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Biotic Filtering in Endophytic Fungal Communities

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ABSTRACT

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Plants can be colonized by complex communities of endophytic fungi. This thesis presents two studies, both of which investigate biotic filtering in endophytic fungal communities. Chapter 1. Endophytic fungi can be acquired horizontally via propagules produced in the environment such as in plant litters of various species. Given that litters from different plant species harbor distinct endophytic fungal communities and that endophytic fungi may be dispersal-limited, the structure of the endophytic fungal community of a given plant may be determined by proximity to particular inoculum sources. Community assembly may also be affected by biotic filtering by the plant. Therefore, a plant may be able to select particular fungal taxa from among the available pool. In that case, the structure of the endophytic fungal community in the plant could be somewhat independent of the structure of the inoculum community. We tested the hypothesis that biotic filtering of endophytic fungal communities occurs in *Bromus tectorum* by exposing it to a variety of inoculum sources including litters from several co-occurring plant species. The inoculum sources differed significantly from each other in the structures of the communities of endophytic fungi they harbored. We characterized the structures of the resulting leaf and root endophytic fungal communities in *Bromus tectorum* using high-throughput sequencing. All tested inoculum sources successfully produced complex communities of endophytic fungi in *Bromus tectorum*. There was significantly more variation in the structures of the communities of endophytic fungi among the inoculum sources than in the resultant endophytic fungal communities in the leaves and roots of *Bromus tectorum*. These results suggest that biotic filtering by *Bromus tectorum* played a significant role in the assembly of the endophytic fungal communities in tissues of *Bromus tectorum*. Because endophytic fungi influence plant fitness, it is reasonable to expect there to be selective pressure to develop a uniform, desirable endophytic fungal community even from disparate inoculum sources via a process known as biotic filtering. Chapter 2. Frequently one finds that different plant species harbor communities that are distinct. However, the nature of this interspecific variation is not clear. We characterized the endophytic fungal communities in six plant species from the eastern Great Basin in central Utah. Four of the species are arbuscular mycorrhizal (two in the Poaceae and two in the Asteraceae), while the other two species are nonmycorrhizal (one in the Brassicaceae and one in the Amaranthaceae). Our evidence suggests that both host mycorrhizal status and phylogenic relatedness independently influence endophytic fungal community structure.

Keywords: community assembly, biotic filtering, endophytic fungi, mycorrhizal fungi, natural selection, phylogeny

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INTRODUCTION

Many organisms host communities of microbial symbionts (Brucker & Bordenstein, 2013; Gordon, Knowlton, Relman, Rohwer, & Youle, 2013; Vandenkoornhuyse, Quaiser, Duhamel, Le Van, & Dufresne, 2015). Because microbial symbionts can influence host fitness (Brucker & Bordenstein, 2013; Peay, Garbelotto, & Bruns, 2010; Vandenkoornhuyse et al., 2015), herein I address some of the factors that influence the assembly of their communities.

One important group of plant symbionts is the endophytic fungi, which have been found in all plant species investigated thus far (Arnold et al., 2003; Arnold, Maynard, Gilbert, Coley, & Kursar, 2000; Rodriguez, White, Arnold, & Redman, 2009). These range from mutualists (Redman, 2002; Rodriguez & Redman, 2008; Rodriguez et al., 2009) to latent pathogens (Delaye, García-Guzmán, & Heil, 2013; Saikkonen, Faeth, Helander, & Sullivan, 1998) and latent saprotrophs (Promputtha et al., 2007; Szink, Davis, Ricks, & Koide, 2016). Plants can be colonized by complex communities of endophytic fungi composed of dozens of species (Arnold, 2007; Arnold & Lutzoni, 2013; Rodriguez et al., 2009).

In this thesis, I present two studies investigating biotic filtering of the available pool of endophytic fungi by a host plant. Biotic filtering refers to the active control of the structure of the endophytic fungal community by the host plant (Koide, Fernandez, & Petprakob, 2011). While plants are exposed to the spores of numerous endophytic fungal species (Arnold & Herre, 2003; Christian, Whitaker, & Clay, 2015; Kaneko & Kakishima, 2001) if biotic filtering of the endophytic fungal community occurs only a fraction of that available pool of endophytic fungi will be able to colonize the plant due to filters created by the plant (Violle et al., 2012). These filters would prevent colonization by other endophytic fungal species.

Given that some endophytic fungal species significantly influence plant fitness (Redman, Dunigan, & Rodriguez, 2001; Rodriguez & Redman, 2008), biotic filtering of endophytic fungal inocula by host plants may be the result of natural selection for the assembly of particular endophytic fungal communities. It seems reasonable for plants of a given plant species to have evolved a mechanism that permits them to develop a uniform, desirable endophytic fungal community even from rather disparate inoculum communities. In Chapter 1, I present a study investigating biotic filtering within a single plant species. We exposed plants to several disparate inoculum communities and characterized the resulting endophytic fungal communities.

Biotic filters may also vary among plant species. Different endophytic fungi may play different roles within a plant (Rodriguez & Redman, 2008). Because each plant species differs in their traits, the kinds of endophytic fungi that are most helpful to them are likely to differ. Distinct biotic filters employed by different plant species could permit the assembly of specific endophytic fungal communities that are matched to each plant species' requirements. In Chapter 2, I address this topic, presenting a study investigating variation in biotic filtering among plant species, while additionally attempting to determine some of the sources of this variation. Plants of different species were exposed to the same inoculum and we characterized their resulting endophytic fungal communities.

CHAPTER 1: Biotic filtering: evidence of selection for endophytic fungal communities in *Bromus tectorum*

INTRODUCTION

Some endophytic fungi are transmitted vertically, but many are acquired horizontally (Schardl, Leuchtmann, & Spiering, 2004) via propagules produced in the environment (Christian et al., 2015). In a given plant community, there are several sources of endophytic fungal inoculum including soil and plant litters of various kinds (Arnold & Herre, 2003; Christian et al., 2015; Kaneko & Kakishima, 2001), each of which host fungal communities that are distinct in their species composition or in the relative abundance of species (Aneja et al., 2006; Prescott & Grayston, 2013).

Because endophytic fungi can be dispersal-limited (Koide, Ricks, & Davis, 2017), plants are likely to be inoculated mainly by sources closest to them. Because neither the distribution of plant litters nor the distribution of members of a focal plant species are regularly distributed in space, the nearest inoculum sources may be quite different for different members of the focal species. It seems likely, therefore, that different members of the focal species would be exposed to distinct inoculum communities and develop distinct endophytic fungal communities. However, given the fact that endophytic fungi influence plant fitness (Redman et al., 2001; Rodriguez & Redman, 2008), it seems reasonable for plants of a given species to have evolved a mechanism that permits them to develop a uniform, desirable endophytic fungal community even from rather disparate inoculum communities. This mechanism would result in biotic filtering, the active control of the structure of the fungal community (Koide et al., 2011).

Bromus tectorum L. (cheatgrass) is an invasive species throughout the United States, particularly in the arid west, where it frequently outcompetes native species (Cline, Uresk, &

Rickard, 1977; Melgoza, Nowak, & Tausch, 1990; Rafferty & Young, 2002) and can lead to increased fire frequency (Balch, Bradley, D'Antonio, & Gómez-Dans, 2013; D'Antonio & Vitousek, 1992). To test the hypothesis that biotic filtering occurs in *Bromus tectorum*, we grew it in a controlled environment with distinct inoculum communities in the form of soil or litters from several co-occurring plants species, collected from our study site in central Utah. Litter and soil were chosen because previous research suggests that these harbor horizontally-acquired endophytic fungi (Arnold & Herre, 2003; Christian et al., 2015; Kaneko & Kakishima, 2001). The exterior of *Bromus tectorum* seeds was considered another potential inoculum source of endophytic fungi because preliminary culturing experiments demonstrated that seed exteriors were colonized by a diversity of fungi. After growing *Bromus tectorum* plants in the presence of the various inoculum sources, we characterized the endophytic fungal communities in *Bromus tectorum* leaves and roots to determine the extent to which they were influenced by inoculum source.

METHODS

Field sampling of inoculum sources and *Bromus tectorum* seeds

Our study site (40°5'34.7" N, 112°19'37.2" W) is a sagebrush-steppe, located approximately 10 km east of Vernon, UT, on land administered by the United States Department of the Interior, Bureau of Land Management. To characterize the vegetation in the community, we measured the cover provided by the common plant species in our study site. We randomly selected 10 positions along the western edge of the site. From each position, we surveyed along a 30-meter transect to the east. All intersections by plants on this transect (Canfield, 1941) were recorded (Table 1).

Table 1: Percent cover of the plants found in the study site.

Species	% cover
Artemisia tridentata	11.89
Chrysothamnus depressus	4.07
Atriplex canescens	3.09
Elymus elymoides	1.93
Bromus tectorum	1.48
Tetradymia glabrata	0.88
Alyssum alyssoides	0.72
Ceratocephala testiculata	0.33
Unidentified cactus	0.04
Unidentified grass	0.01
Descurainia pinnata	0.01

Within the 0.5 km^2 study site, we established 22 plots, each approximately 16 m^2, from which we sampled each of the various inoculum sources. This level of replication was chosen to provide sufficient statistical power to accurately characterize the fungal communities. On 15 May 2017, from each plot we collected samples of soil (top 5 cm) and litters from the most abundant plant species including *Artemisia tridentata* subsp. *wyomingensis* Nutt. (Wyoming sagebrush), *Elymus elymoides* Raf. (bottlebrush squirreltail), *Chrysothamnus depressus* Nutt. (low rabbitbrush), and *Bromus tectorum*. Although also abundant, *Atriplex canescens* Nutt. (fourwing saltbush) produced little litter and, therefore, we were unable to sample it. All samples were placed on ice in the field. Upon returning to the lab later that day, samples were stored temporarily at 6 °C. DNA was extracted from samples over the course of the following 5 days and prepared for fungal sequencing (see below).

On 14 June 2017, we collected *Bromus tectorum* seeds from 12 locations throughout the study site in order to capture site variation. Seeds were then pooled. The seed collection locations were different from the 22 plots from which inoculum sources were sampled and were chosen

for their high density of *Bromus tectorum* plants. Seeds were cold stratified at 6 °C in preparation for the inoculation experiment.

Bromus tectorum inoculation experiment

The purpose of this experiment was to characterize the leaf and root endophytic fungal communities of *Bromus tectorum* exposed to the various inoculum sources collected previously (see above) in order to test the hypothesis that biotic filtering occurs in the assembly of endophytic fungal communities. For example, if there were significantly less variation among endophytic fungal communities developing in tissues of *Bromus tectorum* from the various inoculum sources than in the inoculum sources themselves, we would conclude that biotic filtering occurred.

