A Culture-independent Approach to Understanding the Role of Soil Fungal Communities in *Bromus tectorum* Stand Failure

Nathan J. Ricks[1], Taryn Williamson[1], Susan E. Meyer[2], John M. Chaston[1], Craig E. Coleman[1]

[1]Department of Plant and Wildlife Sciences, Brigham Young University, Provo, UT, 84602

[2]US Forest Service Rocky Mountain Research Station, Shrub Science Lab, Provo, UT 84606

Keywords: *Bromus tectorum*, metagenomics, stand failure, cheatgrass, downy brome

Originality-Significance Statement

Stand failure or die-off is a phenomenon that has been observed in cheatgrass stands throughout the western United States. To provide support for the hypothesis that fungal pathogens are responsible for cheatgrass stand failure, we have identified communities of ascomycete fungi in the soil associated with cheatgrass infestations. We compare the composition of soil communities associated with stands that have experienced a die-off with the soil communities in stands where a recorded die-off has not occurred. The knowledge gained may aid efforts to slow the spread of cheatgrass in invaded habitats where cheatgrass continues to disrupt native ecosystems and to provide fine fuel for frequent rangeland wildfires.

Summary

Bromus tectorum (cheatgrass) is an invasive annual grass that has colonized large portions of the Intermountain Western United States. Cheatgrass stand failures have been observed throughout the invaded region, the cause of which may be related to the presence of several species of pathogenic fungi in the soil or surface litter. In this metagenomic study we compared the fungal communities between sites that have and have not experienced stand failure. Samples were taken from the soil and surface litter near Winnemucca, Nevada and in Skull Valley, Utah. Our results show distinct fungal communities associated with stand failure based on both geography and sample type. In both the Winnemucca and Skull Valley surface litter, there was an elevated abundance of the endophyte *Ramimonilia apicalis* in samples that had experienced a stand failure. Winnemucca surface litter stand failure samples had increased abundance of a potential pathogen in the genus *Comoclathris*. Skull Valley surface litter stand failure samples had increased abundance of the known cheatgrass pathogen *Clarireedia capillus-albis* while the soils had increased abundance of potential pathogens in the genera *Olpidium* and *Monosporascus*.

INTRODUCTION

Cheatgrass (*Bromus tectorum*) is an invasive annual grass that has colonized large portions of Intermountain Western North America. Native grass stands depleted by overgrazing have been replaced by this invader (Mack 1981). Originating in Eurasia, cheatgrass has spread quickly in the dry climate found in the Intermountain West. Cheatgrass will often establish itself in the open spaces between native plants, (Ziska *et al.* 2005) where it provides a flammable layer of plant litter in midsummer that drastically increases the frequency and intensity of rangeland wildfires (Brooks *et al.* 2004). Historically, in sagebrush ecosystems, fire intervals ranged between 60 and 110 years; however, once an area is invaded by cheatgrass, increased fuel loads shorten the fire interval to 3-5 years (Whisenant 1990). Following a burn, enough cheatgrass seeds survive that in the following years cheatgrass comes to dominate the community (Beckstead *et al.* 2011). As cheatgrass spreads, more landscapes are converted to cheatgrass monoculture in areas that were once dominated by sagebrush (Ziska *et al.* 2005). By accelerating the fire cycle, and displacing native plants, the invasion of cheatgrass represents a major threat to the biological diversity in the regions it invades (D'Antonio *et al.* 1992).

