for resources, although other species interactions are likely also important (Figure 1) (Boulangeat et al. 2012).

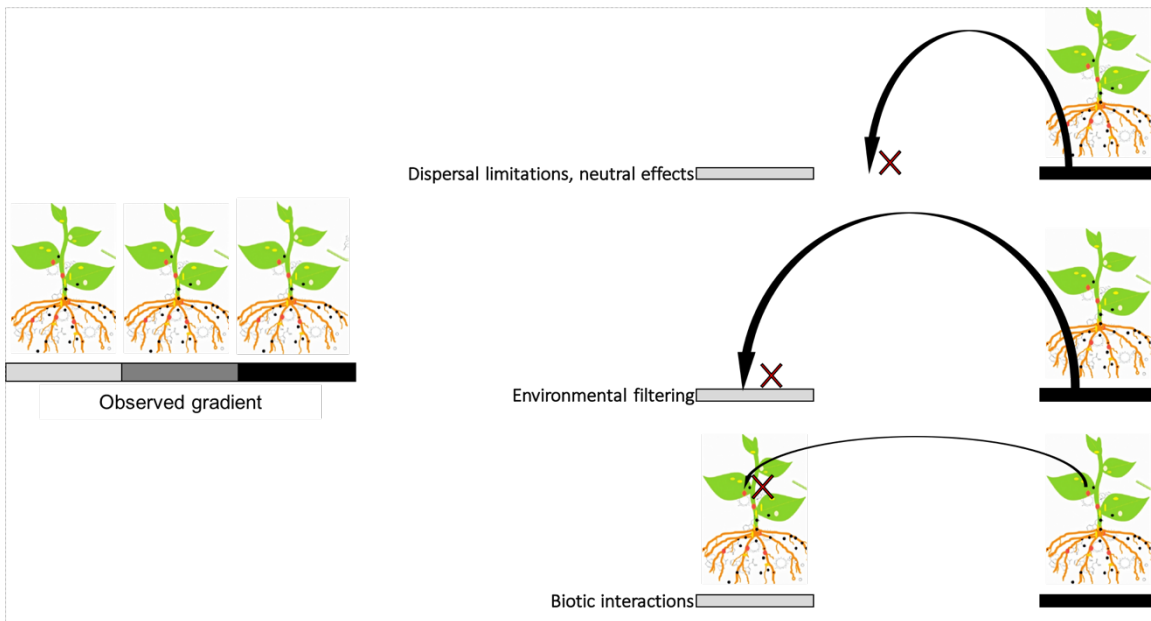


Figure 1: Conceptual model of community assembly based on Kraft et al. 2015.

Many of these ideas on assembly were developed by studying communities macroscopic organisms, namely plants and animals (Martiny et al. 2006). While general patterns of species co-occurrence hold true for microbial communities, the processes that govern these patterns may be vastly different (Horner-Devine et al. 2007). Indeed, there are several points where the divergence of these characterizations may be important.

The abiotic environment is undoubtedly important in plant community assembly but has further impacts on fungal endophytes. The fact that these fungal communities exist within the tissue of another organism suggests that there are two distinct habitat filters – the exterior and interior environment of host tissue (Saunders et al. 2010). Foliar endophytes likely evolved from plant pathogens, and thus share many of the same methods of tissue colonization via appressoria, or the direct penetration via hyphae

(Rodriguez et al. 2009, Saikkonen et al. 2015, Wani et al. 2015). Importantly, both temperature and precipitation are intrinsically linked to the transmission of fungal endophytes. Indeed, both the formation of fungal spores and tissue colonization require nearly 100% humidity (Harvell 2002). In addition to humidity, the colonization of fungi seems to be dependent on periods of leaf wetness. Many endophytes require a minimum period of leaf wetness, while in some species leaf wetness is associated with more efficient colonization (Huber and Gillespie 1992). Overwintering (or over-summering) is a major determinant of many pathogens ranges, and in areas with cold, prolonged winters, temperature filters the annual species pool. Perhaps because of this, the local severity of certain pathogens is also limited by winter minimum temperatures (Harvell 2002). In contrast to the climatic pressures felt by plant communities, the internal tissue of plants is a much more stable environment, especially considering water stress (Hardoim et al. 2015). For this reason, host stress will likely be linked to the impact that climate has on endophytes. Soils can indirectly affect the plant host, but also directly affect saprotrophs, which spend part of their life cycle in the soil (Giauque and Hawkes 2013).

Endophyte dispersal depends upon sporulation, climate and spatial distance. Many non-systemic endophytes have slow rates of sporulation within healthy host plants, but will emerge and sporulate at host senescence (Rodriguez et al. 2009). Endophytes rely upon external forces to disperse their spores, and even at small spatial scales, active spore dispersal may limit the distribution of fungi (Norros et al. 2012). The two most important abiotic factors in spore dispersal are precipitation and wind. Precipitation acts not only to trigger sporulation in many species, but also as a short-distance dispersal aid (Fitt et al. 1989). At larger distances, wind becomes a major contributor to the spread of spores. Interestingly, wind dispersal is thought to be more of a dry-dispersal method, and more efficient in the absence of rain. However, species-specific exceptions to these generalizations do occur (Fitt et al. 1989). When considering the spatial component of dispersal limitation, it can be useful to borrow ideas from island biogeography. Plant populations can be conceptualized as islands in the sense that they represent habitable space, with their own non-random distribution in space. In this sense, the size and



connectivity of plant populations can have large effects on the dispersal of endophytic fungi (Helander et al. 2007).

Similarly, there are several ways to consider the role that biotic interactions play in fungal endophyte community assembly, namely priority effects (or advantages based upon order of arrival), host-microbe interactions, and microbe-microbe interactions (Saunders et al. 2010). Competition involves the struggle for dominance over a limiting resource. Within plant tissue, this can largely be thought of in terms of space and nutrients (Bitas et al. 2013).

### *Local and Regional Effects on Community Assembly*

When viewed through the lenses of global change, community assembly shifts from an academic question to a practical one. Through the impact of land use, climate change, and increased global connectivity, questions of how communities assemble are also indirectly asking how species will react to continued and increased threat of habitat change.

This is especially true for prairies within the Pacific Northwest. The regional climate of these landscapes can be described as Mediterranean, which experience characteristically mild, wet winters, and dry, hot summers (Pfeifer-Meister et al. 2013). Globally, Mediterranean systems contain nearly 20% of the world's plant diversity, while occupying only about 5% of the land area (Cowling et al. 1996). Given the close association between plants and fungi, it logically follows that a large portion of fungal diversity lies within areas with Mediterranean climates. Indeed, studies involving Mediterranean oak forests found a large diversity of fungi, although endophytic fungi are understudied in these systems (Pirttilä and Frank 2011).

Unfortunately, areas with Mediterranean climates are also among those most threatened by land use change. For example, the Willamette Valley is assumed to have once been dominated (49%) by grasslands of various sorts including upland prairie, oak savannah, and wetland landscapes (Bachelet et al. 2011). However, the current distribution of prairies and oak woodlands is estimated to be around 2% of historic levels. Those still in existence are considered to be highly fragmented and degraded (Bachelet et al. 2011). Indeed, nearly every natural resource agency in the Pacific Northwest

classifies prairie/oakland habitat as high priority (Altman 2011). Habitat fragmentation is detrimental to communities through both reduction in habitat and connectivity. For this reason, ecologists tend to compare highly fragmented habitats to islands (Harrison and Bruna 1999). In a study on endophyte frequency among islands, Helander et al. (2007) found that both the distance from the mainland (access to the regional species pool and spatial distance) and the size of the island were the most important predictors of endophyte frequency. This suggests that the dispersal of both endophytes and pathogens may be affected by habitat fragmentation.

In addition to habitat loss, climate change will likely have an effect on local diversity, community composition, and biotic interactions. While there is variation among predicted future climate models for the Pacific Northwest, most models agree that the area will experience an increase in seasonality, with wetter, warmer winters and warmer, drier summers (Mote and Salath 2010). While precipitation is expected to potentially increase by nearly 50%, much of that is expected to occur during the late fall, winter, and early spring (Bachelet et al. 2011), while drought will intensify in duration during the summer months (Garrett et al. 2006). These changes in seasonality are expected to have major impacts not only on plant communities, but also on the dynamic between plants and fungi. In regions characterized by cold winter temperatures, winter acts as a major filter for pathogens. It is projected that up to 99% of a local pathogen population can be lost due to cold winter temperatures (Harvell 2002). With the increase in winter precipitation, we can expect a proportional increase in pathogen incidence and severity (Harvell 2002). Further, warmer annual temperatures are expected to lead to the expansion of pathogen ranges, and could even lead to expansion of host colonization (Roy et al. 2004, Garrett et al. 2006). Studies have found that increases in temperature also lead to decreased microbial diversity, as well as altered community composition (Bálint et al. 2015). This suggests that not only are plant-microbe interactions at risk of change, but also microbe-microbe interactions.

Here, we characterize the diversity and composition of foliar fungal endophytes within Pacific Northwest prairies. We attempt to understand community structure in the context of community assembly, and these major filtering processes: Dispersal limitation

(as a function of spatial distance), environmental filtering (local climate and edaphic factors), and biotic interactions (through host plant species and traits).

First, we asked whether there is variation in foliar fungal endophyte diversity and composition in the prairies of the Pacific Northwest. We wanted to know whether there were any observable patterns in community clustering. As there is relatively little known of the endophytic communities within native bunchgrass populations, this is an important first step to understanding these communities. Given the general paradigm of increasing fungal richness as latitude declines (Arnold and Lutzoni 2007), we hypothesized that endophyte species richness will also tend to increase as latitude decreases. Further, we hypothesized that endophyte communities would vary based upon plant host, region, and site, suggesting host specificity and non-random community assembly.

Second, we ask what predictors contribute to the composition of foliar fungal endophyte communities, and more specifically, how do they act to influence these communities. We hypothesized that dispersal, abiotic, and biotic interactions will all contribute to endophyte community composition. Of these, we expected that environment would play the largest role in determining community composition because of the necessity of precipitation in both fungal sporulation and germination, and the consequences of temperature on survival.

## ***Methods***

### *Site Background and Focal Species*

To determine how biotic factors (host species, host traits) and abiotic factors (climate, spatial distance, soils) influence endophyte community composition in prairie grasses, we selected prairies along a latitudinal gradient. The prairies ranged from the Klamath Mountain ecoregion, through the Willamette Valley, and north into the Puget Trough-Georgia Basin (WPG) ecoregions (Figure 2, Bachelet et al. 2011). This area represents the historic distribution of many native prairie species within the Pacific Northwest (Christy and Alverson 2011, Hamman et al. 2011).

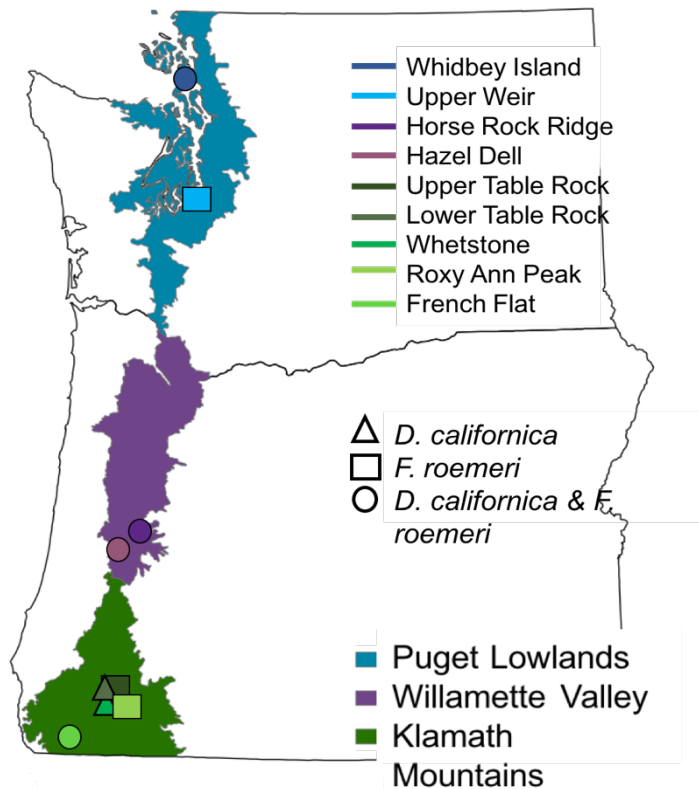


Figure 2: Map depicting ecoregions, and area of study within Washington state and Oregon. This Figure and all others following use the same color scheme: green represents the Klamath Mountain ecoregion, purple represents the Willamette Valley, and blue represents the Puget Lowlands.

For each species, we selected at least two populations within each ecoregion. Populations were only chosen within native, remnant prairies, in which no restorative seeding had been done, and that had an adequate presence of target individuals (200+ within a 30 by 2m transect). We avoided sites that had been seeded because seeded plants, although native, may not be of a local cultivar, and could potentially disrupt endophyte community composition. We further strove to maximize variation in elevation, precipitation and temperature among sites to define a near-continuous climate gradient.