On 1 July 2017, two weeks prior to the start of the experiment, we filled 119 500 mL polyethylene pots with vermiculite moistened with 275 mL complete nutrient solution (Flora series, General Hydroponics, Santa Rosa, CA, USA) to bring it to field capacity. The nutrient solution contained 120 ppm N (as nitrate, 93%, and as ammonia, 7%), 26.2 ppm P as phosphate, and 115.9 ppm K (see Appendix 1-Table 1 for more details). Each pot was then sealed inside a 3.5 L spawn bag with 0.2 μm air filter patches (MycoHaus, Cincinnati, OH, USA) using binder clips. To create an initial sterile growing environment, all pots within their sealed spawn bags were autoclaved for 30 min, two weeks after the application of nutrient solution.

There were 7 inoculation treatments (Table 2), each replicated 17 times. Some of the inoculum consisted of materials collected from the field, including soil and litters from *Bromus tectorum*, *Elymus elymoides*, *Artemisia tridentata* or *Chrysothamnus depressus* (Treatments 1–5). We also included a control treatment with no inoculum (Treatment 7). For Treatments 1–5

and 7, we eliminated the fungi on the outside of the *Bromus tectorum* seeds by heating in 60 °C water for 60 min followed by placing them in 70% ethanol for 30 seconds, 6% sodium hypochlorite for 3 minutes (Bishop, Levine, Kropp, & Anderson, 1997; Shearin et al., 2018) followed by a thorough rinse in sterile water. To examine the role of fungi associated with the seed exterior as a source of endophytic fungi (Treatment 6), seeds were not subjected to that treatment.

Table 2: Inoculation treatments used in the inoculation experiment.

Treatment	Inoculation treatment	Seed surface treatment
1	*Bromus tectorum* litter	Y
2	*Chrysothamnus depressus* litter	Y
3	*Artemisia tridentata* litter	Y
4	*Elymus elymoides* litter	Y
5	Soil	Y
6	Exterior seed fungi	N
7	Control/None	Y

Under a laminar flow hood, spawn bags were unsealed and 8 appropriately treated *Bromus tectorum* seeds were placed in the middle of each pot and pushed slightly below the surface of the vermiculite using sterile forceps. The inocula collected from the field were then added to the appropriate pots (5 mL per pot) and spread evenly across the vermiculite and thus over the seeds (Treatments 1−5). On 14 July 2017 pots were placed in a greenhouse maintained at 25 °C. After one week of growth, each pot was thinned to 1 seedling under the laminar flow hood, then returned to the greenhouse.

After four weeks of growth (17 August 2017) plants had reached an average height of 40 cm and were harvested. Ten plants from each treatment were randomly selected for sampling of their leaf and root endophytic fungal communities. Two disks were sampled from each of the

lowest two leaves on each plant using a 7 mm diameter hole punch. These were placed in 2 mL tubes filled with 95% ethanol for short-term storage. Several root pieces totaling approximately 10 cm length were randomly sampled from each root system and stored temporarily in 95% ethanol. All samples were stored at 6 °C in the ethanol for approximately 2 weeks prior to DNA extraction.

Endophytic fungal sequence library preparation

Inoculum (litter and soil) sources. These samples were extracted using Mo Bio Powersoil Pro DNA extraction kits following the standard protocol for the extraction kits with one exception. Instead of using the Mo Bio Vortex Adaptor as suggested, we agitated the sample by shaking tubes at 1000 rpm for 4 min. using a 2010 Geno/Grinder (SPEX SamplePrep, Metuchen, NJ, USA). All DNA samples were stored at −20 °C until PCR amplification.

Bromus tectorum tissues from inoculation experiment. To remove external (non-endophyte) fungal DNA from the experimental *Bromus tectorum* leaf and root samples, we placed samples in 3% sodium hypochlorite (NaClO) and 1% Tween-20 for 20 minutes, after which tissue was rinsed thoroughly in sterile water (Arnold, Henk, Eells, Lutzoni, & Vilgalys, 2007; Fonseca-García et al., 2016; Khan, Hamayun, Kim, Kang, & Lee, 2011). Plant tissue samples were placed in Mo Bio Powerplant Pro DNA extraction tubes (Mo Bio Laboratories Inc., Carlsbad, CA, USA), and DNA was extracted following the standard protocol except for the alteration described above. All DNA samples were stored at −20 °C until PCR amplification.

Samples were prepared for high-throughput sequencing using a two-step PCR amplification. In the first step, the ITS2 region from the fungal ITS region was amplified using ITS4 FUN and 5.8S FUN primers (Taylor et al., 2016). The thermal cycling program was: hot-

start activation at 95 °C for 15 min, 27 cycles of 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 2 min with final elongation at 72 °C for 10 min. In the second step, barcodes and Illumina flowcell adapters were appended to the PCR1 amplicons. The thermal cycling program for the second thermal cycling program was: hot-start activation at 95 °C for 15 min, 12 cycles of 95 °C for 30 s, 55 °C for 40 s, and 72 °C for 40 s with final elongation at 72 °C for 10 min. We used Apex Hot start PCR Master Mix (Apex Bioresearch Products, North Liberty, IA, USA).

Identical volumes of PCR2 product from each sample were pooled together to create the sequence library prior to sequencing. Sequencing was done at the Institute for Bioinformatics and Evolutionary Studies (iBEST) genomics resources core at the University of Idaho (http://www.ibest.uidaho.edu/, Moscow, ID). Amplicon libraries were sequenced using 2 × 300 paired-end reads on an Illumina MiSeq sequencing v3 (600 cycles) platform (Illumina Inc., San Diego, CA, USA).

Bioinformatics

The initial bioinformatic processing was accomplished using the DADA2 pipeline (Callahan et al., 2016), including quality filtering parameters as recommended. Paired reads were assembled using mergePairs function with a minimum overlap of 20 bp and allowing a maximum mismatch of 5% within the region of overlap. Non-overlapping reads were joined with a 10 bp sequence of Ns. Using the UNITE database (Abarenkov et al., 2010) as a reference, sequence variants produced by the pipeline were assigned taxonomy using a Ribosomal Database Project Naïve Bayesian Classifier algorithm (Wang, Garrity, Tiedje, & Cole, 2007) with kmer size of 8, and 50% bootstrap threshold required to assign taxonomy.

We grouped all sequence variants into genera based on their assigned taxonomy (Arumugam et al., 2011). As 34% of the totals reads could not be assigned to a genus, these reads were clustered into genera based on a 94% similarity criterion (Cai, Ye, Tong, Lok, & Zhang, 2013; Edgar, 2010; Mende, Sunagawa, Zeller, & Bork, 2013). Previously identified genera were used as cluster centers, and additional cluster centers were generated de novo. To minimize errors in the selection of cluster centers, we iteratively re-selected centers until we approached a minimum asymptote for the number of cluster centers. This entire bioinformatic pipeline, while implemented using the DADA2 package in the R statistical environment (R Development Core Team, 2018), was similar to the open reference OTU picking strategy implemented in the QIIME pipeline (Edgar, 2010).

Data analysis

While endophytes can be found in plant litter and soil (Christian et al., 2015; Promputtha et al., 2007; Szink et al., 2016), not all fungi found in our various inoculum sources are necessarily endophytes. However, in another, related study at the same site, we had sampled the endophytic fungal communities in the leaves of *Artemisia tridentata*, *Elymus elymoides*, *Chrysothamnus depressus*, *Bromus tectorum, Atriplex canescens,* and *Alyssum alyssoides*. Using these data, as well as data from the *Bromus tectorum* inoculation experiment, all fungal genera found in any surface-treated leaf or root sample were classified as endophytic fungi. All other fungal genera were removed from our analyses of the various inoculum sources. Ninety six percent of the fungal genera identified from the various inoculum sources were categorized as endophytic. We thus compared endophytic fungal communities among inoculum sources, performing permutational multivariate analyses of variance (PERMANOVA) in the R statistical environment

(R Development Core Team, 2018) with the Vegan package (Oksanen et al., 2018) using Bray-Curtis dissimilarities (Anderson, 2001). To make specific comparisons between inoculum sources, we performed pairwise PERMANOVAs and, to protect against false positives, we used Benjamini-Hochberg false discovery rate adjustments on all P values (Benajmini & Hochberg, 1995). Variation in community structure was visualized using ordination (non-metric multidimensional scaling, NMDS) using Bray-Curtis dissimilarities, 25 perturbations and three axes, and displayed the ordinations using the first two axes. We identified the common endophytic fungal genera in the leaves and roots of the inoculation experiment and compared their sequence read numbers among the various inoculum sources, protecting against potential false positives using Benjamini-Hochberg adjustments on all P values. For a genus to be considered common, it had to occur in at least 2% of the sequence reads of either the leaves or the roots from the entire inoculation experiment. We did not sequence the communities of exterior seed fungi, and therefore this community was excluded from all analyses.

We compared variation in the structure of communities of endophytic fungi among the inoculum sources to variation in the structure of communities of endophytic fungi that developed from those inoculum sources in the leaves and roots of *Bromus tectorum*. Variation was characterized using beta diversity, which was calculated as the distance of each sample to the centroid of its treatment group (Anderson, Ellingsen, & McArdle, 2006). We excluded the control treatment from this analysis as these plants were grown without external inoculum. We also excluded the treatment in which plants were grown with seed exterior fungi as an inoculum source, as we did not characterize those fungi via high-throughput sequencing.

We determined whether inoculum source had a significant effect on leaf and root endophytic fungal communities of *Bromus tectorum* with PERMANOVA, and variation in

community structure was visualized using ordination as above. To characterize the diversity of the leaf and root endophytic fungal communities of *Bromus tectorum* among inoculation treatments, we calculated the effective number of genera (Jost, 2006) for each leaf and root sample. The effective number of genera was calculated as it is a more intuitive measure of diversity than other diversity metrics as it scales linearly (Jost, 2006). We identified common genera as those occurring in at least 2% of the sequence reads of either the leaves or the roots from the entire inoculation experiment. We compared for leaves and roots the sequence read numbers for all common genera in all inoculation treatments, protecting against potential false positives using Benjamini-Hochberg adjustments on all P values.

RESULTS

Illumina sequencing yielded 251 endophytic fungal genera in the inoculum sources, and 180 in the tissues of *Bromus tectorum* grown in the inoculation experiment, for a total of 259 unique endophytic fungal genera.

Inoculum source (soil and litters from *Bromus tectorum*, *Elymus elymoides*¸ *Artemisia tridentata* and *Chrysothamnus depressus*) was a significant factor in determining community structure of endophytic fungi among the various inoculum sources ($P < 0.001$, $R^2 = 0.269$; Figure 1, Appendix 1-Table 2). Moreover, all inoculum sources possessed significantly different endophytic fungal community structures from each other according to the pairwise comparisons by PERMANOVA (all $P < 0.001$; Table 3).

Table 3: Pairwise PERMANOVAs between inoculum sources used in the inoculation experiment.