Stand failure is a common but poorly understood naturally occurring phenomenon in cheatgrass monocultures. Also known as ‘die-off’, stand failure occurs when complete mortality of both germinating seeds and preemergent seedlings prevents all seedling establishment. When stand failures occur, large areas previously occupied by a cheatgrass monoculture become largely empty of any visible vegetation. Stand failures represent a natural form of cheatgrass control and can provide an opportunity for native plant restoration (Meyer *et al.* 2014). For example, when native grass seeds were planted in a stand failure area, native grasses were able to outcompete cheatgrass in the following years (Baughman *et al.* 2016). Hypotheses for the occurrence of stand failures have ranged from abiotic factors such as weather, to a number of different fungal agents such as *Microdochium nivale* and

Ustilago bullata (Piemeisel 1938; Klemmedson *et al.* 1964; Meyer et al. 2010). Several fungal species have been identified that act as pathogens towards cheatgrass, including *Pyrenophora semeniperda, Epicoccum nigrum,* an undescribed species of *Fusarium* belonging to the *Tricinctum* group (*Fusarium* Link sp. n., FTSG) and *Clarireedia capillus-albis*, a newly described species responsible for so called bleach blonde syndrome (Meyer *et al.* 2016). *Pyrenophora semeniperda*, *E. nigrum* and FTSG are pathogens that kill seeds in the seed bank and are potential stand failure causal agents (Beckstead *et al.* 2007; Stewart *et al.* 2009; Meyer *et al.* 2016). Nevertheless, Baughman and Meyer (Baughman *et al.* 2013) suggested that *P. semeniperda* may not be a direct cause of stand failure because of its inability to kill rapidly germinating seeds. They concluded that it could play a role in the rate of post-stand failure recovery through its impact on dormant seeds in the carry-over seed bank. Both FTSG and *E. nigrum* can kill rapidly germinating, nondormant seeds, especially under conditions of low water potential, and have been demonstrated to significantly reduce stand emergence under field conditions (S. Meyer, unpublished data). *Clarireedia capillus-albis* is a crown-infecting pathogen that leaves cheatgrass plants stunted and straw-colored, with inflorescences that fail to mature. When disease reaches epidemic levels in stands, it can cause the plants to collapse en masse and form a mat of thick dense litter. As *C. capillus-albis* does not impact seeds or seedling emergence, if it is a causal agent in stand failure, its effects must be indirect. It is possible that the dense litter left behind by the disease could create an environment which promotes the attack of other pathogenic fungi (Meyer *et al.* 2016).

The ability of known fungal pathogens to cause cheatgrass mortality suggests they may play a role in stand failure. Despite the work done on specific cheatgrass pathogens, the fungal community associated with stand failures and with cheatgrass seed beds in general is poorly understood. The objective of the present research was to use a metagenomic approach to understand the fungal community structure in soils where cheatgrass dominates and where stand failures have occurred. Our goal was to elucidate the causal agents of stand failures and the potentially complex interactions among plant pathogens and non-pathogenic fungi that may influence their impact. We wished to test the

hypothesis that whatever causes stand failure persists in the soil and is manifested as a difference in fungal community composition between stand failure and non-stand failure sites. We chose sampling sites in Skull Valley, Utah and near Winnemucca, Nevada based on modeling using remote sensing technology (Weisberg *et al.* 2017). We reasoned that community differences common to the Utah and Nevada study areas, separated by hundreds of miles, would reflect shared, biologically important differences between stand failure and non-stand failure sites. Our strategy was to combine PacBio long-read sequencing of the ITS1 and 2 region for maximizing taxonomic identification capability with high-yield Ilumina sequencing of the ITS1 region alone for maximizing depth of coverage.

RESULTS

Soil Fungal Communities Vary with Soil Type, Geographic Location, and History of Stand Failure