At each site, we measured including spatial, edaphic, and climatic. To quantify spatial factors we measured latitude, longitude, and elevation at both ends of each transect using GPS. To quantify edaphic factors, we dug shallow holes in the soil (10 by

10 cm cylinder) at the start and end of each transect, and determined the volume of soil using water displacement. Soil volume was used to estimate soil bulk density, measured as soil weight/soil volume (Goodman and Ennos 1999). Soil samples were analyzed for inorganic nitrogen availability, soil depth, soil bulk density, soil pH, total soil nitrogen and carbon, and soil texture. Inorganic nitrogen (ammonium  $\text{NH}_4^-$  and nitrate  $\text{NO}_3^-$ ) was measured using ion exchange resin membranes (Western Ag Innovations, Saskatoon, SK, Canada). Resin membranes were incubated in the soil for approximately four months (April to August), which encompasses the majority of the plant growing season. Soil depth was measured to obstruction using an 18 Volt drill with a 90cm steel rod (0.5cm diameter) to probe the soil at the start, middle, and end of each transect. Depths greater than the 90cm cutoff were recorded as + 90cm. After measuring the field-moist mass, each sample was dried at 60 °C for a minimum of 48 hours to determine oven-dry mass. Soil pH was measured using a 1:1 fresh soil to deionized water slurry by mass. Total soil nitrogen and carbon were measured using a 4010-elemental combustion analyzer (Costech Analytical Technologies, Valencia, CA, USA). We measured soil texture following the USDA NRS method (Gee and Bauder 1986). This involved creating soil slurries with the dispersant Sodium Hexametaphosphate (HMP). Percentages of clay, silt, and sand are determined by the specific gravity of the slurries over a given period of time. We expect that edaphic factors will affect foliar endophyte communities indirectly through the host, as well as directly (for saprotrophic fungi).

Climate data included 30 year averages (1981-2010) from the Parameter elevation Regression on Independent Slopes Model (PRISM) 800m resolution dataset of precipitation and temperature (PRISM Climate Group, Oregon State University). Temperature data included monthly mean, maximum, minimum, and dew point data. Precipitation data included monthly accumulation as well as average annual accumulation. To best represent our regional climate, which is strongly Mediterranean ((Bachelet et al. 2011), these data were grouped into three 'seasons' corresponding to winter (November to February), spring (March to June), and summer (July to October).

For focal host species, we chose two native, cool-season C3 bunchgrasses: *Danthonia californica* (Bol.) and *Festuca roemerii* (Yu E, Alexeev). These grass species are relatively long-lived, able to survive up to several decades, and are among those

species considered to be a historic cornerstone of Pacific Northwest prairies (Christy and Alverson 2011, Stanley et al. 2011). While grasses have been extensively studied from the perspective of clavicipitaceous endophyte fungal associations, relatively little is known about their greater endophyte diversity (Porrás-Alfaro and Bayman 2011). *D. californica* is a widespread, perennial bunchgrass whose natural range extends from southern California into British Columbia, and east from the Pacific Ocean toward the Rocky Mountains (Darris and Gonzalves 2007). *D. californica* can grow in a large variety of soil types, although it cannot withstand severe drought. It is important to ecosystem functioning through erosion control, as a re-vegetative species, wildlife habitat, and forage. This grass flowers between May and early June (Darris and Gonzalves 2007). *F. roemerii* is another native bunchgrass, with a distribution from central California into British Columbia, growing only on the west side of the Cascade and Sierra Nevada mountain ranges. This grass favors moderately moist ecosystems, although it is relatively drought resistant. Flowers emerge around late May, and seeds mature sometime near early July (Darris et al. 2012). *F. roemerii* is associated with upland prairie and oak savannah, where it often acts as important habitat for birds, insects and mammals (Darris et al. 2012).

### *Site Descriptions*

All sites follow the general pattern of Mediterranean climate systems: Wet, cool winters and dry, hot summers (Bachelet et al. 2011). This swing in seasonality causes a switch from the ‘expected’ growing season, where winter and spring are more important for plant biomass accumulation (Bachelet et al. 2011). There was, however, variation in these patterns among sites, particularly in both monthly and annual precipitation (Figure 3, Table 1). While we observed differences in temperature among sites, the variation is much less pronounced than for precipitation. Interestingly, neither temperature nor precipitation data strictly followed latitude, longitude, or elevation (Figure 4, Table 1), potentially allowing us to decouple these factors. Edaphic factors are shown for sites where *F. roemerii* (Table 2) and *D. californica* (Table 3) are present.

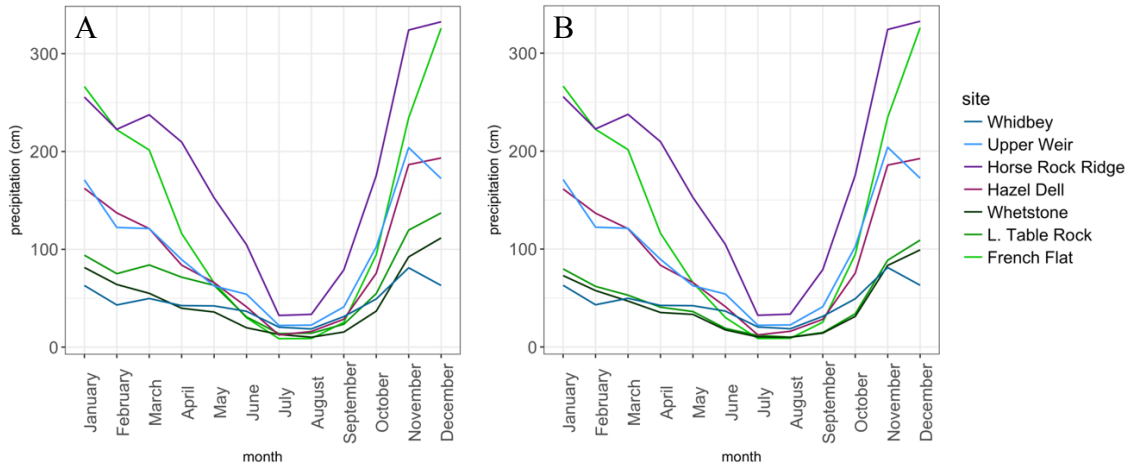


Figure 3: Monthly precipitation accumulation among sites where A) *F. roemerii* and B) *D. californica* are present. Data are based upon 30-year normals (1980-2010).

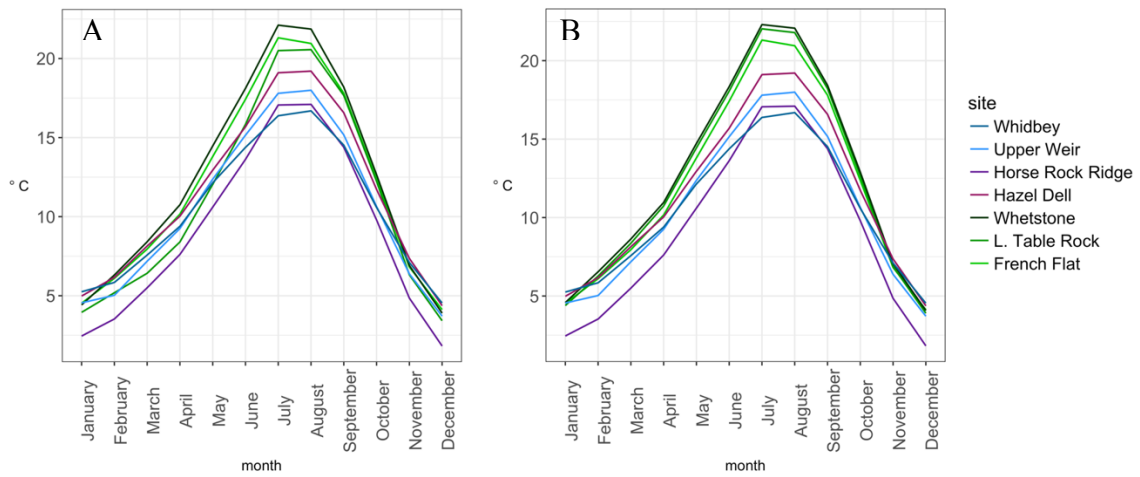


Figure 4: Mean monthly temperature among sites where A) *F. roemerii* and B) *D. californica* are present. Data are based upon 30-year normals (1980-2010).

Table 1: 30-year normals in annual precipitation (MAP), maximum (Max), mean (Mean), minimum (Min), and dewpoint (Dewpoint) temperature.

Site	Range Group	Species Present	Latitude	Longitude	Elevation (m)	MAP (mm)	Max (°C)	Mean (°C)	Min (°C)	Dewpoint (°C)
French Flat	SOR	Both	42.1	-123.63	448	1599.87	19.9	11.94	3.95	4.25
Roxy Ann peak	SOR	<i>F. roemerii</i>	42.35	-122.79	949	781.73	16.8	11.04	5.27	3.21
Whetstone Preserve	SOR	<i>D. californica</i>	42.42	-122.91	382	511.32	20	12.55	5.11	5.35
Upper Table Rock	SOR	<i>F. roemerii</i>	42.47	-122.88	418	575.23	19.9	12.34	4.74	4.88
Lower Table Rock	SOR	<i>D. californica</i>	42.47	-122.95	427	558.49	19.9	12.31	4.76	4.81
Hazel Dell 3b	COR	<i>D. californica</i>	44.02	-123.22	168	1119.22	17.3	11.36	5.4	6.65
Hazel Dell 3a	COR	<i>F. roemerii</i>	44.03	-123.22	169	1124.22	17.3	11.36	5.4	6.64
Horse Rock Ridge	COR	Both	44.3	-122.88	627	2159.97	14.6	9.03	3.49	4.39
Upper Weir Prairie	WA	Both	46.91	-122.71	162	1185.22	15.5	10.44	5.34	5.94
Smith Prairie	WA	Both	48.21	-122.62	64	540.75	14.3	10.37	6.43	6.28



Table 2: soil descriptions for sites where *D. californica* and *F. roemerii* are present

Site	Roxy Ann Peak	French Flat	Whetstone Preserve	Lower Table Rock	Upper Table Rock	Hazel Dell 3b	Hazel Dell 3a	Horse Rock Ridge	Upper Weir Prairie	Smith Prairie
Soil texture	clay loam	sandy clay loam	sandy loam	sandy clay loam	clay loam	clay	clay	sandy loam	sandy loam	loamy fine sand
Percent Sand	33.1	51.9	61.6	46.5	28.9	33.9	29.5	74	69.3	84.4
Percent Clay	35.4	26	19.2	33.9	38.2	49.9	42.6	6.5	6.7	5.2
Percent Silt	31.5	22.1	19.2	19.6	32.9	16.2	27.9	19.5	24	10.4
soil bulk density g/cm <sup>3</sup>	1.26	1.46	1.23	1.23	0.95	0.96	0.99	0.62	0.62	1.02
Soil Depth cm	87	47.33	42.66	11	14	70.33	66.33	17.5	23.33	35.6
Soil pH	6.93	7.53	6.34	6.95	7.31	6.15	6.52	5.92	5.2	6.01
Percent Nitrogen	0.2	0.15	0.12	0.19	0.2	0.3	0.3	0.5	1.06	0.9
Percent Carbon	3.05	2.25	1.62	3.01	2.5	3.68	3.73	6.91	14.84	11.9
NO3 mg/cm <sup>2</sup>	14.02	32.23	36.28	34.08	26.18	21.98	17.58	5.71	16.68	109.52
NH4 mg/cm <sup>2</sup>	6.81	1.44	5.34	2.08	2.62	3.4	4.2	9.24	10.2	4.6

### *Demographic Data Collection*

Our sampling design involved the use of transects to define each plant population. Transects were 50 cm wide, spanning 15-30 m in length, depending upon plant density. Each transect included ~ 200 individuals per target species, with variation in the number of individuals from each size class, from seedling to mature adult to encompass the full life-history variation on a per site basis. Each plant was marked with a colored permanent marker (usually a painted nail) and mapped, creating the possibility to return to specific individuals. For each individual within a population we measured plant size, fecundity, and density. Reproductive fitness was measured by estimating the number of seeds produced by each plant. For *F. roemerii* we counted the number of flowering stems and measured the length of floret producing spikelets. On a subset of stems per site we counted the number of spikelets per axis length, and used this to create a regression for seed number per unit length of axis. *D. californica* was more complicated because it produces two kind of flowers: chasmogamous flowers, which are out in the open and potentially outcrossed, and cleistogamous flowers that always self-pollinate and remain hidden in the leaf sheaths (Clay 1983). We addressed this issue by counting the number of chasmogamous spikelets, and estimated the number of cleistogamous spikelets by destructively sampling ten nearby plants at each site and counting their cleistogamous seeds. The number of cleistogamous seeds were correlated to the number of flag leaves on flowering stems, and so we counted these to help estimate reproduction. Size was estimated by calculating basal area (length X width) of grass tussocks. We used target species' density (plants/m<sup>2</sup>, measured at the site level) to calculate an estimate of intra-specific competition. To assess damage by herbivores and pathogens, we scored each individual for the presence/absence of damage. Pathogen damage was defined as spots and infection structures. Herbivore damage included invertebrate damage (including galls, seed herbivory, leaf chewing, leaf mining, leaf sucking) and damage by large herbivores (grazing).

### *Endophyte Sample Collection*

Fresh plant material was collected from 12 randomly sampled adult (flowering) plants from each population. We preferentially sampled for larger, reproductive individuals in an attempt to control for plant age, which may confound endophyte load. (Clay and Schardl 2002), and therefore older plants would be more likely to host complex communities. At the time of collection, measurements of size and reproduction were conducted for each individual host, as well as location along the transect. We collected five asymptomatic leaves from each individual using dissecting scissors. The scissors were wiped clean with sterile water after each sample was taken, to reduce cross contamination among samples. Each sample was wrapped in paper towel dampened with sterilized deionized water and sealed within clean plastic Ziploc bags. We stored samples on ice while in the field, and placed them in refrigeration as soon as possible (within two days of collection). The samples were stored in refrigeration (4 °C) for up to two days post field work.