	Bromus tectorum litter	*Chrysothamnus depressus* litter	*Artemisia tridentata* litter	*Elymus elymoides* litter	Soil
Bromus tectorum litter					
Chrysothamnus depressus litter	$P < 0.001$ $R^2 = 0.256$				
Artemisia tridentata litter	$P < 0.001$ $R^2 = 0.235$	$P < 0.001$ $R^2 = 0.148$			
Elymus elymoides litter	$P < 0.001$ $R^2 = 0.163$	$P < 0.001$ $R^2 = 0.177$	$P < 0.001$ $R^2 = 0.182$		
Soil	$P < 0.001$ $R^2 = 0.152$	$P < 0.001$ $R^2 = 0.225$	$P < 0.001$ $R^2 = 0.196$	$P < 0.001$ $R^2 = 0.146$	

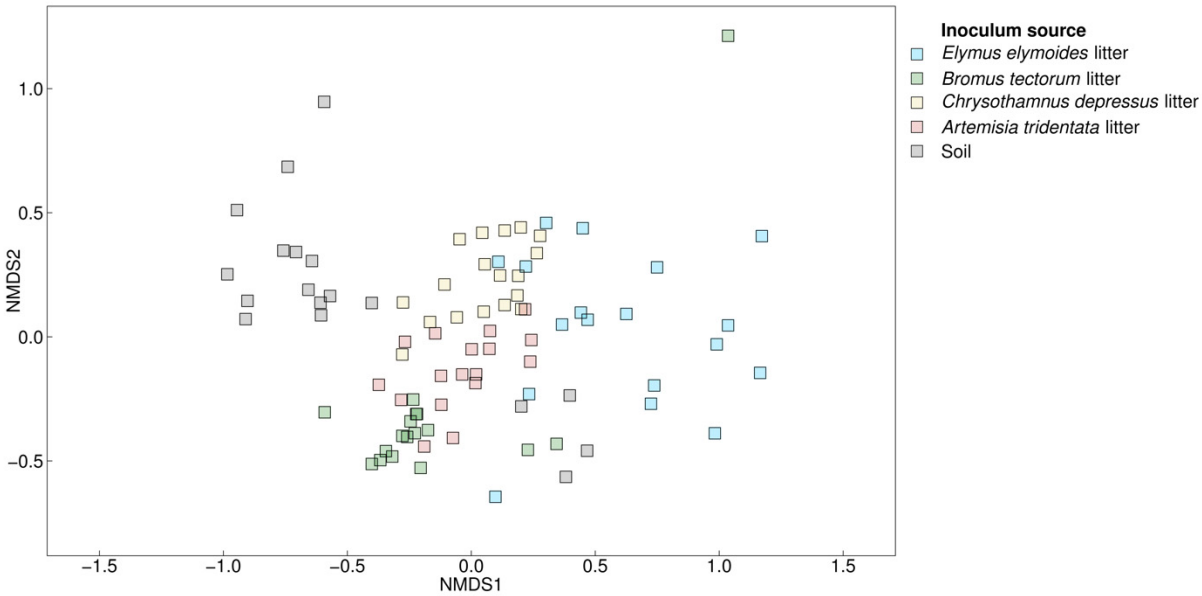

Figure 1: NMDS ordinations visualizing the endophytic fungal communities in the various inoculum sources.

There was significantly more variation in the structures of the communities of endophytic fungi among the inoculum sources than in the resultant endophytic fungal communities in the leaves and roots of *Bromus tectorum* of the inoculation experiment (Figure 2). The beta diversity of the endophytic fungal communities in the inoculum sources was significantly larger than for the resultant endophytic fungal communities that assembled in the leaves and roots of *Bromus*

tectorum, and there was no significant difference between the beta diversities in leaves and roots (Figure 3).

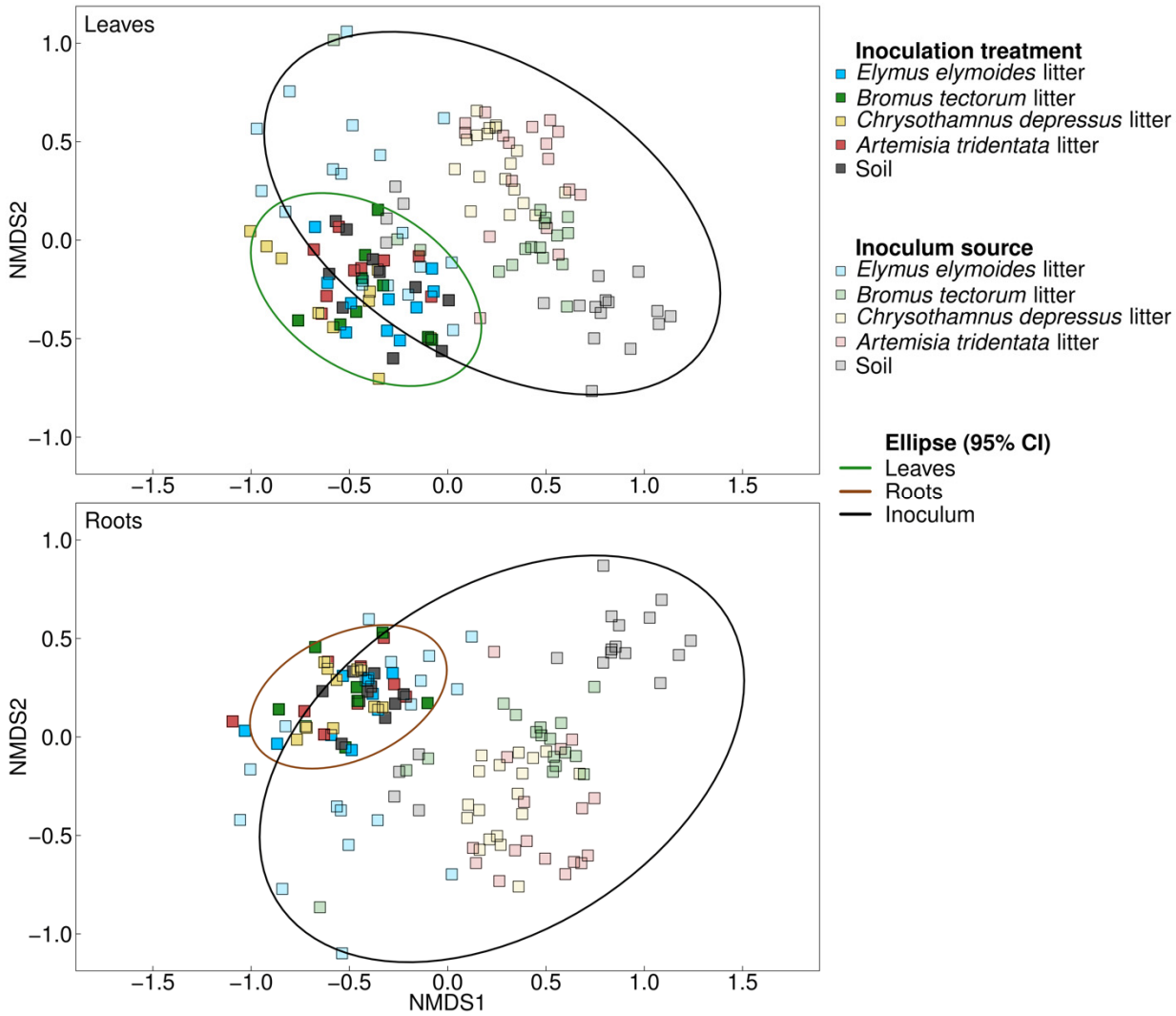

Figure 2: NMDS ordinations visualizing the endophytic fungal communities in the various inoculum sources and in the resultant endophytic fungal communities in the leaves and roots of *Bromus tectorum* in the inoculation experiment. Ellipses are drawn to include 95% of the variation for each group. A. Leaf endophytic fungal communities. B. Root endophytic fungal communities.

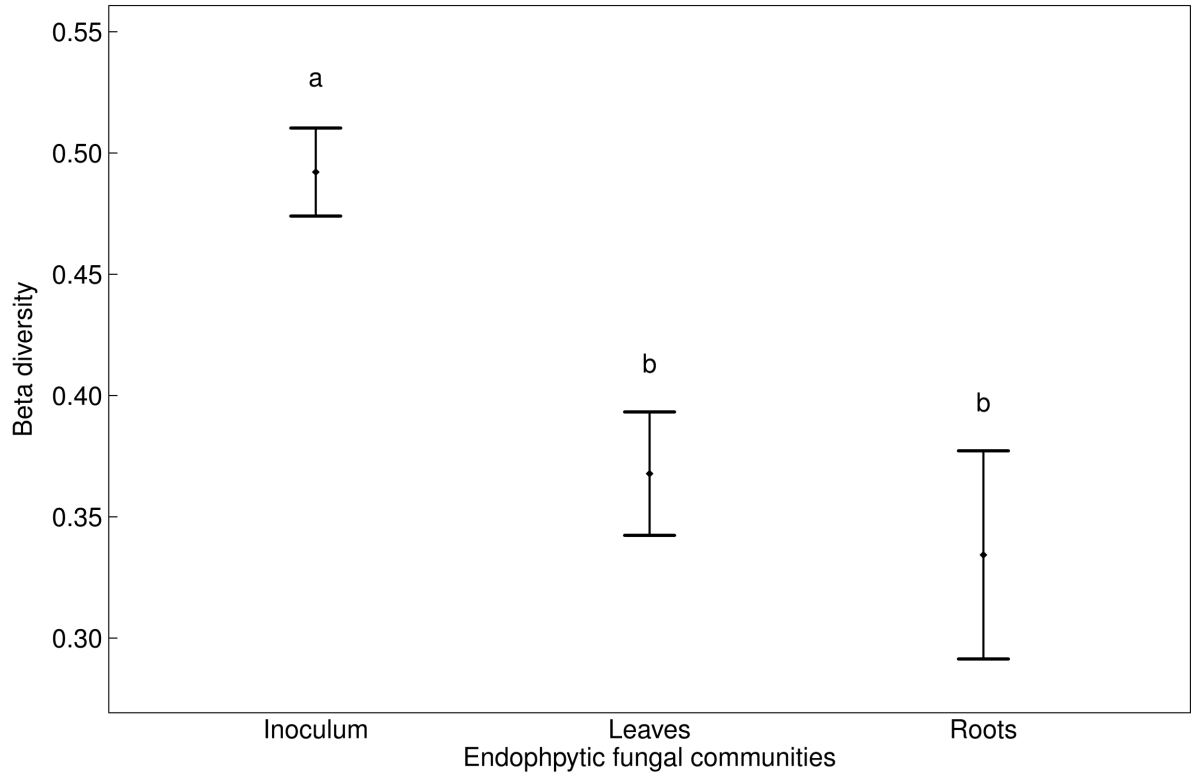

Figure 3: Beta diversity of endophytic fungal community samples from the inoculum, and leaves and roots from the inoculation. Beta diversity was measured using the distance to centroid method (Anderson et al., 2006). Different letters represent significant differences according to Tukey HSD. Bars represent 95% confidence intervals of beta diversity.