We used a two-step approach to sequencing the fungal DNA in the sampled soils. First, we created a reference library of sequences in the samples by sequencing an amplicon of the fungal ITS1 and ITS2 regions as a single read using PacBio sequencing technology. Ten pools of samples from the surface litter and ten pools of samples from the soil were generated from 10 of the 19 sampling locations indicated in Table S1. Sequencing of the ITS amplicons from these pools yielded 123,664 reads (per pool mean 6,182 +/- 1,440 reads; median 6,319 reads) and 614 fungal operational taxonomic units (OTUs). Using the UNITE database, taxonomic assignments were made to the species level for 28% of OTUs (Table S2). In the second step, we sequenced the ITS1 amplicons for each soil sample individually on the Illumina HiSeq platform yielding 13,000,017 reads (per pool mean 28,509 +/- 67,274 reads; median 8,677 reads). After quality filtering, the reads were assigned to a total of 525 amplicon sequence variants (ASVs). Use of the ITS1/2 reference set increased assignment of reads at the species level from 37.99% to 43.82% (Table S2). Rarefaction curves suggested adequate saturation of the sampling (Fig. S1). Of all ASVs, 84% were assigned to just 30 taxonomic groups, primarily from

the Ascomycota and Basidiomycota (Table S3), and just 3 ASVs were 'core', or present in all rarefied samples (Table S4). Some ASVs were also detected that corresponded to *Clarireedia capillus-albis*, FTSG, *Epiccocum nigrum*, *Pyrenophora seminiperda, Ustilago bullata*, and *Microdochium* sp., all of which are known pathogens of cheatgrass (Piemeisel 1938; Klemmedson and Smith 1964; Meyer et al 2016). Overall, the taxa identified by the analysis follow expected norms and included candidate species that could potentially have been responsible for cheatgrass stand failures in the affected areas.

PERMANOVA and principal coordinate analysis (PCoA) were used to define the factors that contributed to variation in the sampling site fungal communities (Fig. 1). Fungal microbiota composition varied significantly with each of sample type (soil or surface litter), location (Skull Valley, UT, USA or Winnemucca, NV, USA), and history of stand failure (yes or no) according to both of the weighted and unweighted Unifrac distance metrics examined (Table 1). Because sample type and location were each significant covariates in the analysis the data were split into four sampling groups to focus on the variation in fungal communities arising from stand failure history (Table 2). These individual analyses showed significant differences in fungal community composition of surface litter with stand failure in both Skull Valley and Winnemucca, except for the Skull Valley samples when analyzed by weighted Unifrac distance. In contrast, there were no significant differences in fungal composition of soil samples from either Winnemucca or Skull Valley by either metric. Together, these results suggest that in areas that experienced a cheatgrass stand failure, the fungal communities of surface litter are more strongly impacted by the causal conditions than are the soil communities.

Analysis of composition of microbiomes (ANCOM) revealed specific ASVs that varied in abundance with stand failure in surface litter at each site (Table 3). Among these, just two ASVs were more abundant in stand failure sites versus no-stand failure litter samples at both Winnemucca and Skull Valley: one assigned to the class *Tremellomycetes* and another to the species *Ramimonilia*

apicalis. None of the known cheatgrass pathogens varied significantly between stand failure and non-stand failure sites. We also used ANCOM to identify fungal ASVs that varied with sample type and location, independent of stand failure, revealing 103 and 30 ASVs that varied significantly with location (Table S5), and sample type (Table S6), respectively. These included FTSG having a greater abundance in soils, and Winnemucca having a greater abundance of *C. capillus-albis*.

α-Diversity varies minimally with sample type, but not location or stand failure history

Faith and Shannon diversity metrics were used to test for differences in α-diversity in the fungal communities within year, sample type, location, and history of stand failure (Figs. S2 and S3). The soil samples had larger Faith and Shannon diversity index values than surface litter, indicating a greater diversity of fungi present in the soil compared to the surface litter. Also, Winnemucca samples had a higher Shannon diversity values than did samples from Skull Valley. All other differences, including with history of stand failure, were non-significant. Together, these data reveal greater taxonomic diversity in soil versus surface litter samples, and greater diversity in the Winnemucca samples than Skull Valley.