### *DNA Extraction and High-Throughput Sequencing*

Upon returning to the lab, samples were surface sterilized and then frozen within three days of collection to minimize any community changes that could have taken place after collections are taken. The surface sterilization methodology was: immersion in 75% ethanol for 1 min, transfer to a 1% sodium hypochlorite solution for 2 min, and 75% ethanol for an additional minute. The leaves were then rinsed in sterilized DI water, and blotted dry. Using a flame-sterilized scalpel and tweezers, leaves were cut into small sections, and 0.5 g of leaf tissue was transferred into sterilized 2ml flat-bottomed tubes containing approximately 0.3g of sterile zirconium beads. After being labeled, each sample was frozen at -20 °C until DNA extraction.

To extract DNA, we followed a modified protocol from the Qiagen DNeasy plant kit (Qiagen, Hilden, Germany). Before going through the extraction protocol, we subjected each sample to two flash freeze/thaw cycles to help breakdown plant tissue (Roy et al. 1998). After adding the cellular lysis buffer, each sample was homogenized for two cycles of 30 seconds, using a Biospec Mini Beadbeater-8 (Biospec Products,

Bartlesville, OK, USA). From this point, the protocol Qiagen was followed to completion. DNA was stored at -20 C until further processing.

To screen the samples for the presence of fungal DNA, the DNA was amplified by PCR using ITS1 (5' TCC GTA GGT GAA CCT TGC GG 3') and ITS2 (5' GCTGCGTTCTTCATCGATGC 3') primers (White et al. 1990). The internal transcribed region includes the ITS1, 5.8, and ITS2 (Vancov & Keen 2009). The two ITS regions are hypervariable, non-coding strands of DNA, while the 5.8S gene is highly conserved, making the region ideal for taxonomic identification within the fungal kingdom (Schoch et al. 2012). PCR was carried out in 10 µl reactions. The reagents included 2 µl genomic DNA, 0.4 µl MgCl<sub>2</sub> (25 mM), 0.3 µl of each 10mM primer, 2 µl Milli-Q Ultrapure Water (MilliporeSigma, Darmstadt, Germany), and 5 µl 2x PCR Super Master Mix (100 U/ml of Taq DNA polymerase, 0.5mM dNTPs, 4mM MgCl<sub>2</sub>, stabilizers and dye) (Bimake, Houston, Texas, USA). Reactions were assembled on ice to reduce non-specific amplification and primer dimerization. PCR conditions include an initial five min of denaturation at 95 °C; 35 cycles of: 1 min at 95 °C, 1 min at 55 °C, 1 min at 72 °C; final elongation of 10 min at 72 °C. PCR products were visualized using gel electrophoresis, and successful samples were again stored at -20 °C for Illumina processing. Along with our samples, we also prepared positive and negative controls. These were included to examine, quantify, and address biases associated with PCR, Illumina sequencing, and the bioinformatics pipeline we use to create our OTU table.

Once all sample were screened for the presence of fungal DNA, we were able to begin Illumina prep. We chose to use the ITS1 region as the target sequence for several reasons. The entire ITS region has a range of roughly 450-800 base pairs (bp), and while most current high-throughput sequencing technologies are unable to provide full coverage over this range, ITS1 has been widely used for taxonomic assignment (Blaalid et al. 2013). In contrast to the complete ITS region, the average length of the ITS1 region is approximately 350 bp (Vancov and Keen 2009), and still maintains regions of conserved and highly variable DNA. Illumina next generation sequencing (NGS) relies upon the inclusion of unique tags to differentiate samples from one another. For paired end sequencing, these are unique combinations of 8 bp tags ligated to both forward and reverse primers. Using Illumina-tagged ITS1 and ITS2 primers (Integrated DNA

Technologies, Coralville, IA, USA), we performed three PCR replicate reactions on each sample, although there is evidence that PCR replicates are unnecessary (Smith and Peay 2014). DNA replicates were pooled, and products were cleaned and purified using Mag-Bind RxnPure Plus beads to remove primers and other impurities before sequencing (Omega bio-tech, Norcross, GA, USA). The University of Oregon Genomics and Cell Characterization Core Facility (GCCCF) completed the remaining Illumina prep protocols necessary before sequencing, including sample pooling, fragment analysis, and size selection. Fragment analysis (Advanced Analytical, Ankeny, Iowa, USA) was used to quantify the quality of DNA samples. Size selection (Blue Pippin, Sage Science, Beverly, MA, USA) was used to exclude DNA fragments less than 200 bp and greater than 1200 bp. The final concentration of DNA was 5.213 nM. Pooled DNA was sequenced using the Miseq Standard v.3 2x300bp sequencing platform at the Oregon State University Core facility. This sequencing platform is capable of a depth of >25 million reads.

### *Bioinformatics*

Throughout the bioinformatics process, we used the Usearch pipeline as much as possible. This pipeline is applicable to our data for many reasons, including the fact that it makes no assumptions about the size of marker genes (Edgar 2013). This is preferable for work using the ITS region, as it is quite variable in length (Porrás-alfaro et al. 2013). The general workflow included several quality filtering steps, removal of singleton sequences, clustering to a specified cutoff (with included quality steps), using UTX to assign taxonomy to OTUs (Operational Taxonomic Units), and the creation of a BIOM format table, which includes species data, sample data, and site metadata.

### *Initial Quality Filtering*

We strove to attain high sequence quality through several steps. These include the removal of low-quality reads, removal of probable chimeras, and clustering at thresholds higher than the expected sequencing error frequency. For an initial quality optimization step, we chose to trim low quality reads from our fastq files using the fastx trimmer. After visually assessing our reads, we chose to use an average phred quality

score of 34 as our cutoff. Forward reads were trimmed to 250 bp, while reverse reads were trimmed to 220 bp. This should give adequate sequence overlap to appropriately meet the assumptions of the Usearch software (Edgar 2010). We assembled paired end reads using the Usearch algorithm (-fastq\_mergepairs). This merging algorithm is useful in that it uses a Bayesian approach to calculating the q-scores of paired reads. This means that agreements on base calls between forward and reverse sequences improve Q scores, while disagreements reduce them (Edgar and Flyvbjerg 2015). Further quality filtering involved expected error filtering (fastq\_filter). This algorithm calculates expected error for each sequence, and uses that information to further filter based upon read quality (Edgar and Flyvbjerg 2015).

#### *Removal of Primer Artifacts*

Sequences may contain multiple primer occurrences, where primer sequences are inserted, sometimes multiple times, within the ITS target sequence (Bálint et al. 2014). In total, 312 sequences (or 0.003% of total sequences) were removed.

#### *Chimera Checking*

Chimeric sequences are non-biological sequences that often arise from PCR amplification errors. They are created when two or more parent sequences give rise to a new, yet non-original sequence. Left alone, they can be misinterpreted as novel OTUs, and must therefore be removed. We used the uchime algorithm to find and remove chimeric sequences (Edgar et al. 2011).

#### *Extraction of Fungal ITS1*

It is recommended that only the highly variable regions be included in OTU clustering (Bálint et al. 2014). Despite ITS1 being the target sequence for our study, our primers extended past this region, into the SSU (small ribosomal subunit) and 5.8S gene regions, and therefore needed to be removed. Using the ITSx extractor (<http://microbiology.se/software/itsx/>), we identified and removed fragments from the SSU and 5.8S regions.

### *OTU Clustering*

OTU clustering was based upon uparse pipeline (Edgar 2013) from the usearch V8.1. First, we de-replicated the sequences, in effect keeping each unique sequence and data for their abundances (-derep\_fulllength). We sorted sequences based upon size, or number of sequences (-derep\_fulllength), and removed singleton sequences (sequences with only one representative). For OTU clustering, we chose a 97% similarity radius. The algorithm considers high abundance sequences as more biologically correct, and thus examines sequences in order of decreasing abundance. Each input sequence is compared to existing OTU bank, and is be added to existing OTU, discarded, or given new OTU status whether they are > 97% similar, chimeric, or  $\leq$  97% similar, respectively (Edgar 2013).

### *Taxonomy Assignment*

Taxonomy was assigned using the UNITE (User-friendly Nordic ITS Ectomycorrhiza) Database (Abarenkov et al. 2010). Before assigning taxonomy, we curated the most recent UNITE database ([https://unite.ut.ee/sh\\_files/utax\\_reference\\_dataset\\_22.08.2016.zip](https://unite.ut.ee/sh_files/utax_reference_dataset_22.08.2016.zip)) to include only accessions that contain taxonomic resolution at least up to the class level. We did this so that any sequence will at least blast to a higher taxonomic ranking than phylum. To assign taxonomy to our OTU database we used the -utax command, using our curated UNITE database as a reference. Once taxonomy was assigned to our reads, we used the -usearch\_global command to create a json, human readable biom table. Using the biom command add-metadata, we appended sample and site metadata to the biom table. This table was imported into R statistical software for use in all following statistical analyses.

### *Statistical Analyses*

#### *Biom Trimming and Optimization*

All manipulation of biom tables was carried out using the R package *phyloseq* (McMurdie and Holmes 2013). Due to the greater sensitivity of NGS, the inclusion of both positive and negative controls are important in removing potential contaminants (Nguyen et al. 2015). There is a fine line between removing ecologically meaningful

observations and allowing contaminants into downstream analyses. We subtracted the number of sequences from each OTU in our negative control from each sample (Nguyen et al. 2015). To deal with sequence biases, we chose to use methodology adapted from RNA-Seq analysis. The common practice of rarefying presents the problem of discarding a large portion of data, and thus we chose to use variance stabilization methodology from the R package *DESeq* (Love et al. 2014). This technique provides the means to compare microbial samples with widely different read abundances, while retaining the majority of data (McMurdie and Holmes 2014). Since sequence abundance has been shown not to necessarily reflect actual cellular abundance (Nguyen et al. 2015), we chose to perform all analyses on incidence based (i.e., presence/absence) microbial data.

### *Fungal Diversity*

All diversity metrics were calculated using Hellinger transformed community matrices to reduce the weight of rare OTUs (Legendre and Gallagher 2001). We examined microbial diversity at the sample, site, and regional species pool among host species. As a preliminary assessment, we were interested in the taxonomic overlap among host species. We looked at the number of fungal OTUs at both the class and phylum level. To calculate the regional species pool (gamma diversity), we used the function *specpool*, which calculates observed gamma diversity, as well as extrapolated values for Chao and Jackknife1 indices. Since we are using presence/absence data in all our analyses, we used species richness as a measure of alpha diversity. We calculated the number of OTUs present and compared sites among host using one-way ANOVA and Tukey's HSD post hoc tests to compare groups.

### *Fungal Community Structure*

We used permutational multivariate analysis of variance (PERMANOVA) to investigate how fungal communities compared among host, ecoregion, and site. We performed analyses on site and ecoregion for both total communities as well as separated by host plant species. To visualize differences in fungal community composition we used non-metric multidimensional scaling (NMDS). We used the function *metaMDS()* to examine the composition and relationships among fungal communities. We used Jaccard



distance index because it is more appropriate for use with presence/absence data (Oksanen et al. 2017).

### *Predictors of Fungal Communities*

A difficult problem in many ecological systems is how to deal with the complex interactions of environmental variables and spatial distance (Koenig 1999). Further, when using direct gradient analyses such as multivariate redundancy analysis (RDA), the use of Euclidian distance is inappropriate (Legendre and Gallagher 2001). For these reasons, we chose to model spatial distance using principal coordinates of neighbor matrices (PCNM). This technique allows for the creation of orthogonal spatial variables, which can be used directly in hypothesis testing (Dray et al. 2006) It does this by using principal coordinate analysis (PCoA) of a truncated distance matrix amongst points. The resulting PCNM axes with positive eigenvalues can be used as spatial components. Our analysis involved the use of source code from the R package *PCNM* (available from [www.r-forge.r-project.org/R/?group\\_id=195](http://www.r-forge.r-project.org/R/?group_id=195)).

We used variation partitioning to assess the relative contribution of host traits, climate, edaphic factors, and spatial distance on fungal community composition. Variation partitioning has been developed as a technique aimed at establishing relationships among community data and environmental predictors, and has the advantage that up to four explanatory matrices can be tested against a response matrix. It does this through the use of direct gradient analysis (in this case RDA) to test the partial, linear effect of each explanatory matrix on the response data (Borcard et al. 1992). Despite the robust nature of this technique, the results are still sensitive to multicollinearity, which can lead to distorted results. Multicollinear variables have the negative effect of inflating  $R^2$  values, clouding interpretation of analyses (Buttigieg & Ramette 2014). Because of this, we needed to reduce the multicollinearity among explanatory variables. We chose to deal with this problem by running principal components analysis (PCA) on our environmental variables (climate and edaphic factors). We selected the first few two axes, which explained the majority of variance, and used them as variables in our variation partitioning model. We created matrices for each major filter category (climate and edaphic, host traits, spatial distance), and ran them

against our community matrix. We used the function *varpar()* to perform the analysis. This function gives an output listing adjusted  $R^2$  values for all combinations of explanatory matrices, including host traits (a), spatial distance (b) and abiotic (c), as well as shared partitioning for host and spatial (a & b), environment and spatial (b & c), etc. To test our reduced model, we also ran PCA on each matrix, and used axis scores within the variation partitioning model. We used RDA to examine each individual fraction, and used Monte Carlo global permutation tests of significance of canonical axes (implemented with the function *anova.cca()*). We plotted the results to visualize the contribution of each fraction on endophyte community composition. As a visual representation of the effect that all environmental variables had on community composition, we created and overlaid environmental vectors on our NMDS figures. These figures are for visual interpretation only, as they are fit on unconstrained ordinations.

All statistical analyses were performed in R 3.3.1 (R Core Team 2016) using the *vegan* package (Oksanen et al. 2017).