In *Bromus tectorum* leaves of the inoculation experiment, inoculation treatment was a significant factor determining endophytic fungal community structure ($P < 0.001$, $R^2 = 0.193$; Figure 4A, Appendix 1-Table 3). The NMDS plot indicated that the control treatment (no external inoculum source) clustered far from all the other treatments. By removing the control treatment (no external inoculum source) from the PERMANOVA, inoculation treatment was no longer a significant factor ($P = 0.624$, $R^2 = 0.077$, Appendix 1-Table 4). The effective numbers of fungal genera in leaf endophytic fungal communities were significantly different among inoculation treatments ($P < 0.001$; Figure 5A). The fungal communities in the control treatment had significantly fewer effective genera than in all other treatments, while all the other treatments were not significantly different from each other. The total number of sequence reads

per sample was significantly different among inoculation treatments ($P < 0.001$; Figure 6A). Leaf samples in the control treatment had significantly fewer reads than in all other treatments, and all other treatments were not significantly different from each other.

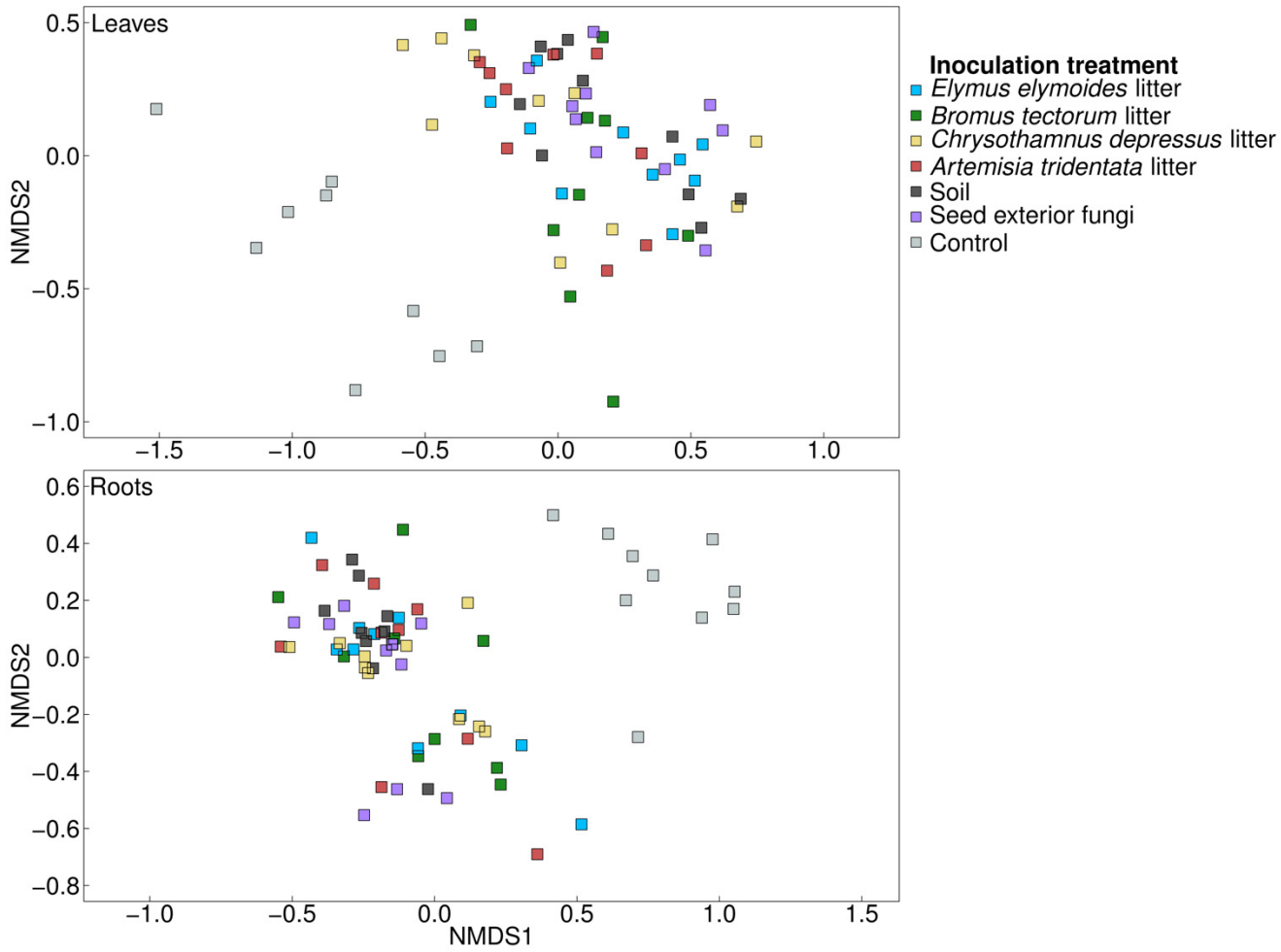

Figure 4: NMDS ordinations visualizing the endophytic fungal communities in Bromus tectorum tissues among the various inoculation treatments in the inoculation experiment. A. Leaf endophytic fungal communities. B. Root endophytic fungal communities.

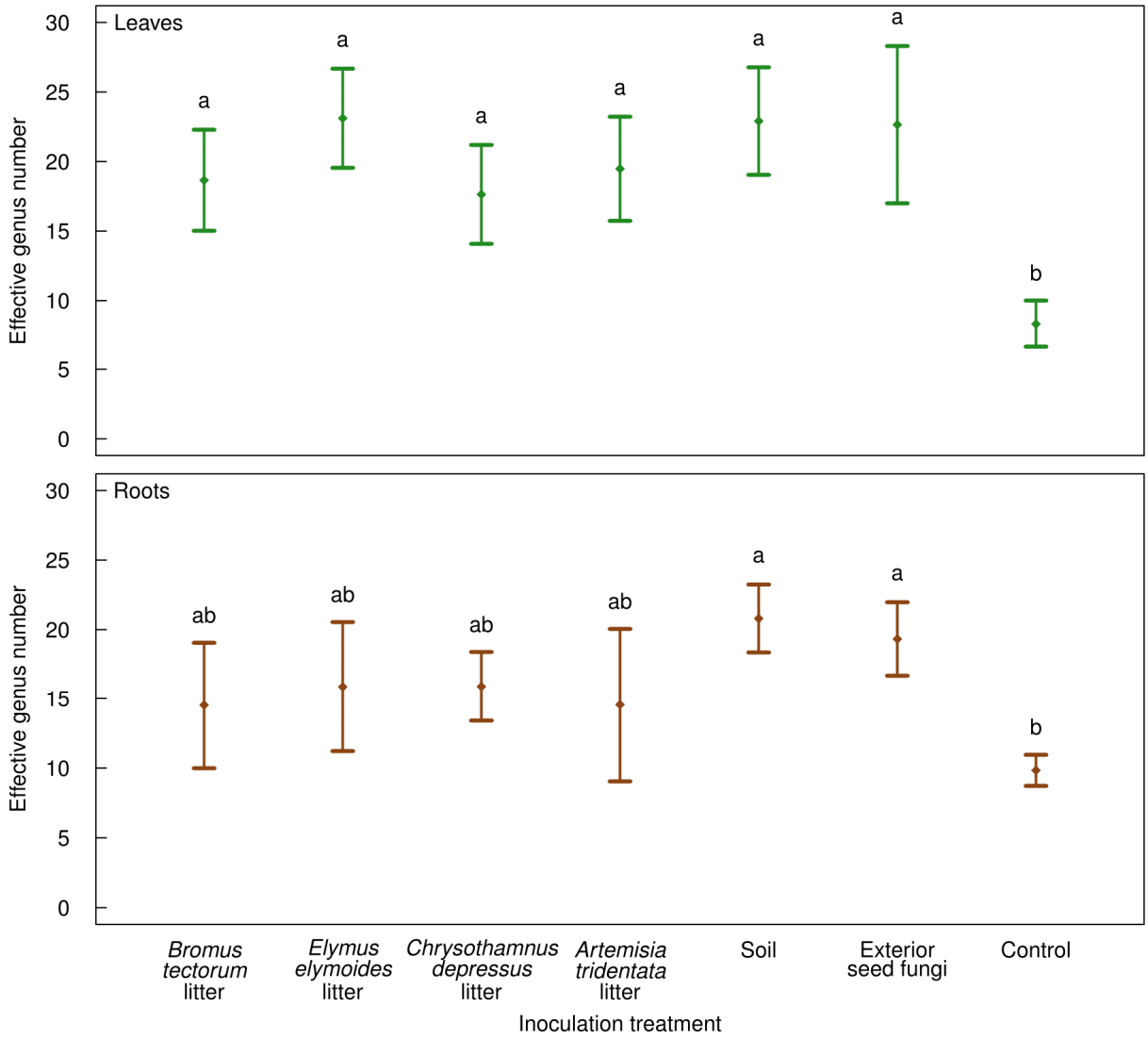

Figure 5: Effective genus number in Bromus tectorum tissues among inoculation treatments. Different letters represent significant differences according to Tukey HSD. Bars represent 95% confidence intervals of the mean effective genus number. A. Leaf endophytic fungal communities. B. Root endophytic fungal communities. n = 10

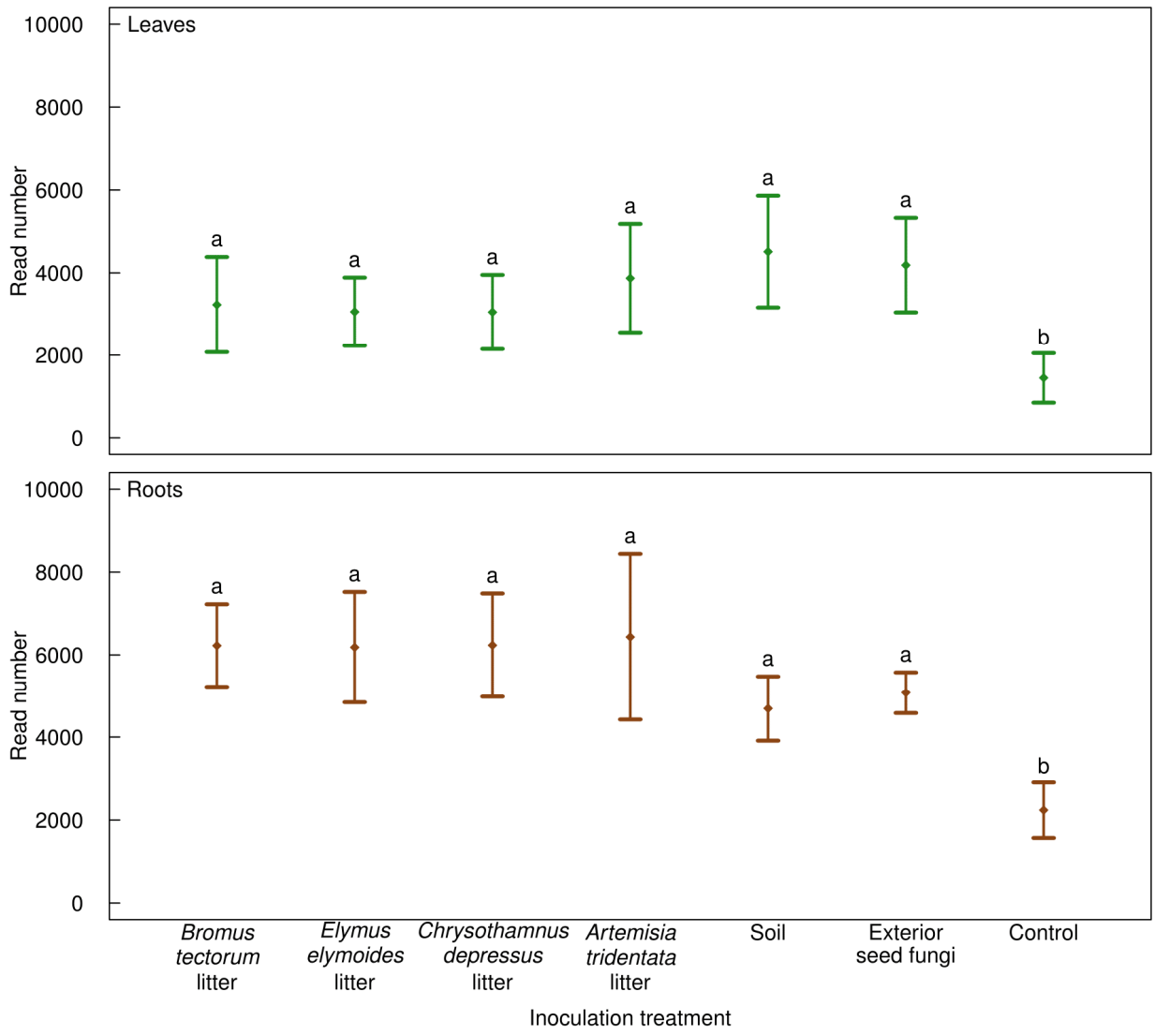

Figure 6: Total sequence read numbers for endophytic fungal communities in tissues of Bromus tectorum among inoculation treatments. Different letters represent significant differences according to Tukey HSD. Bars represent 95% confidence intervals of the mean read number. A. Leaf endophytic fungal communities. B. Root endophytic fungal communities. n = 10

In *Bromus tectorum* roots of the inoculation experiment, inoculation treatment was a significant factor determining endophytic fungal community structure ($P < 0.001$, $R^2 = 0.334$; Figure 4B, Appendix 1-Table 5,). As with the leaf communities, the control treatment clustered far from the other treatments in the NMDS plot. However, when we removed the control treatment from the analysis, inoculation treatment was still significant in determining community structure, but with a lower R^2 ($P < 0.001$, $R^2 = 0.173$; Appendix 1-Table 6). The effective number of genera in root endophytic fungal communities was significantly different among inoculation treatments ($P < 0.001$; Figure 5B). Endophytic fungal communities in the control treatment had significantly fewer effective genera than the soil inoculum and seed exterior fungi treatments, while all other treatments were not significantly different from each other. The total sequence reads per sample in the root communities was significantly different among inoculation treatments ($P < 0.001$; Figure 6B). The control treatment had significantly fewer reads than all other treatments, and all other treatments were not significantly different from each other.

We identified 24 common genera (accounting for at least 2% of the sequence reads of either the leaves or the roots from the entire experiment) in the tissues of *Bromus tectorum* grown in the inoculation experiment. For each of these common genera, when excluding the control treatment there were no significant differences in the read numbers among all inoculation treatments in leaves (Figure 7A). In roots, after excluding the control treatment there were only two genera, *Coprinopsis* and *Chaetomium*, that differed significantly in sequence reads among inoculation treatments: plants grown with litter from *Artemisia tridentata* had more reads of *Coprinopsis* than all other inoculation treatments, while plants grown with soil inoculum or seed exterior fungi had fewer reads of *Chaetomium* than all other inoculation treatments (Figure 7C). In each treatment provided with an inoculum source (Treatments 1−6), all 24 of these common

genera occurred in at least one leaf sample and in at least one root sample. In the control treatment, however, 15 of these genera did not occur in any root or leaf sample (Treatment 7; Figures 7B, 7D).

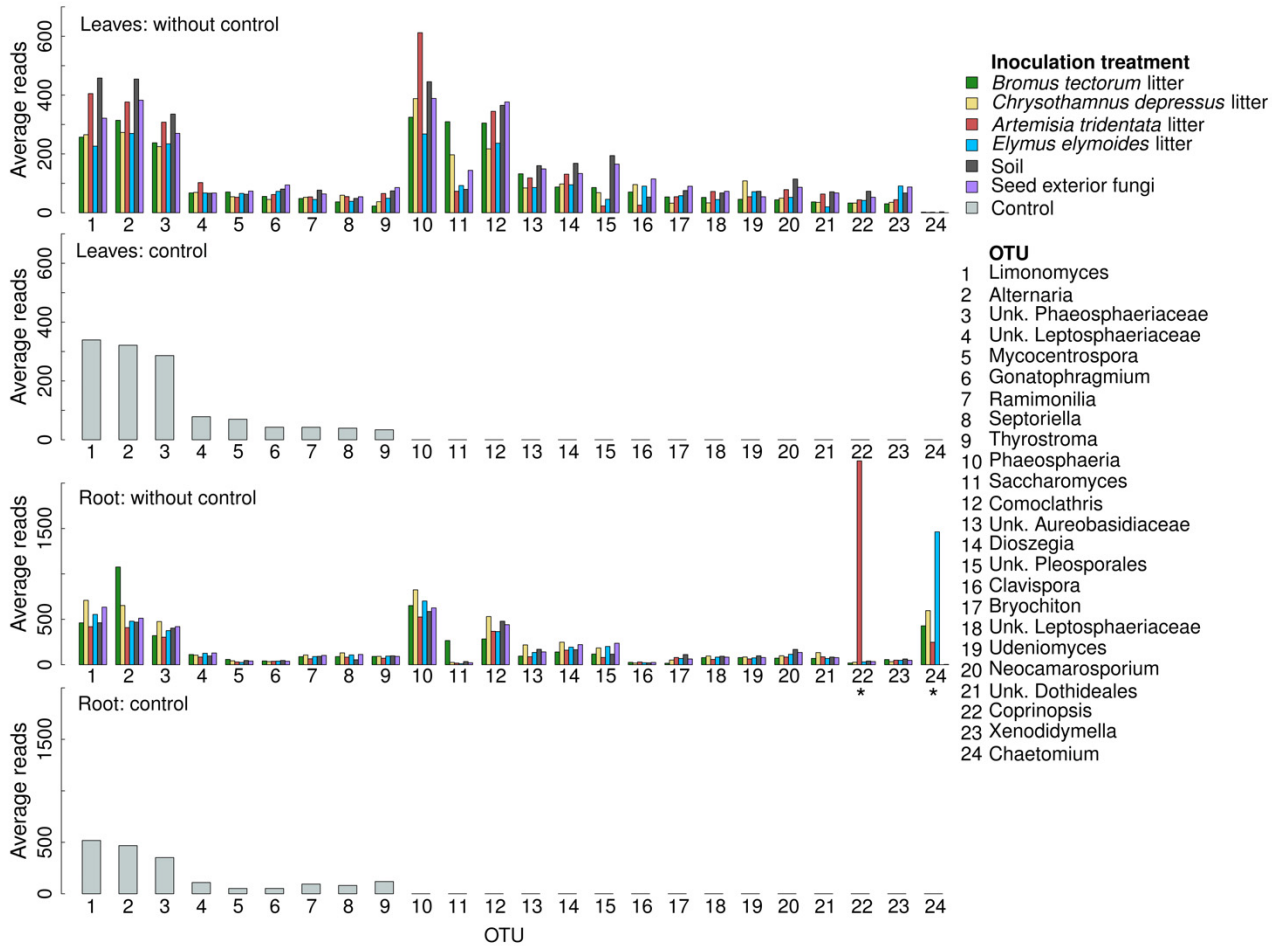

Figure 7: Frequency histogram of the average sequence read numbers of the common genera (at least 2% of the sequence reads of either the leaves or the roots from the entire inoculation experiment) in the leaves and roots of Bromus tectorum receiving the various inoculation treatments. A. Leaf endophytic fungal communities excluding control treatment. B. Leaf endophytic fungal community from control treatment. C. Root endophytic fungal communities excluding control treatment. D. Root endophytic fungal community from control treatment. Genera that differed significantly between inoculation treatments (excluding the control) are marked with *. The abbreviation 'Unk' in the legend refers to unknown genera that have been clustered at 94% similarity. n = 10

The 24 common genera were nearly all found in all the inoculum sources used in the inoculation experiment; 18 of the genera occurred in all inoculum sources, while the remaining 6 genera occurred in 4 of the 5 inoculum sources, but not necessarily in the same 4 (Table 4). Thirteen of the 24 genera differed significantly in read numbers among inoculum sources (Figure 8).