## **Results**

### *Bioinformatics*

A total of 11,033,436 raw sequences were returned from the Illumina MiSeq sequencing effort. After quality filtering, removal of primer artifacts, and chimera filtering, a total of 9,432,696 sequences remained, 1,182,213 of which were unique. Following our clustering steps, we were left with 3922 OTUs. The final OTU count after variance stabilization and removal of negative controls was 3090.

### *Fungal Diversity and Composition*

The total fungal community was comprised of seven phyla, 27 classes, 103 orders, 220 families, and 599 genera. When subsetted by host species, *D. californica* was host to 1884 OTUs. Of these, the two most prevalent phyla were Ascomycota (75% of OTUs), and Basidiomycota (23% of OTUs) (Figure 5). Meanwhile, *F. roemerii* was host to 2235 OTUs. Again, the most common phyla were Ascomycota (73% of OTUs), and Basidiomycota (25% of OTUs) (Figure 6). For both host species, the five most common

classes were Agaricomycetes (*F. roemerii* = 9%, *D. californica* = 11%), Dothideomycetes (*F. roemerii* = 35%, *D. californica* = 37%), Eurotiomycetes (*F. roemerii* = 8%, *D. californica* = 7%), Sordariomycetes (*F. roemerii* = 18%, *D. californica* = 15%), and Tremellomycetes (*F. roemerii* = 8%, *D. californica* = 9%) (Figure 6). Fungal orders among host are shown in Figure 7.

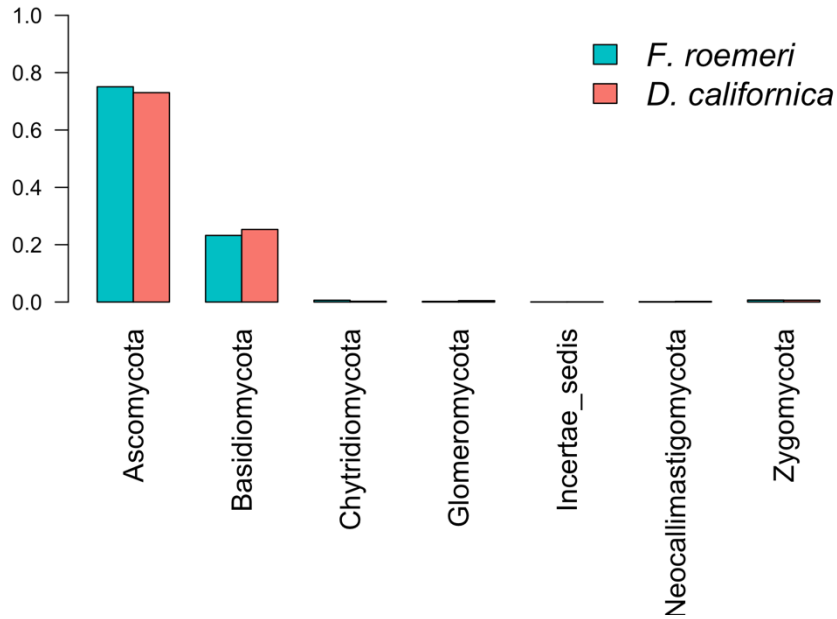


Figure 5: Major phyla recovered from both host species.

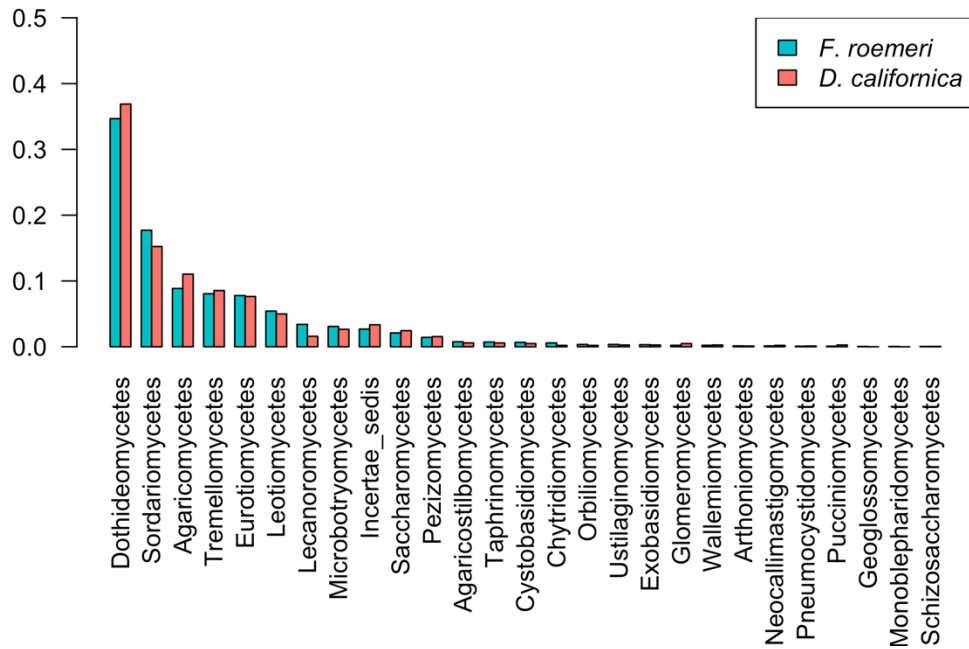


Figure 7: Major classes recovered from both host species.

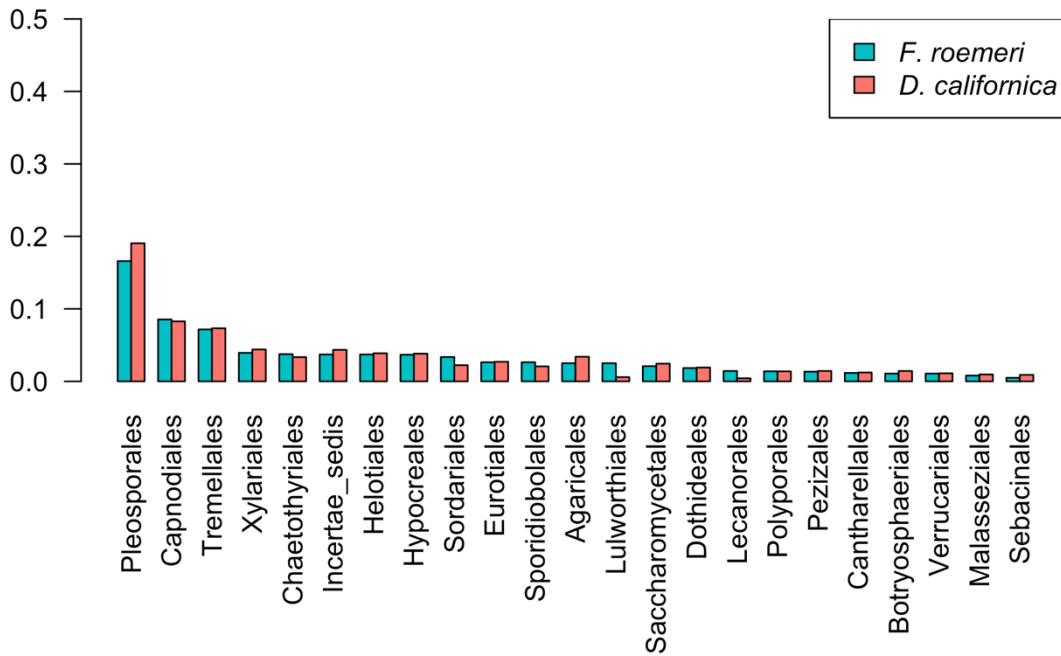


Figure 6: Major orders recovered from both host species.

Examination of a species accumulation curves suggests that we did not completely describe the regional species pool for either host species (Figure 8). Chao and Jackknife species richness estimators both predicted higher diversity than was observed for each host, although they differed in their estimation of sequencing depth. Despite predicting almost twice the diversity for *F. roemerii* (Chao = 3021 +/- 72 SE; Jackknife1 = 3042 +/-101 SE), the predicted abundance curves suggest a relatively adequate sequencing depth (supplemental Figure 1). On the other hand, *D. californica* was predicted to house an even greater diversity of endophytes (Chao = 3598 +/- 160 SE, Jackknife1 = 2812 +/- 131 SE). Further, the accumulation curves suggest that we did not come close to reaching the regional endophyte diversity (supplemental Figure 2).

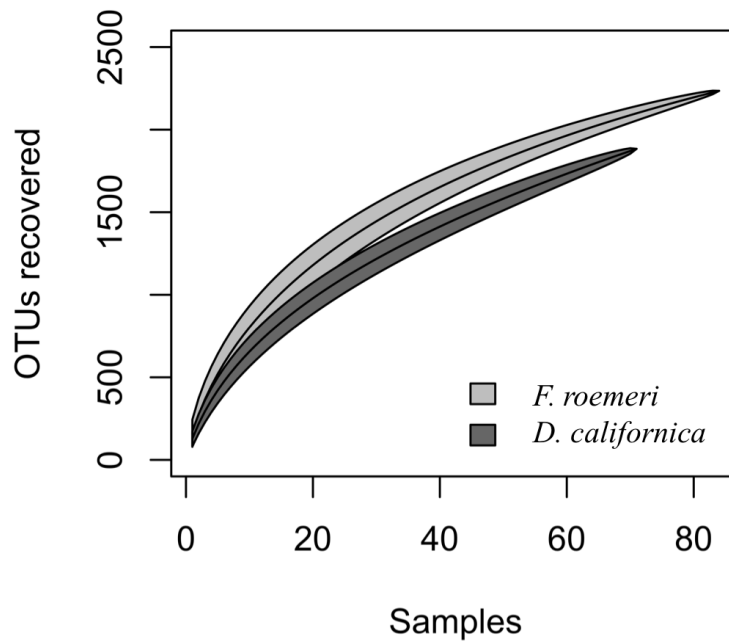


Figure 8: Species accumulation curve for host species with increasing sample size. Shaded region represents SE.

Alpha diversity also differed among sites and species. Communities within *F. roemerii* had very different alpha diversity among sites ( $F_{6,77} = 2.94$ ,  $p = 0.012$ , Figure 9A). Interestingly, our southern-most site showed the lowest species richness (French Flat  $204.4 \pm 15.9$  SE), while the two sites with the highest richness were from separate ecoregions (Upper Table Rock, Klamath Mountains,  $288.7 \pm 27.5$  se; Upper Weir, Puget

Trough,  $281.2 \pm .17.8$  se). In contrast, communities isolated from *D. californica* showed no difference in richness ( $F_{5,65} = 21.84$ ,  $p = 0.12$ , Figure 9B), although there was a trend of increased richness with the two northern sites (Horse Rock Ridge, Willamette Valley,  $224.8 \pm 10.6$  se; Whidbey, Puget Trough,  $228.5 \pm 10.9$  SE). Since French Flat was our only serpentine site, we also performed these analyses excluding this site. Any differences in alpha diversity we saw for *F. roemerii* were no longer present ( $F_{5,66} = 1.74$ ,  $p = 0.14$ ), while communities among *D. californica* still show no differences, although they become marginally more significant ( $F_{5,54} = 2.14$ ,  $p = 0.09$ ).

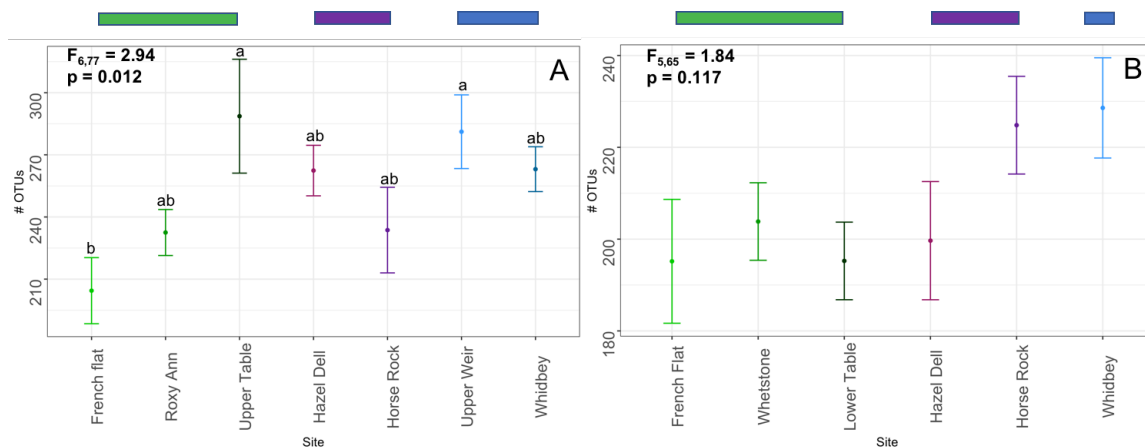


Figure 9: OTU richness for A) *F. roemerii* and B) *D. californica* among sites. Lettering represents differences among site richness (Tukey’s HSD). Error bars represent SE. Colors represent ecoregions: Klamath Mountains (green), Willamette Valley (purple), and Puget Trough (blue).