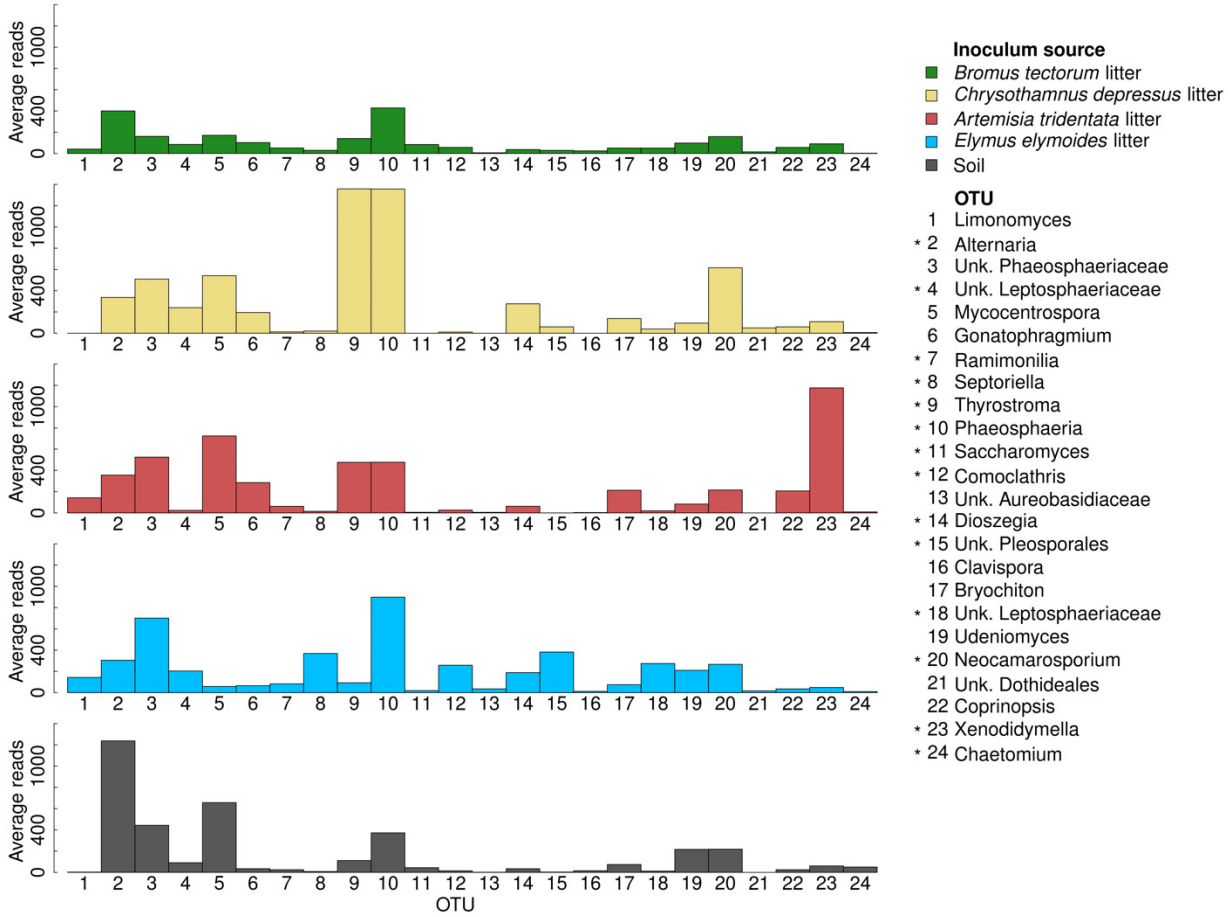

Figure 8: Frequency histogram of the average sequence read numbers of the common endophytic fungal genera in the various inoculum sources. Genera are ordered from left to right as in Figure 7. Genera that differ significantly in read numbers among the various inoculum sources are marked with *. n = 22

Table 4: Common genera (at least 2% of the sequence reads of either the leaves or the roots from the entire inoculation experiment) identified in the *Bromus tectorum* inoculation experiment, and their presence in the various inoculum sources. Ordering of OTUs from left to right is identical to Figures 5 and 6. Columns with 'Unknown' refers to unknown genera that have been clustered at 94% similarity.

Inoculum source	*Limonomyces*	*Alternaria*	Unknown *Phaeosphaeriaceae*	Unknown *Leptosphaeriaceae*	*Mycocentrospora*	*Gonatophragmium*	*Ramimonilia*	*Septoriella*	*Thyrostroma*	*Phaeosphaeria*	*Saccharomyces*	*Comoclathris*	Unknown *Aureobasidiaceae*	*Dioszegia*	Unknown *Pleosporales*	*Clavispora*	*Bryochiton*	Unknown *Leptosphaeriaceae*	*Udeniomyces*	*Neocamarosporium*	Unknown *Dothideales*	*Coprinopsis*	*Xenodidymella*	*Chaetomium*
Bromus tectorum litter	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Chrysothamnus depressus litter		X	X	X	X	X	X	X	X	X	X	X		X	X		X	X	X	X	X	X	X	X
Artemisia tridentata litter	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X		X	X	X
Elymus elymoides litter	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Soil	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

DISCUSSION

Because different sources of inoculum possessed unique endophytic fungal communities, as demonstrated herein, it is reasonable to expect a host exposed to different inoculum sources to develop different endophytic fungal communities. Alternatively, it also seems reasonable for mechanisms to have evolved that permit hosts to develop a common endophytic fungal community from disparate inoculum sources through the process of biotic filtering (Koide et al., 2011), particularly because the fitness of the host plant can be strongly affected by the composition of its endophytic fungal community (Arnold et al., 2003; Fonseca-García et al., 2016; Redman et al., 2001; Rodriguez & Redman, 2008). The results from *Bromus tectorum* leaves and roots were consistent with biotic filtering because the endophytic fungal communities that assembled in the tissues of *Bromus tectorum* in the inoculation experiment were significantly less variable (lower beta diversity) than the communities of endophytic fungi in the various inoculum sources themselves. As further support for biotic filtering, irrespective of the inoculum source the plants were exposed to, the endophytic fungal communities that assembled within the leaves were not significantly different from each other. The communities that assembled in the roots of *Bromus tectorum* roots were significantly different among the various inoculation treatments, but the impact of the inoculation treatment on root endophytic fungal community structure was small, and the differences were largely driven by two OTUs: *Coprinopsis* and *Chaetomium*. Moreover, the ordination plot for the root samples suggests no clear trend in the grouping of the endophytic fungal communities from the treatments other than the control. Biotic filtering is suggested by previous research by Vincent et al. (2016), who showed interspecific variation in endophytic fungal communities, in part, could be attributed to leaf traits.

Our results are consistent with the hypothesis that biotic filtering occurs as a consequence of the impact of endophytic fungal community structure on host plant fitness. However, our inoculation experiment was performed under unnatural conditions in a greenhouse, and plants were rooted in vermiculite supplied with all necessary plant nutrients. Conditions are very different in the field and the strength of selective forces may be different there.

How might biotic filtering of potential endophytic fungi operate? A fungus must first successfully make its way into the leaf interior (Arnold & Lutzoni, 2013; Edward Allen Herre et al., 2007), either by penetrating the cuticle or by growing into a stomate. After finding itself in the leaf interior, a fungus must then successfully obtain nutrition from the leaf and compete successfully with other fungi. Because competitive hierarchies are controlled by the environment (Koide et al., 2011), each of these steps is likely to represent opportunities for filtering by the host that only selected fungi can overcome. Among all the fungal species available in the various inoculum sources, the genera comprising the endophytic fungal communities are presumably among the few that were able to successfully navigate the filtering process.

Biotic filtering may result in a consistent endophytic fungal community in *Bromus tectorum* across a landscape, irrespective of variation in the nearest available sources of inoculum. While the fungi associated with the surface of the seeds were not sequenced, they produced leaf and root endophytic fungal communities that had sequence read numbers and effective numbers of genera that were not significantly different from those of communities produced by the soil and litter inocula. Thus, in addition to inoculum supplied by environmental sources, the exterior of the seed coat itself may be sufficient to produce the core members of the community of endophytic fungi. Our results suggest that for *Bromus tectorum*, the availability of appropriate endophytic fungal inoculum may not represent a bottleneck as its distribution

expands. The success of *Bromus tectorum* as an invasive species may be due, in part, to the ability to assemble a common endophytic fungal community from a variety of inoculum sources.

The soil, the various plant litters, and even the exterior of the seed clearly functioned as inoculum for endophytic fungi of *Bromus tectorum*. Both the total sequence read numbers and the effective number of genera were significantly increased with those inoculum sources when compared to the control (seeds treated to remove surface fungi, Treatment 7). It is, perhaps, not surprising that all the inoculum sources harbored a set of common endophytic fungal genera. After all, all inoculum sources were collected from the same field site. Inoculum sources from different field sites, however, may differ to a greater degree.

Our sequencing effort was largely insufficient to assign taxonomy below the level of genus. Therefore, we cannot say for sure that those communities we found not to differ significantly in structure actually did not differ in species composition. For example, there could have been multiple species of a given endophytic fungal genus distributed among the various inoculum sources, but we would not have been able to distinguish among them in the endophytic fungal communities assembling in *Bromus tectorum* leaves or roots. Nevertheless, at the level of genus, it would appear that some biotic filtering existed in the assembly of leaf and root endophytic fungal communities.

CHAPTER 2: Potential sources of variation in endophytic fungal communities: host phylogenetic relatedness and mycorrhizal status

INTRODUCTION

Endophytic fungi have been found in all plant species investigated thus far (Arnold et al., 2003, 2000; Rodriguez et al., 2009). They frequently form complex communities composed of dozens of species (Arnold, 2007; Arnold & Lutzoni, 2013; Rodriguez et al., 2009). Frequently one finds that different plant species harbor distinct communities of endophytic fungi (Arnold et al., 2003; Vincent, Weiblen, & May, 2016), however the nature of this interspecific variation is not clear. To our knowledge, no one has determined the impact of either phylogenetic relatedness or mycorrhizal status (whether the plant species is mycorrhizal or non-mycorrhizal) on the structure of endophytic fungal communities. These were our goals in this chapter.

Phylogenetic relatedness has been shown to influence partner choice in a number of symbioses including, to name a few, ant-fungal mutualisms (Chapela, Rehner, Schultz, & Mueller, 1994; Currie et al., 2003), host-parasite interactions (Boeger & Kritsky, 1997; Hafner & Nadler, 1988), gut microbial symbioses (Brucker & Bordenstein, 2013; Hongoh et al., 2005), plant-herbivore interactions (Farrell & Mitter, 1990, 1998), and plant-pollinator interactions (E.A. Herre et al., 1996; Lopez-Vaamonde, Rasplus, Weiblen, & Cook, 2001). Therefore, plant phylogeny seems to be a likely factor determining the degree of variation among plant species in their endophytic fungal communities. Among the plant species in the eastern Great Basin, therefore, we chose for study plant species that varied in their phylogenetic relatedness, including two members of the Poaceae (*Elymus elymoides*, *Bromus tectorum*), two members of the Asteraceae (*Chrysothamnus depressus, Artemesia tridentata*), one member of the Brassicaceae (*Alyssum alyssoides*) and one member of the Amaranthaceae (*Atriplex canescens*).

Our expectation is that phylogenetic distance is correlated with endophytic fungal community dissimilarity.

Members of the Poaceae and Asteraceae are capable of forming arbuscular mycorrhizas, but neither *Alyssum alyssoides* nor *Atriplex canescens* can (Brundrett, 1991, 2009; Malloch, Pirozynskit, & Raven, 1980; Tester, Smith, & Smith, 1987). Because nonmycorrhizal status may be caused by the production of antifungal compounds (Schreiner & Koide, 1993a, 1993b), mycorrhizal status may influence endophytic fungal communities independent of phylogenetic relatedness.

Another source of variation among plant species in the structure of their endophytic fungal communities may be location. Because endophytic fungal inoculum can be dispersal-limited (Koide et al., 2017), plant species with different spatial distributions may possess different endophytic fungal communities simply because the fungal taxa comprising the available inoculum vary with location. If, however, one samples a variety of *co-occurring* plant species, location cannot contribute to the variation. Because we did not want the results of our study to be confounded by location, we set out to explore variation in the structure of endophytic fungal communities among *co-occurring* plant species in the eastern Great Basin of the United States.

METHODS

Field sampling of endophytic fungal communities

Our study site (40°5'34.7" N, 112°19'37.2" W) is a sagebrush-steppe, located approximately 10 km east of Vernon, UT, on land administered by the United States Department of the Interior, Bureau of Land Management. We chose six species to sample for their endophytic fungal

communities. Four of these are arbuscular mycorrhizal species including *Artemisia tridentata* subsp. *wyomingensis* (Wyoming sagebrush), *Elymus elymoides* (bottlebrush squirreltail), *Chrysothamnus depressus* (low rabbitbrush), and *Bromus tectorum* (cheatgrass). Among the mycorrhizal species, *Artemisia tridentata* and *Chrysothamnus depressus* are members of the Asteraceae, while *Bromus tectorum* and *Elymus elymoides* are members of the Poaceae (Figure 1). The remaining two species, *Atriplex canescens* Nutt. (fourwing saltbush, Amaranthaceae), and *Alyssum alyssoides* L. (yellow alyssum, Brassicaceae), are nonmycorrhizal species.