### Fungal Community Structure

There was strong separation in fungal communities between hosts along the second NMDS axis ( $F_{1,153} = 5.97$ ,  $p < 0.001$ ,  $R^2 = 0.04$ , Figure 10). Interestingly, when both hosts were present at the same site, in most cases their communities had no overlap (Figure 11). When examining fungal communities among ecoregions, there was strong separation along the first axis ( $F_{3,153} = 5.08$ ,  $p < 0.001$ ,  $R^2 = 0.06$ ). The southern sites formed a distinct cluster, while the central and northern sites clustered together (Figure 12). Communities isolated from *F. roemerii* showed strong clustering by site, suggesting

different community composition among site ( $F_{5,70} = 3.54$ ,  $p < 0.001$ ,  $R^2 = 0.22$ , stress = 0.20). While there was clear separation between the southern and central/northern sites, there does not seem to be any additional clustering by latitude (Figure 13). Communities from French Flat appear to be distinct, while communities from Roxy Ann and Upper Table Rock appear to be more similar. Likewise, there is great overlap among Horse Rock Ridge and Upper Weir communities and among Hazel Dell and Whidbey communities, respectively. Similarly, communities from *D. californica* showed strong clustering by site ( $F_{5,70} = 4.8$ ,  $p < 00.1$ ,  $R^2 = 0.27$ , stress = 0.19). However, in contrast with those from *F. roemerii*, the *D. californica* communities showed stronger clustering by latitude. Communities clustered by region along the first axis, but also by latitude within those groupings along the second axis (Figure 14).

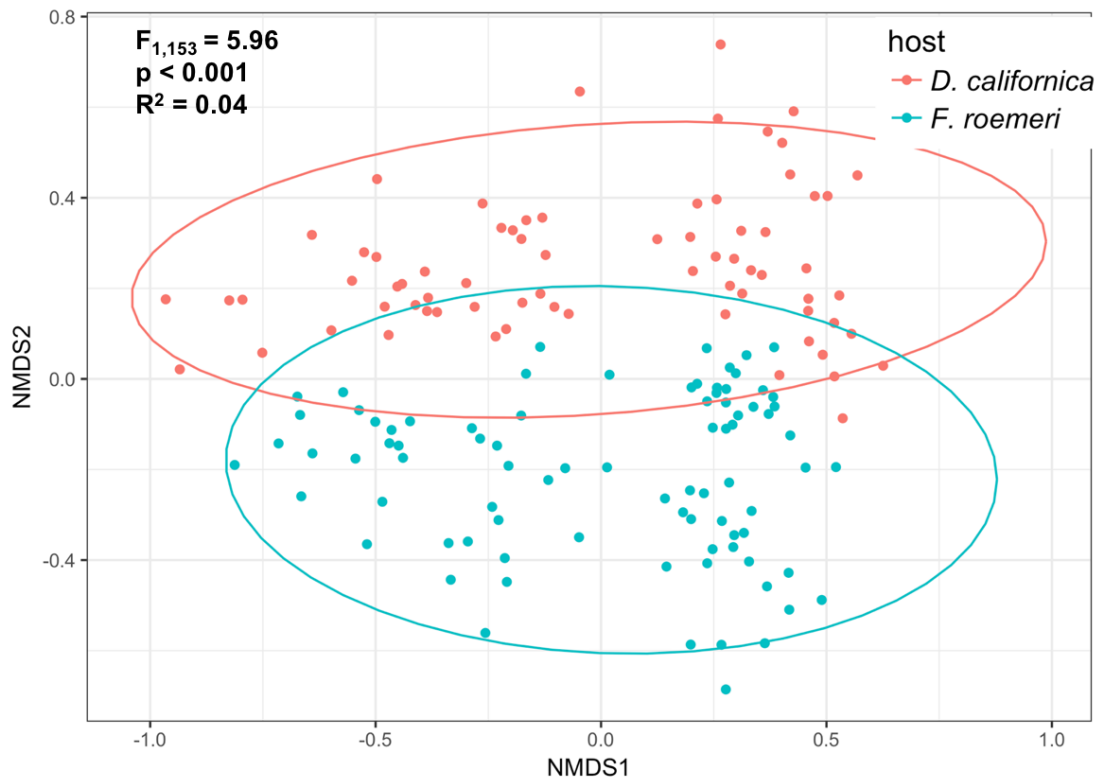


Figure 10: NMDS ordination of endophyte communities grouped by host species. Ellipses represent 95% confidence intervals.

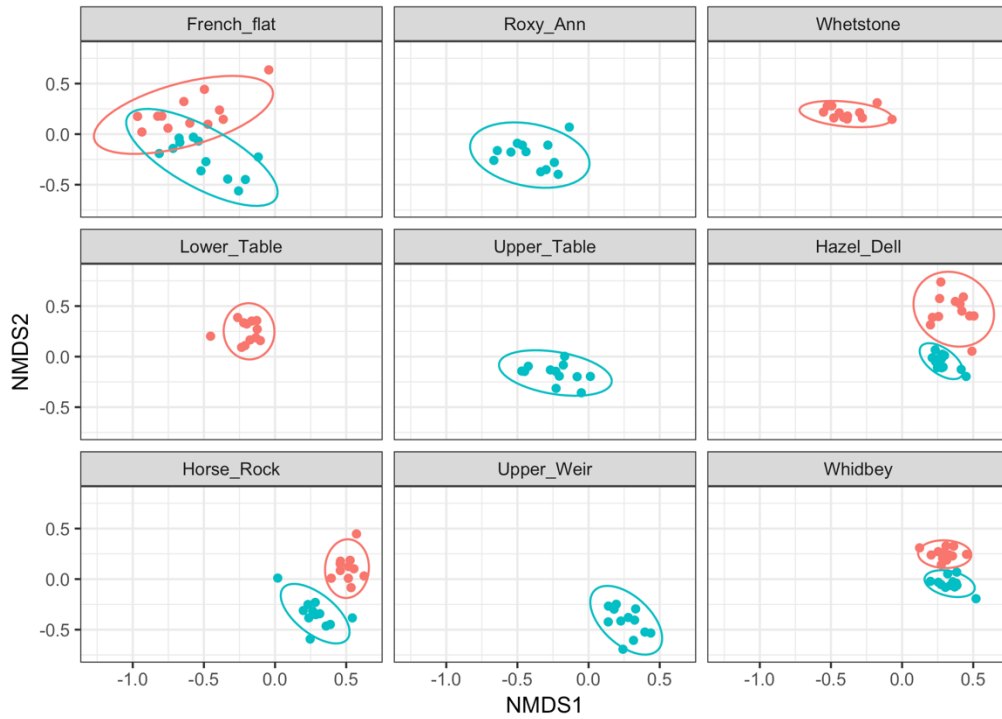


Figure 11: NMDS ordination showing separation by host. This figure is the same ordination as Figure 10, except that each site is separated to show overlap among site and host.

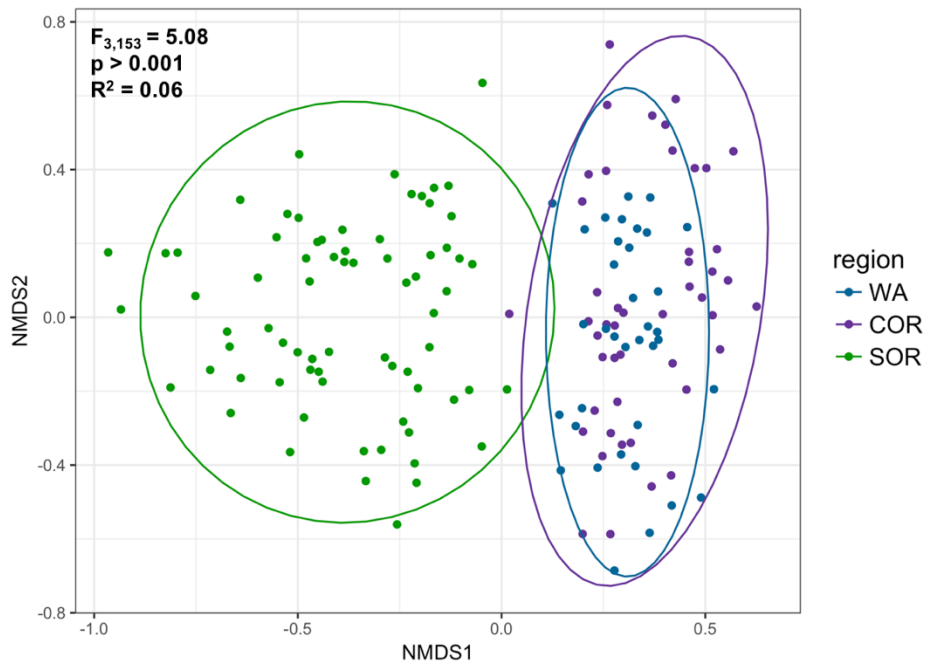


Figure 12: NMDS ordination of endophyte communities grouped by region (both hosts). Ellipses represent 95% confidence intervals.



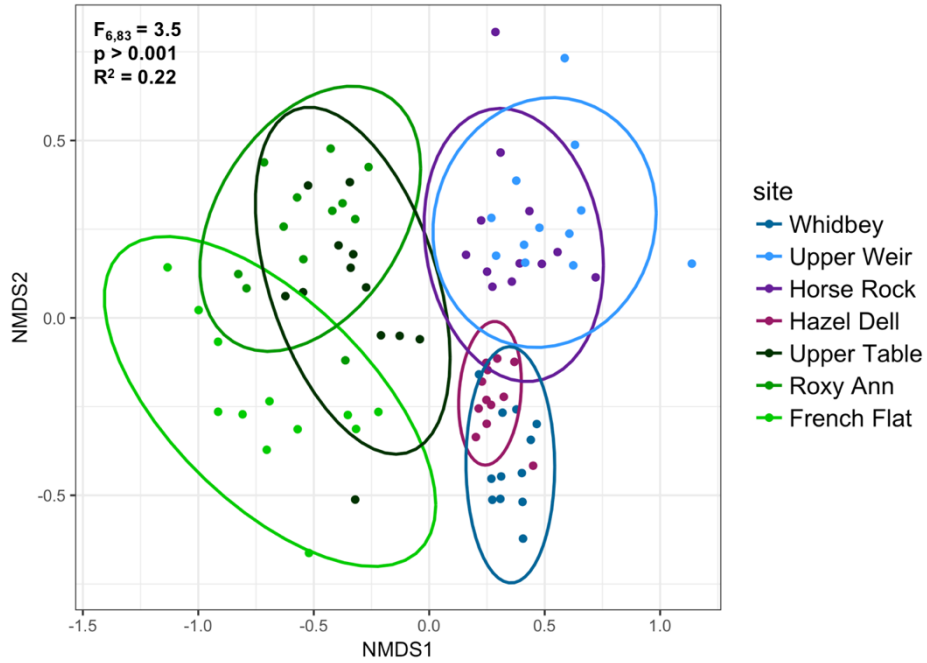


Figure 13: NMDS ordination of *F. roemeri* communities grouped by site. Ellipses represent 95% confidence intervals.

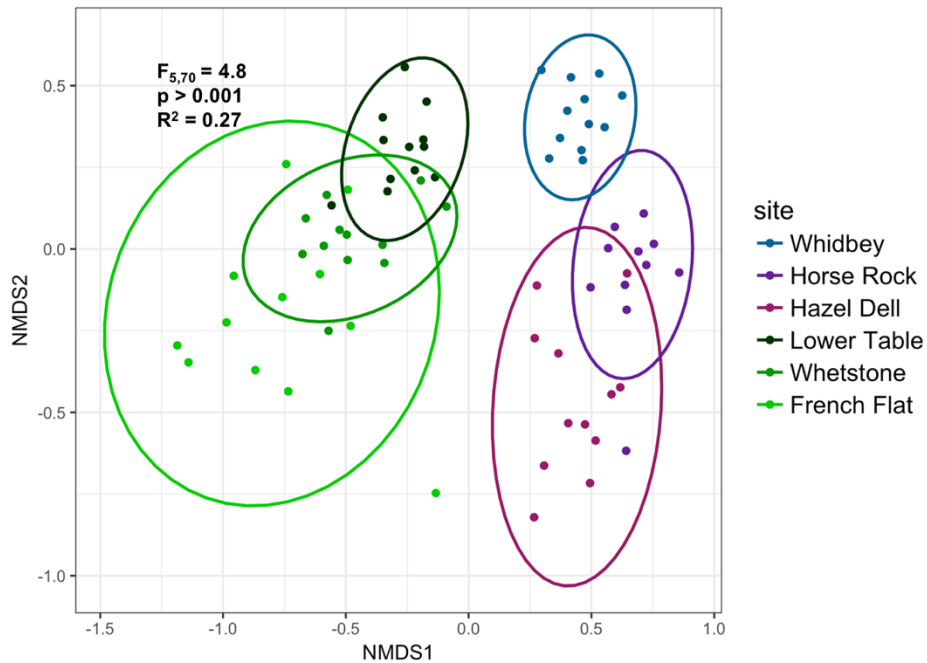


Figure 14: NMDS ordination of *D. californica* communities grouped by site. Ellipses represent 95% confidence intervals.

### Predictors of Fungal Communities

Given the spatial structure of our sampling scheme, we were only able to pick up on mid- and broad-scale spatial structures. For communities of *F. roemeri*, we found two PCNM variables that were positive, although one of them was only marginally significant (PCNM1  $F_{1,80}=5.05$ ,  $p < 0.001$ ; PCNM2  $F_{1,80} = 1.27$ ,  $p = 0.054$ , Figure 15). PCNM1 indicates a spatial structure in which greater similarity exists between the Klamath and Puget Trough regions, while the PCNM2 shows structuring which separates Klamath from the both Willamette Valley and Puget Trough (Figure 15). Similarly, for *D. californica* communities, we found three positive PCNM variables, two of which were significant (PCNM1  $F_{1,67} = 7.63$ ,  $p < 0.001$ ; PCNM2  $F_{1,67} = 3.50$ ,  $p < 0.001$ , figure 16). Again, both PCNM variables map to broad scale spatial structures. Interestingly, PCNM1 seems to reflect the same split between Klamath and Willamette Valley/Puget Trough regions. PCNM2 again suggests a return to similarity between the Klamath and Puget Trough regions (Figure 16).