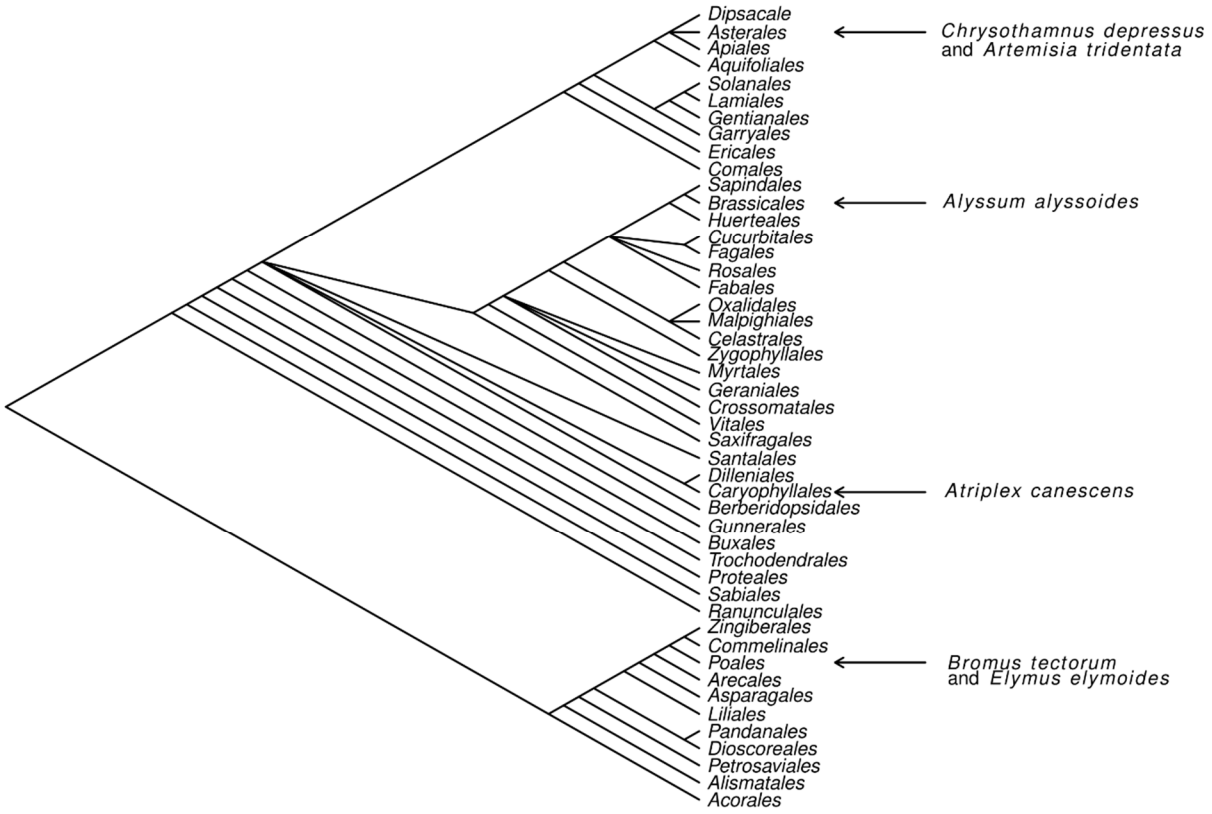

Figure 1: Phylogeny of major angiosperm orders (Bliss et al., 2013) with the locations of the six species in this study.

Within the 0.5 km^2 study site, we established 22 plots from which we sampled leaves of the six species. Plots were approximately 16 m^2 and included all plant species. This level of replication was chosen to provide sufficient statistical power to accurately characterize the endophytic fungal communities. On 15 May 2017, from each plot we sampled 5 leaves from of each of the plant species. The leaves from each species in a plot were later pooled into a single sample for DNA extraction. All sampled leaves appeared to be disease-free. All samples were placed on ice in the field. Upon returning to the laboratory later in the day, samples were stored temporarily at 6 °C. DNA was extracted from samples during the next 5 days and prepared for fungal sequencing (see below).

Endophytic fungal sequence library preparation

To remove external (non-endophyte) fungal DNA from samples, we placed samples in 3% sodium hypochlorite (NaClO) and 1% Tween-20 for 20 minutes, after which tissue was rinsed thoroughly in sterile water (Arnold et al., 2007; Fonseca-García et al., 2016; Waqas et al., 2015). Approximately 0.5 g of plant tissue from each sample were placed in Mo Bio Powerplant Pro DNA extraction tubes (Mo Bio Laboratories Inc., Carlsbad, CA, USA), and DNA was extracted following the standard protocol for the extraction kits with one exception. Instead of using the Mo Bio Vortex Adaptor, we agitated the sample by shaking tubes at 1000 rpm for 4 min. using a 2010 Geno/Grinder (SPEX SamplePrep, Metuchen, NJ, USA). All DNA samples were stored at −20 °C until PCR amplification.

Samples were prepared for high-throughput sequencing using a two-step PCR amplification. In the first step, the ITS2 subregion from the fungal ITS region was amplified using ITS4 FUN and 5.8S FUN primers (Taylor et al., 2016). The thermal cycling program was:

hot-start activation at 95 °C for 15 min, 27 cycles of 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 2 min with final elongation at 72 °C for 10 min. In the second step, barcodes and Illumina flowcell adapters were appended to the PCR1 amplicons. The thermal cycling program for the second thermal cycling program was: hot-start activation at 95 °C for 15 min, 12 cycles of 95 °C for 30 s, 55 °C for 40 s, and 72 °C for 40 s with final elongation at 72 °C for 10 min. We used Apex Hot start PCR Master Mix (Apex Bioresearch Products, North Liberty, IA, USA).

Identical volumes of PCR2 product from each sample were pooled together to create the sequence library prior to sequencing. Sequencing was done at the Institute for Bioinformatics and Evolutionary Studies (iBEST) genomics resources core at the University of Idaho (http://www.ibest.uidaho.edu/, Moscow, ID). Amplicon libraries were sequenced using 2 × 300 paired-end reads on an Illumina MiSeq sequencing v3 (600 cycles) platform (Illumina Inc., San Diego, CA, USA).

Bioinformatics

The initial bioinformatic processing was accomplished using the DADA2 pipeline (Callahan et al., 2016), including quality filtering parameters as recommended. Paired reads were assembled using mergePairs function with a minimum overlap of 20 bp and allowing a maximum mismatch of 5% within the region of overlap. Non-overlapping reads were joined with a 10 bp sequence of Ns. Using the UNITE database (Abarenkov et al., 2010) as a reference, sequence variants produced by the pipeline were assigned taxonomy using a Ribosomal Database Project Naïve Bayesian Classifier algorithm (Wang et al., 2007) with kmer size of 8, and 50% bootstrap threshold required to assign taxonomy.

We grouped all sequence variants into genera based on their assigned taxonomy (Arumugam et al., 2011; Fonseca-García et al., 2016). As 34% of the totals reads could not be assigned to a genus, these reads were clustered into genera based on a 94% similarity criterion (Cai et al., 2013; Edgar, 2010; Mende et al., 2013). Previously identified genera were used as cluster centers, and additional cluster centers were generated de novo. To minimize errors in the selection of cluster centers, we iteratively re-selected centers until we approached a minimum asymptote for the number of cluster centers. This entire bioinformatic pipeline, while implemented using the DADA2 package in the R statistical environment (R Development Core Team, 2018), was similar to the open reference OTU picking strategy implemented in the QIIME pipeline (Edgar, 2010).

Data analysis

To compare endophytic fungal communities among plant species, we performed permutational multivariate analyses of variance (PERMANOVA) in the R statistical environment (R Development Core Team, 2018) with the Vegan package (Oksanen et al., 2018) using Bray-Curtis dissimilarities (Anderson, 2001). To make specific comparisons between plant species, we performed pairwise PERMANOVAs and, to protect against false positives, we used Benjamini-Hochberg false discovery rate adjustments on all P values (Benajmini & Hochberg, 1995). We additionally visualized variation in endophytic fungal community structure among plant species using ordination (non-metric multidimensional scaling, NMDS) using Bray-Curtis dissimilarities, 25 perturbations and three axes, and displayed the ordinations using the first two axes.

A statistical test such as a PERMANOVA can only identify significant differences in endophytic fungal community structure among plant species, but we also wanted to correlate phylogenetic distance with endophytic fungal community dissimilarity. Therefore, we calculated the Bray-Curtis distances between the centroids of the replicate endophytic fungal communities of each plant species as a measure of community dissimilarity, and generated standard errors of these distances using jackknife resampling (Efron, 1981). We then correlated the distances between centroids with the divergence time for each species pair estimated with TimeTree (Kumar, Stecher, Suleski, & Hedges, 2017). TimeTree estimates divergence time between species pairs by utilizing phylogenetic trees from relevant publications.

RESULTS AND DISCUSSION

Illumina sequencing yielded 214 unique endophytic fungal genera. In the analysis of mycorrhizal plant species only, plant species was a significant factor determining endophytic fungal community structure ($P < 0.001$, $R^2 = 0.160$; Figure 2A, Appendix 2-Table 1). When examining all plant species, including both mycorrhizal and nonmycorrhizal plants, plant species was, again, a significant factor determining community structure ($P < 0.001$, $R^2 = 0.304$; Figure 2B, Appendix 2-Table 2).