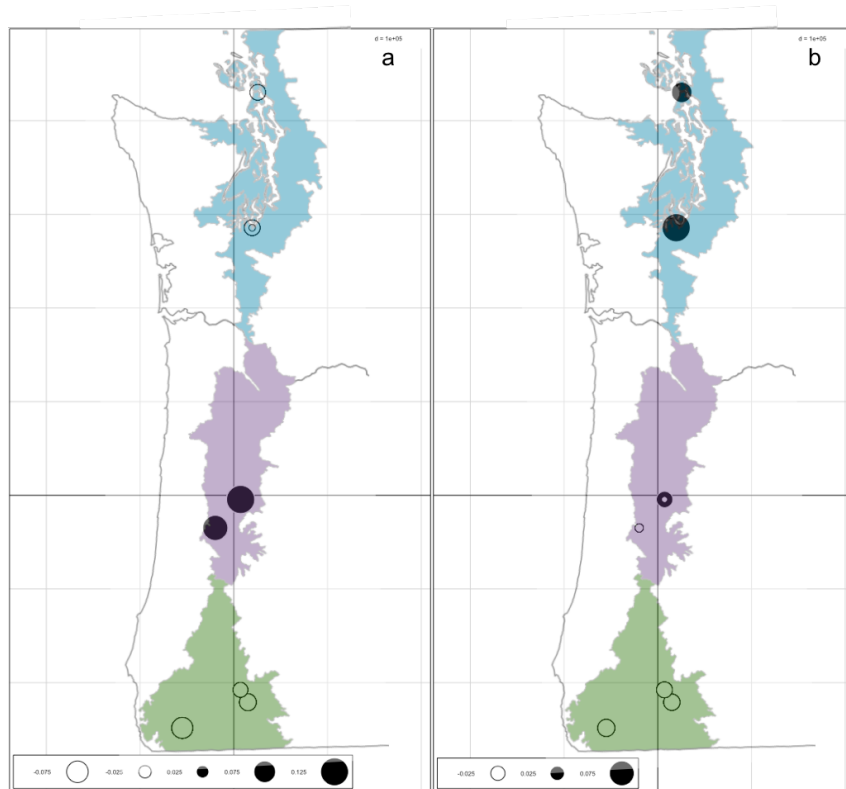


Figure 15: Graphical representation of a) PCNM1 and b) PCNM2 variables for *F. roemeri* endophyte communities. Circles of the same size and color are most similar, while circles of the same size and different color are most different.

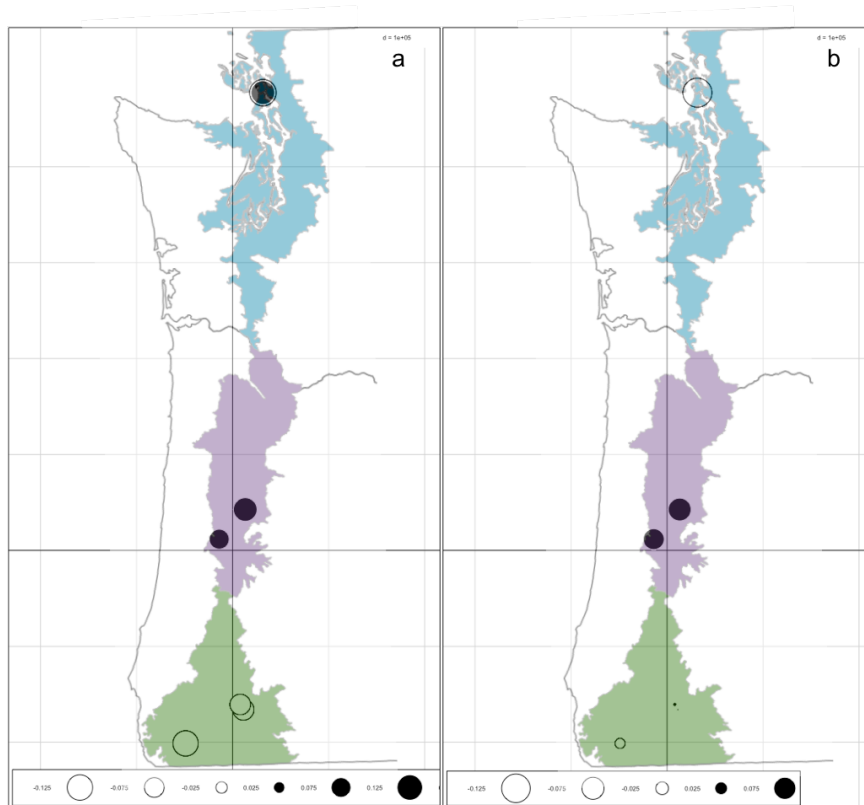


Figure 16: Graphical representation of a) PCNM1 and b) PCNM2 variables for *D. californica* endophyte communities. Circles of the same size and color are most similar, while circles of the same size and different color are most different.

Seasonal climate data were very highly multicollinear, and so we chose to build our model based upon winter data (again, from November through February), because it has been established that fungal spore formation in Mediterranean environments peaks with winter precipitation (Peay and Bruns 2014). Even with reducing the climatic variable into one season, there was a high degree of multicollinearity among environmental variables. For this reason, we ran a PCA and used the first two axes that explained the most variance as predictor variables. For communities within *F. roemerii*, 11 percent of the total community variation was explained among our explanatory matrices. The test output returns partitioned fractions for 1) each matrix alone (as if it were the only explanatory matrix provided to the test), 2) variation described by a combination of explanatory matrices (as if these combinations, i.e., environment and space were both given together), and as a result 3) variation explained only by each

individual matrix (as if the variation for all other matrices were being controlled for) (Figure 16). Spatial distance explained the largest portion of variation six percent ( $F_{3,67} = 3.16, p < 0.001$ ), while environmental traits only contributed three percent of unique variation explained ( $F_{3,67} = 2.56, p < 0.001$ ). Host traits (outside of host identity) explained five percent of unique variation ( $F_{3,67} = 1.76, p < 0.001$ ). Interestingly, four percent of variation was explained jointly by spatial distance and environment, while two percent was explained jointly by both environment and host traits. Three percent of the variation was explained by both host traits and spatial distance. The shared variation suggests that we were not able to completely disentangle environment from spatial distance, and may retain some spatial autocorrelation in the system.

For communities associated with *D. californica*, 17 percent of the total community variation was explained by our predictor matrices. In this case, each matrix explained a small portion alone (Figure 17). Environment explained two percent ( $F_{3,67} = 4.11, p < 0.001$ ), spatial distance explained six percent ( $F_{3,67} = 5.57, p < 0.001$ ), and host traits explained one percent ( $F_{3,67} = 2.10, p < 0.001$ ). Four percent of variation was explained by both host traits and spatial distance, three percent was shared by spatial distance and abiotic environment, and two percent was shared between environment and host traits (this shared variation is not testable through RDA).

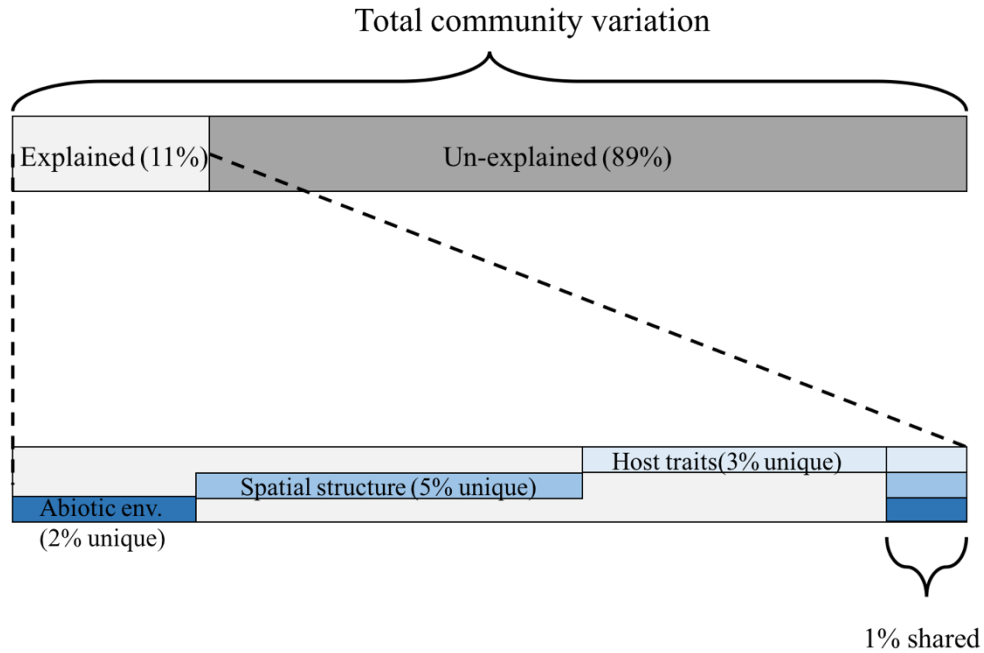


Figure 17: Variation partitioning for *F. roemeri* showing both unique and shared variation explained among filter categories.

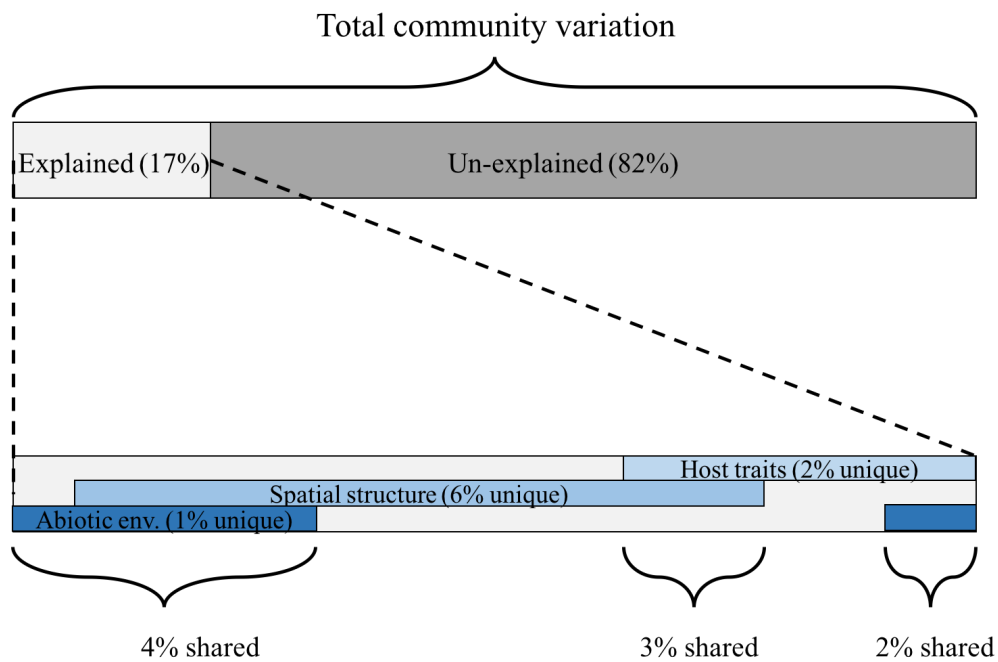


Figure 18: Variation partitioning for *D. californica* showing both unique and shared variation explained among filter categories.

Examination of each explanatory matrix individually provides a deeper understanding of how each variable within the broad filter categories may be affecting the community composition. The strongest climatic variables affecting *F. roemerii* communities were winter precipitation and winter minimum temperature, while the strongest edaphic factors were NO<sub>3</sub> and NH<sub>4</sub> availability ( $F_{3,67} = 2.56$ ,  $p < 0.001$ , Figure 19). Both spatial variables affected community composition, although the second spatial variable seems to have had a stronger effect ( $F_{3,67} = 3.16$ ,  $p < 0.001$ , Figure 20). All host traits seem to have influenced composition as well ( $F_{3,67} = 1.76$ ,  $p < 0.001$ , Figure 21). For *D. californica*, winter mean temperature had the strongest effect on community composition, while winter precipitation was weaker. The strongest edaphic factors were NO<sub>3</sub> and NH<sub>4</sub> concentrations in the soil ( $F_{3,67} = 4.11$ ,  $p < 0.001$ , Figure 22). Again, both spatial variables align quite well with the first two RDA axes ( $F_{3,67} = 5.57$ ,  $p < 0.001$ , Figure 23). Host reproductive output and host density appear to be the strongest host trait predictors of community composition ( $F_{3,67} = 2.10$ ,  $p < 0.001$ , Figure 24).

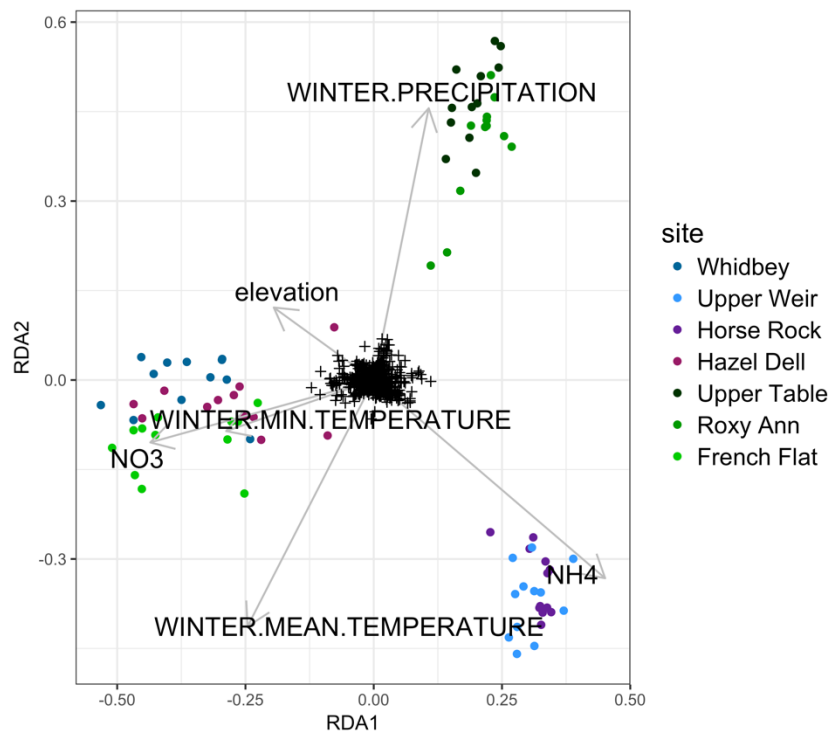


Figure 19: Tri-plot showing the effect of environmental variables on community composition of *F. roemerii* fungal endophytes.