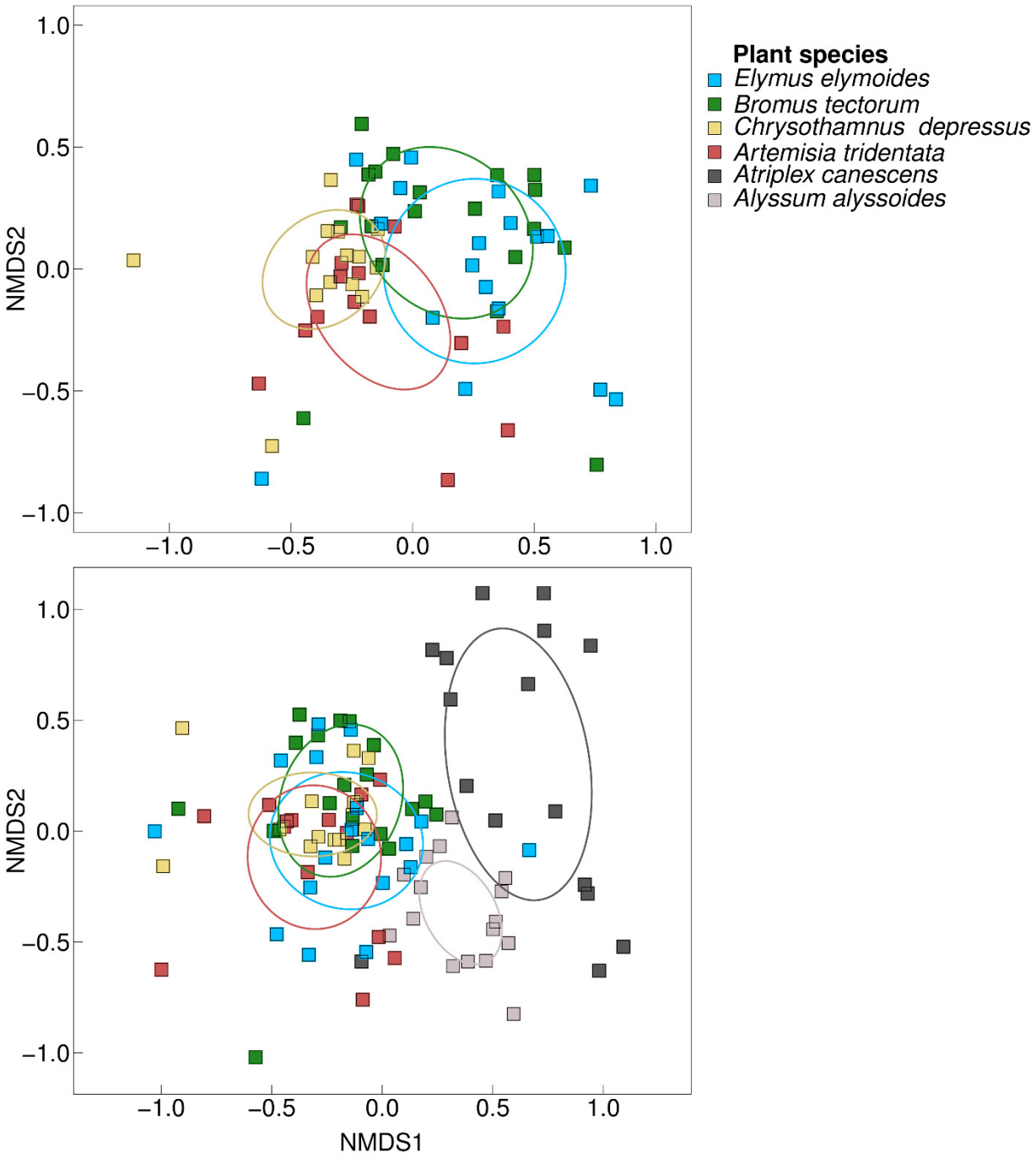

Figure 2: NMDS ordinations visualizing the leaf endophytic fungal communities from various plants species. Ellipses are drawn to include 95% of the variation for each group. A. Endophyte fungal communities of mycorrhizal plant species only (excluding Atriplex canescens and Alyssum alyssoides), B. Endophyte fungal communities of all plant species.

The pairwise PERMANOVAs comparing the fungal communities of particular pairs of plant species indicated that the endophytic fungal communities of the two grasses, *Bromus tectorum* and *Elymus elymoides*, were not significantly different (P = 0.352), and that the endophytic fungal communities of the two members of the Asteraceae, *Artemisia tridentata* and *Chrysothamnus depressus,* were not significantly different (P = 0.173). However, all of the comparisons among the remaining plant species were significantly different (P < 0.001; Table 1).

Table 1: Results of pairwise PERMANOVAs among plant species and distances between centroids of fungal communities for specific plant species. P values are displayed in the first row. Distance to centroid of each plant species is displayed in the second row. Shown in parentheses are the standard errors for distances, calculated by jackknife resampling.

	Bromus tectorum	*Chrysothamnus depressus*	*Artemisia tridentata*	*Elymus elymoides*	*Atriplex canescens*
Bromus tectorum					
Chrysothamnus depressus	P < 0.001 D = 0.378 (0.05)				
Artemisia tridentata	P < 0.001 D = 0.326 (0.05)	P = 0.173 D = 0.171 (0.05)			
Elymus elymoides	P = 0.364 D = 0.259 (0.05)	P < 0.001 D = 0.433 (0.06)	P < 0.001 D = 0.382 (0.06)		
Atriplex canescens	P < 0.001 D = 0.630 (0.07)	P < 0.001 D = 0.760 (0.05)	P < 0.001 D = 0.737 (0.05)	P < 0.001 D = 0.630 (0.07)	
Alyssum alyssoides	P < 0.001 D = 0.648 (0.03)	P < 0.001 D = 0.603 (0.03)	P < 0.001 D = 0.593 (0.04)	P < 0.001 D = 0.599 (0.03)	P < 0.001 D = 0.651 (0.05)

The distances between endophytic fungal community centroids ranged from 0.171 to 0.760 (Table 1) and, using data from all six species, the correlation between centroid distance and time since divergence was nearly significant (P = 0.066, R^2 = 0.235). After removing the two nonmycorrhizal species from this analysis, the correlation was significant (P = 0.045, R^2 = 0.672). It may be important to remove the nonmycorrhizal plant species from the correlation between divergence time and centroid distance because nonmycorrhizal plant species may have different communities of endophytic fungi for reasons in addition to phylogenetic distance; at least some produce antifungal compounds (Schreiner & Koide, 1993a, 1993b). These results are,

therefore, consistent with our prediction that phylogenetic distance is correlated with endophytic fungal community dissimilarity.

We also found that the structures of the endophytic fungal communities of *Alyssum alyssoides* (nonmycorrhizal, Brassicaceae) were quite different from those of *Atriplex canescens* (nonmycorrhizal, Amaranthaceae), and that these two nonmycorrhizal species had endophytic fungal communities that differed significantly from the four mycorrhizal plant species. In fact, the endophytic fungal communities associated with *Atriplex canescens* and *Alyssum alyssoides* were more distant from the endophytic fungal communities of the mycorrhizal plant species than those of the mycorrhizal plants were from each other. Phylogenetic distance alone may not have produced this pattern because both nonmycorrhizal species are more closely related to the *Asteraceae* than *Poaceae* is to the *Asteraceae.* Therefore, the results are consistent with our expectation that mycorrhizal status, independent of phylogeny, significantly influences the structure of endophytic fungal communities.

We note that the distance between the endophytic fungal communities of the two nonmycorrhizal plant species is similar to the distance between the communities of the two nonmycorrhizal plant species combined and the four mycorrhizal plant species combined. Thus, the endophytic fungal communities of the nonmycorrhizal plant species are as dissimilar from each other as they are from the communities of the mycorrhizal plant species. It is not surprising to find that the structure of the endophytic fungal communities differs significantly between *Alyssum alyssoides* and *Atriplex canescens* despite the fact that both are nonmycorrhizal. They are members of quite distantly related families and likely use different mechanisms for maintaining their non-mycorrhizal status (Tester et al., 1987).

We have herein presented evidence consistent with the hypotheses that phylogenetic relatedness and mycorrhizal status contribute significantly to variation in the structure of communities of endophytic fungi. While our study was rather limited in its scope and replication, our results suggest that these hypotheses are worthy of further exploration.

CONCLUSIONS AND FUTURE DIRECTIONS

In this thesis I demonstrated the presence of biotic filtering of endophytic fungal communities by a plant host. We showed that biotic filter of a single plant species can produce a uniform endophytic fungal community from rather disparate inoculum communities. We additionally showed variation in biotic filters among contrasting plant species, resulting in species that vary in their endophytic fungal communities even when provided with the same inoculum community. We further showed that variation among plant species had a phylogenetic origin and may be additionally linked to the mycorrhizal status of the species.

Given the fact that endophytic fungi can influence plant fitness, we hypothesized that the biotic filtering of endophytic fungal inoculum communities may be the result of natural selection because it could insure the development of a desirable endophytic fungal community irrespective of the inoculum source. It is also possible, however, that biotic filtering was not the result of natural selection for a specific endophytic fungal community *per se*, but rather was the consequence of selection for other traits. For example, leaf traits such as cuticle thickness, stomatal density, or leaf chemistry may be selected for by a range of abiotic environmental factors and may only incidentally select for a specific endophytic fungal community. Therefore, future research should identify the extent to which specific endophytic fungal communities are adaptive.

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APPENDIX 1

Table 1: Concentration of nutrient solution used in the inoculation experiment

Nutrient	Concentration (ppm)
N	120
K	115.9
Ca	100
P	26.2
S	10
Mg	20
B	0.2
Fe	2
Mn	1
Zn	0.3
Cu	0.2
Mo	0.016
Co	0.01

Table 2: PERMANOVA table of endophytic fungal communities from the inoculum as influenced by inoculum sources.

Factor	df	SS	MS	F	R^2	P
Inoculum source	4	6.589	1.647	7.603	0.268	<0.001
Residuals	83	17.973	0.217		0.732	
Total	87	24.559			1.000	

Table 3: PERMANOVA table of leaf endophytic fungal communities from *Bromus tectorum* in the inoculation experiment as influenced by the inoculation treatment.

Factor	df	SS	MS	F	R^2	*P*
Treatment	6	2.419	0.403	2.384	0.193	<0.001
Residuals	60	10.146	0.169		0.807	
Total	66	12.565			1.000	

Table 4: PERMANOVA table of leaf endophytic fungal communities from *Bromus tectorum* in the inoculation experiment as influenced by the inoculation treatment. The control treatment was excluded from this analysis

Factor	df	SS	MS	F	R^2	*P*
Treatment	5	0.762	0.152	0.864	0.077	0.624
Residuals	52	9.176	0.176		0.923	
Total	57	9.937			1.000	

Table 5: PERMANOVA table of root endophytic fungal communities from *Bromus tectorum* in the inoculation experiment as influenced by the inoculation treatment. The control treatment was excluded from this analysis

Factor	df	SS	MS	F	R^2	*P*
Treatment	6	3.056	0.509	4.928	0.334	<0.001
Residuals	59	6.098	0.103		0.666	
Total	65	9.154			1.000	

Table 6: PERMANOVA table of root endophytic fungal communities from *Bromus tectorum* in the inoculation experiment as influenced by the inoculation treatment. The control treatment was excluded from this analysis

Factor	df	SS	MS	F	R^2	*P*
Treatment	5	1.233	0.247	2.171	0.173	<0.001
Residuals	52	5.909	0.114		0.827	
Total	57	7.142			1.000	

APPENDIX 2

Table 1: PERMANOVA table for endophytic fungal communities of mycorrhizal plants only (excluding Atriplex canescens and Alyssum alyssoides), as influenced by host plant species.

Factor	df	SS	MS	F	R^2	P
Plant species	3	2.093	0.698	3.927	0.160	<0.001
Residual	62	11.012	0.178		0.840	
Total	65	13.105			1.0000	

Table 2: PERMANOVA table for endophytic fungal communities all plant species, as influenced by host plant species

Factor	df	SS	MS	F	R^2	P
Plant species	5	7.454	1.491	8.038	0.304	<0.001
Residual	92	17.063	0.186		0.696	
Total	97	24.516			1.0000	