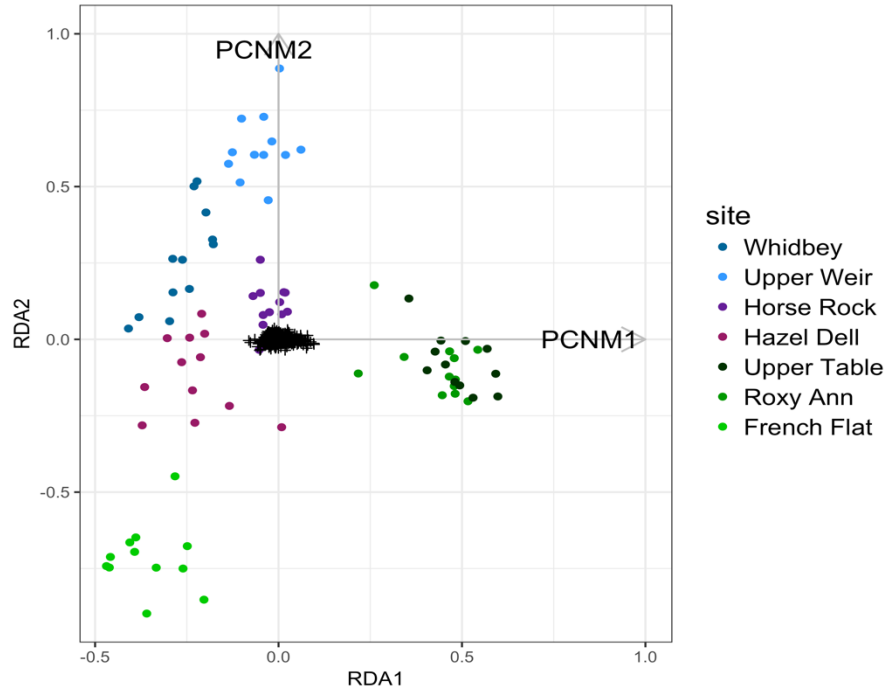


Figure 20: Tri-plot showing the effect of spatial variables on community composition of *F. roemeri* fungal endophytes.

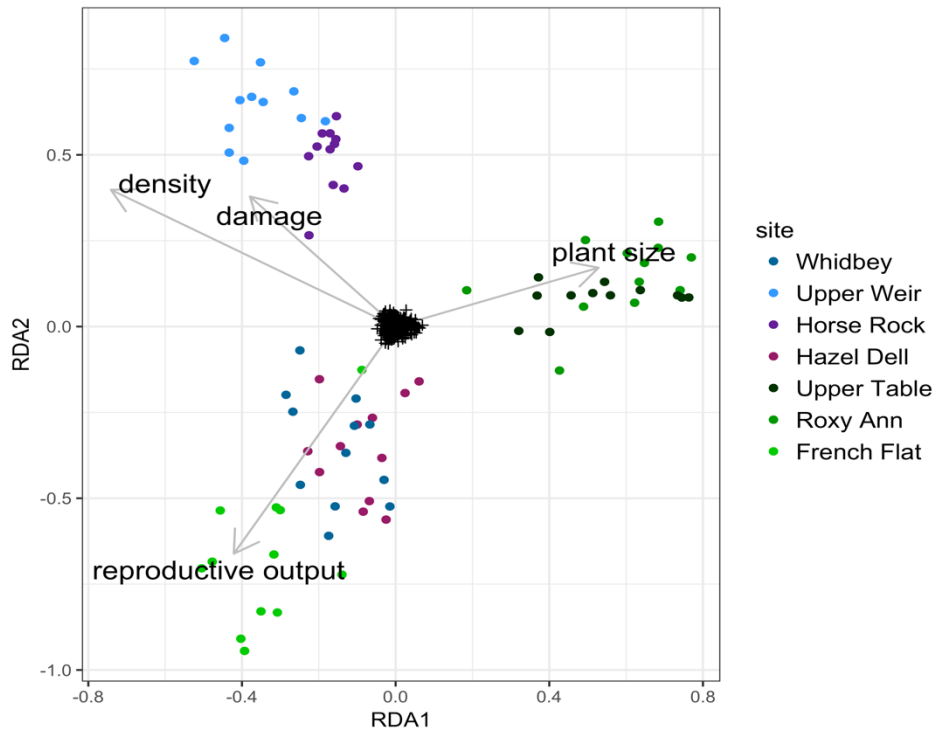


Figure 21: Tri-plot showing the effect of host variables on community composition of *F. roemeri* fungal endophytes.

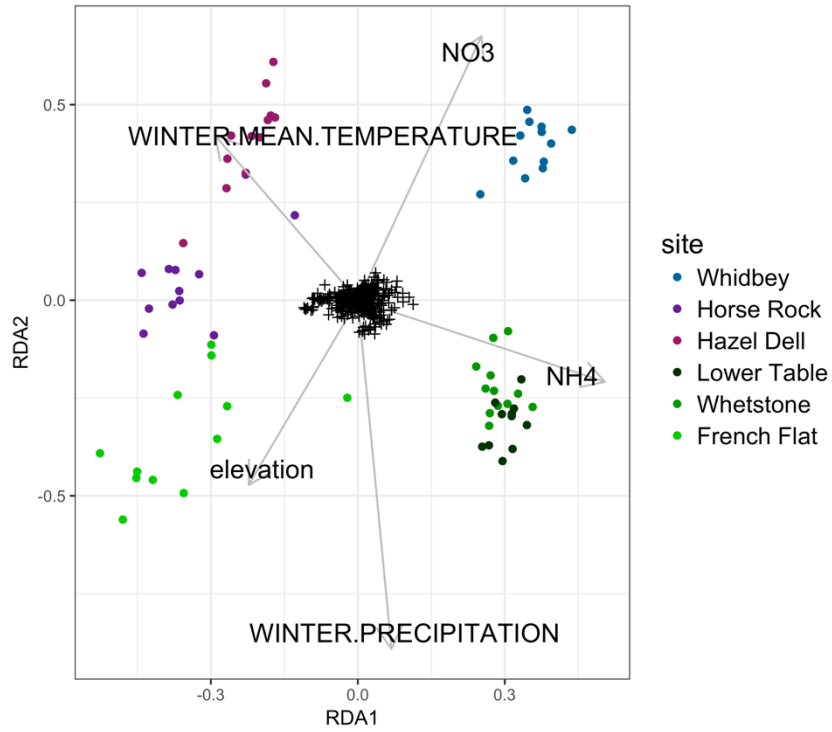


Figure 22: Tri-plot showing the effect of environmental variables on community composition of *D. californica* fungal endophytes.

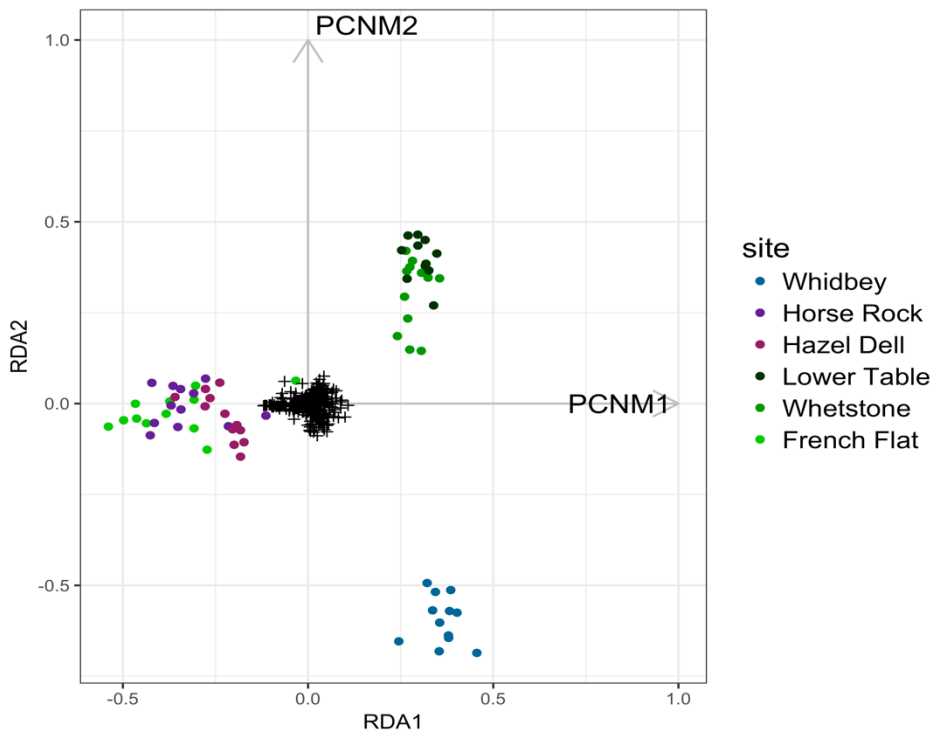


Figure 23: Tri-plot showing the effect of spatial variables on community composition of *D. californica* fungal endophytes.



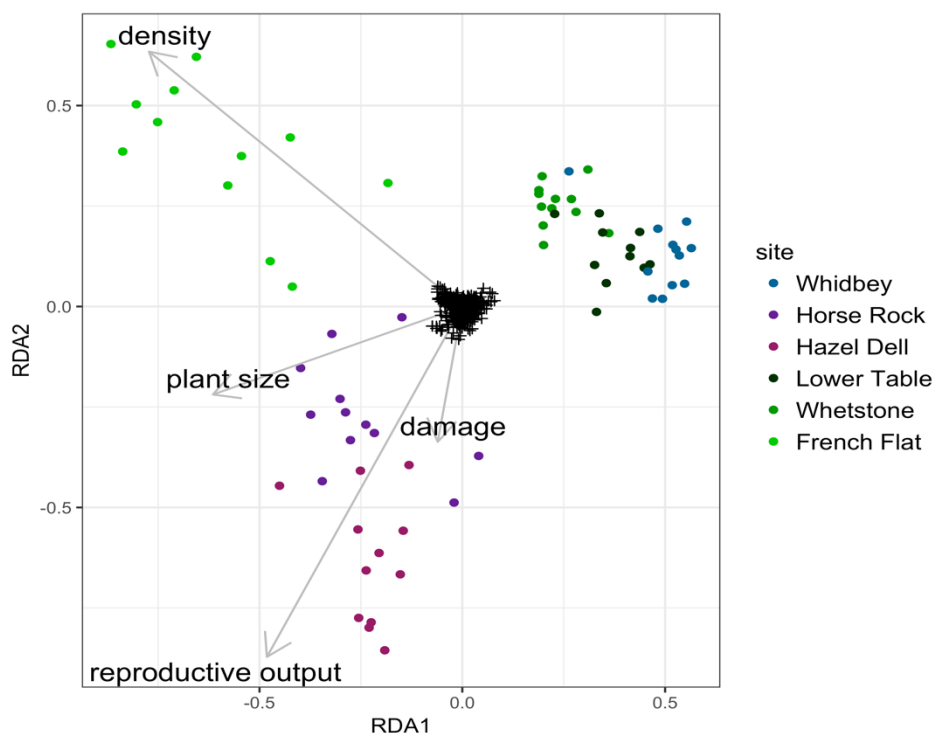


Figure 24: Tri-plot showing the effect of host variables on community composition of *D. californica* fungal endophytes.

### ***Discussion***

As we expected, the phylosphere of native Pacific Northwest bunchgrasses is a very diverse habitat. We found over three thousand distinct OTUs among two grass hosts, and this was estimated to be on the low side of the actual fungal diversity within Pacific Northwest prairies. Indeed, despite our best effort to describe the regional diversity of foliar fungal endophytes among prairie grass species, we found only about half of the estimated diversity. While we had only 12 fewer samples from *D. californica*, we found about 300 fewer OTUs than from *F. roemerii* communities. Interestingly, when we examined the higher-level taxonomy among these host species, there were few differences. Of the two most prominent phyla, Ascomycota and Basidiomycota, we only observed a 2% difference in relative proportion among host. On the level of class, Dothidiomycetes was by far the most abundant taxon, followed by Sordariomycetes, Agaricomycetes, Tremellomycetes, Eurotiomycetes, and Leiotiomycetes. These results

generally agree with other studies (Kembel and Mueller 2014). Again, although there were small differences in abundances, the two host species had remarkably similar taxonomic composition at the phylum, class, and order levels, but the actual species in each host were markedly different. An investigation on these differences will be the subject of another paper.

Interestingly, species diversity did not follow a latitudinal gradient as we expected. For communities of *F. roemerii*, we found the lowest diversity at the furthest southern site (French Flat), while the highest diversity was shared between a different southern Oregon and Washington site (Upper Table Rock and Upper Weir). These sites didn't share region, similar precipitation, temperature or edaphic patterns. Within *D. californica*, there were no significant differences in diversity among sites, although there seemed to be a trend of increased diversity with increased latitude. It should be mentioned that French Flat was our only site where the plants were growing on serpentine soil. These soils are characterized by high concentrations of Ca and Mg, as well as trace metals, which are inhospitable to most plants. As such, there are often vastly different plant communities on these soils, with higher proportions of endemic species (Whittaker 1954). Because of this, we examined diversity with and without this site. Interestingly, when French Flat was not included in the analyses, we found no difference in diversity among sites. These findings contrast with other studies which found that species diversity increases at lower latitude (Arnold and Lutzoni 2007). However, Arnold & Lutzoni (2007) found that while there was higher species diversity in the tropic, they were dominated by few classes, while the relatively small diversity of species in northern latitudes originated from more diverse classes. There are many differences between our study and that by Arnold and Lutzoni that could contribute to this difference in latitudinal diversity including the limited latitudinal scope of the study (~800 km), host species, and regional species pools. In addition, while their study was culture based, ours was culture independent, which could also affect these results. Studies with smaller scope in distance may be unable to pick up these large-scale diversity patterns (Altman 2011). In contrast, there is the idea that a symbiotic lifestyle can act to shelter the symbiont from external pressures. Since internal tissues are

shielded from desiccation (Thomas et al. 2016), they may insulate symbionts from the effects of latitude (Willig et al. 2003).

It is clear that the foliar fungal endophytes examined in this study display a non-random distribution across Pacific Northwest prairies. Our results indicate that host species, host region, and site all contributed to the composition of fungi. Host specificity is one of our clearest results, yet is contradictory to much of the literature on endophyte assembly (Sanchez Marquez et al. 2012, Higgins et al. 2014). The establishment and maintenance of host specificity is likely a product of many factors, including host traits, host phylogenetic relatedness, and host range (Kembel and Mueller 2014). Host traits encompass a wide range of attributes, including leaf physiology, leaf chemistry, and life histories. While we measured neither leaf chemistry nor quantitative host life histories, there are differences in host physiology. *F. roemerii* leaves are very narrow and recalcitrant, while those of *D. californica* are broad and relatively labile. This may affect the colonization ability and rate of fungal endophytes, and may explain why richness estimators predicted such a larger diversity of fungi within *D. californica*. In addition, our two host species are very distantly related. They both belong to the Family Poaceae, but that is where they diverge. *D. californica* belongs to the PACMAD clade (Sánchez-Ken & Clark), while *F. roemerii* belongs to the BOP clade (Clark et al., 1995)(Soreng et al. 2015). There is evidence that phylogenetic distance may be just as important in determining community composition as is environmental, spatial, or biotic interactions (Kembel and Mueller 2014). While host specificity may act to confine endophytic range, it tends to lead toward a closer co-evolutionary relationship. This has significant implications for pathogens, where this specificity may allow the fungi to escape host defenses, and better exploit plant resources (Barrett and Heil 2012).

Interestingly, spatial structure provided the most explanatory power for communities within each host species. There was the highest degree of similarity in community composition among central and northern sites, which were relatively distinct from southern sites. This is not surprising considering the topography of the area. The southern sites are separated from the two northern sites by the Klamath Mountains. On the other hand, the Columbia River acts as a barrier between the Willamette Valley and Puget Trough ecoregions. The river is likely a small barrier, and indeed, some scientists

don't consider them to be distinct ecoregions (Floberg et al. 2004). However, mountains represent a sizable dispersal barrier for fungi (Peay et al. 2010), and they could be acting to differentiate communities on several levels. Current endophyte distributions likely depend on both dispersal of fungi and host. Besides roadway openings, there is also little to no connectivity of grasslands between the Klamath Mountain Ecoregion and the Willamette Valley. This suggests that for the most part, any movement of endophytes must be mediated by wind, or another external force. Depending upon host specificity of fungal community members, differentials in dispersal abilities could lead to the geographic structuring we uncovered. For *D. californica*, and to a lesser degree for *F. roemerii*, we were unable to disentangle the effects of dispersal limitation from abiotic filters. In some ways, this result is not surprising given the link between sporulation and spore transmission and climate, namely precipitation and wind patterns.

The question then becomes why is there so much unexplained variation in these ecological communities? In some ways, this high degree of unexplained variation is to be expected. Ecological studies are inherently noisy in the sense that there are many levels at which confounding variables can persist. However, we believe there are points in our sampling and environmental data gathering which could have better captured this variation. One large example is in our spatial analysis. Because of our strict guidelines in site selection (a minimum of 200 adult host plants), and the relatively small number of intact, unrestored prairies, we were left with an uneven sampling scheme, where our spatial scale varied from the range of tens of meters to the range of hundreds of kilometers. Unfortunately, there are few to no prairies that could bridge these two scales. Although our spatial analysis method is robust to irregular sampling schemes (Blanchet et al. 2008), it relies on a minimum spanning distance that can effectively connect any two sampling locations. As previously mentioned, our sampling sites were separated by large distances, the largest being several hundred km. Consequently, we were only able to pick up on very broad scale spatial patterns. We also believe that our sampling of abiotic variables was potentially inadequate. The use of 30-year normals in temperature and precipitation captured a very coarse grained representation of climate, but may not adequately describe the weather during the likely period of infection, often in the previous year. There is a lot of evidence that fine scale weather patterns are very

important in determining pathogen transmission (Garrett et al. 2006), and thus also endophytes. Finally, although host traits have been shown to be important in determining endophyte community composition (Kembel and Mueller 2014), they do not encompass the spectrum of biotic interactions that may be affecting communities. We were unable to examine fungal-plant interactions, nor were we able to capture fungal-fungal interactions.

## CHAPTER III

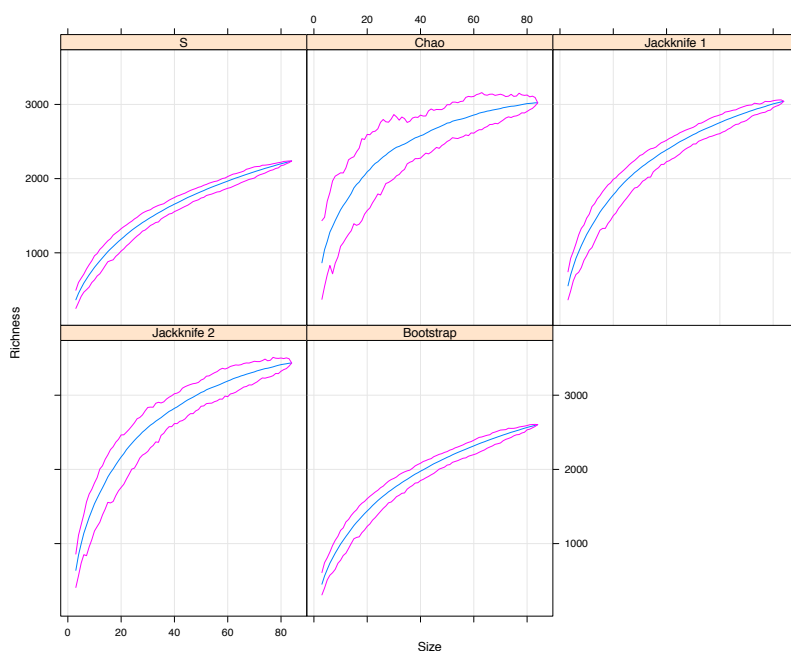
### CONCLUSIONS

We found that there are marked differences in foliar fungal endophyte communities within Pacific Northwest prairies. Host, region, and site all contributed to differentiate fungal community composition along a 700km latitudinal gradient. We found differences in local and regional diversity, community composition, and response to biotic and abiotic factors between the two bunchgrass host species surveyed. A large part of the variation was due to host specificity within Pacific Northwest prairie grasses. While there are differences in communities, we did find that communities appear to be influenced by external filters in similar ways. Environmental, biotic, and spatial filters were all important in describing endophytic communities, but spatial structuring described the largest portion of variance in these communities. For both host species, there was a large portion of unexplained variance. The unexplained variance is likely to be partially explained by medium and fine scale spatial structuring, which we did not measure. Further, the impact of plant-fungal and fungal-fungal interactions likely also play a pivotal role in community assembly.

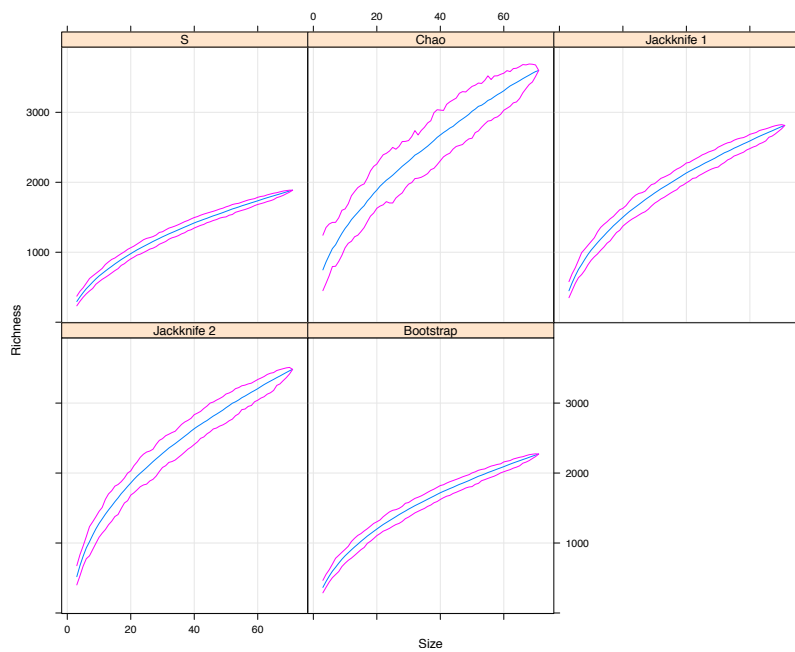
The use of an observational study is a way to begin to understand and characterize the diversity and composition of a relatively unknown system. We found that spatial distance and winter climate are important in endophyte community assembly. While we were unable to completely describe each of the community assembly filters due to the coarseness of our sampling scheme and climate variables, we believe that this study will provide a basis for future studies. The combination of both observational and experimental research, including greenhouse and reciprocal transplant experiments, will be necessary to elucidate the consequence of environmental filtering on foliar fungal endophyte community assembly and structure.

# APPENDIX

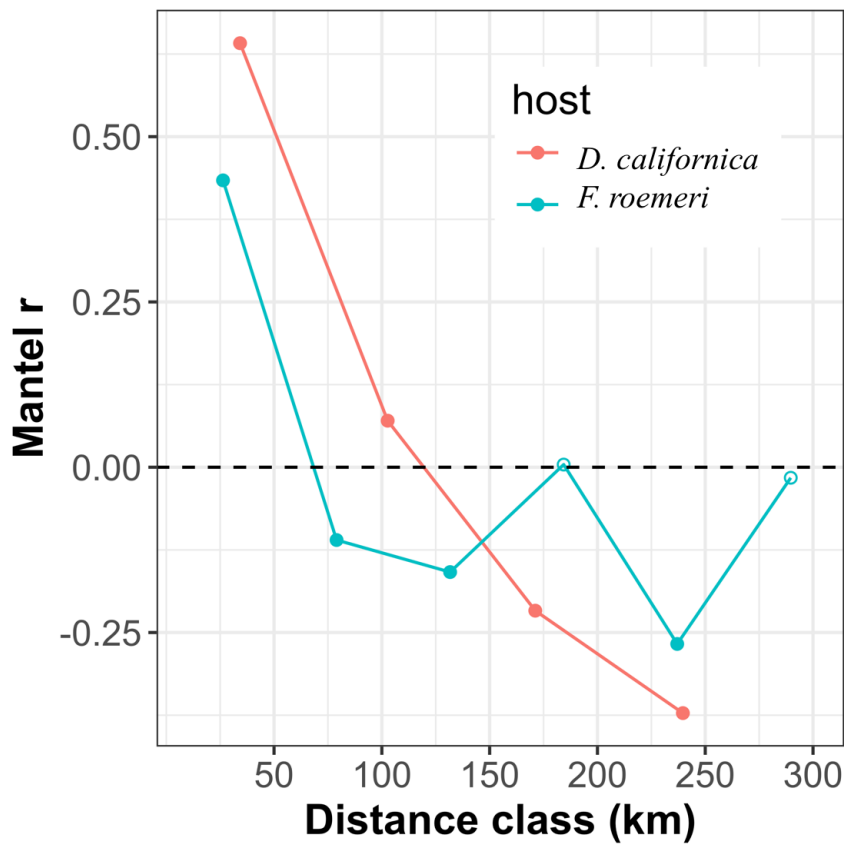
## SUPPLEMENTARY FIGURES



**Supplemental Figure 1:** Observed regional species diversity (S), as well as several diversity indices among *F. roemerii* communities. Solid blue lines represent means, while purple area represents SE.



**Supplemental Figure 2:** Observed regional species diversity (S), as well as several diversity indices among *F. roemerii* communities. Solid blue lines represent means, while purple area represents SE.



**Supplemental Figure 4:** Mantel correlogram comparing community dissimilarity and distance among *F. roemeri* (mantel's  $r = 0.081$ ,  $p < 0.01$ ) and *D. californica* (mantel's  $r = 0.446$ ,  $p < 0.01$ ). Because a dissimilarity matrix was used, positive mantel's  $r$  signifies spatial autocorrelation, while negative  $r$  signifies landscape homogenization. Filled circles represent significant comparisons.



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