Long-term signal in fungal community composition

One hypothetical expectation is that there is a linear change in the abundance of specific, possibly causal, fungal species with time from stand failure. If so, the fungal communities at sites with recent versus distant stand failures might be expected to be very different in composition. We tested if this was the case in our data by examining the difference between each stand failure site, relative to the control no stand failure sites, with time. We used weighted Unifrac distances for this analysis (Fig. S4). Weighted unifrac distances of Surface litter, but not soil, samples from both Skull Valley and Winnemucca varied significantly over time. At Winnemucca, only the 2015 site differed in distance to

the non-stand failure sites, whereas at Skull Valley, all years that had experienced a stand failure differed from the non-stand failure sites.

An alternative hypothesis to linear change with time is that the fungal community is permanently changed following stand failure. If this were the case, all sites that experienced a stand failure would be more closely related to each other than to the sites that had never experienced a stand failure. To test this hypothesis, we compared the Unifrac distances of samples from each year to all other samples, binned into stand-failure or non-stand-failure groups (Fig. S5). Unweighted unifrac distances in Skull Valley, but not Winemucca, sites consistently showed that years affected by a stand failure were more similar to other stand failure sites than to sites that had not experienced a stand failure. Therefore, stand failure can but does not necessarily reshape the fungal composition of both the soil and surface litter in ways that are different from the original composition for at least 25 years after the die-off event.

DISCUSSION

Cheatgrass seed banks contain a wide variety of fungal species. Despite there being a large number of ASVs present, the thirty most abundant taxa comprised 84% of all sequence reads. We conclude that these 30 ASVs represent the numerically abundant cheatgrass seed bed fungal community. While we did not collect any functional information on the taxa detected, we can infer functions for some groups. For example, *Keissleriella, Preussia, Sparticola* and *Didymosphaeriaceae* species most likely act as saprophytes (Cannon *et al.* 2007). Others, such as *C. capillus-albis* and *Olpidium brassicae* are known plant pathogens (Tewari *et al.* 1983; Meyer *et al.* 2016). There are also a large percentage of ASVs, such as *Vishniacozyma globispora, Cryptococcus, Naganishia,* and *Holtermanniella takashimae* within the *Tremellomycetes* class of Basidiomycetes. Many fungi in this class are yeasts that act as parasites towards other fungi. It is unknown why these species are found so abundantly in cheatgrass communities, but it may be that the cheatgrass environment is conducive to their growth.

Effects of Stand Failure on Fungal Community

The significant interaction between stand failure history and location is supported by the finding that different taxa are responsible for the shifts in the fungal microbiota between Skull Valley and Winnemucca. While it is premature to conclude from the PERMANOVA results that the causal agent of stand failures is found in the surface litter, it does suggest that there are major community differences between stand failure and non-stand failure sites found in the surface litter that are not seen in the soil.

Recovery of Fungal community

We detected a significant effect of years since stand failure on the fungal surface litter communities in both locations in our study, with one location showing a partial shift towards the non-die-off community (Winnemucca, NV), and the other displaying long-term divergence from samples collected in areas that never experienced a die-off (Skull Valley, UT) (Fig S4). Because this effect was detected using weighted, but not un-weighted Unifrac distances, this implies there are significant differences in the abundances of fungal species of sites affected by stand failure compared to those not affected by stand failure (Fig. S4). The community effects appear to be limited to the surface litter and more prevalent in Skull Valley, though the reasons for this are unknown and may be related to the soil composition or chemistry, the environment, elevation, or other uncharacterized factors.

In at least some sites that are affected by a stand failure, the fungal community shown changes that persist for long periods of time (at least 28 years) (Fig. S5). As these results were seen in the unweighted, but not weighted distances, they may affect the presence, but not abundance, of key community members.

More abundant ASVs at stand failure sites could be implicated as causal agents of stand failure; or alternatively, as organisms whose growth was promoted by stand failure. Other interpretations are that other fungi differentially abundant in the different locations were separate and independent causes of stand failure; or that fungal communities surveyed in years after stand failure do not directly reflect the causes of stand failure. Despite this, our data still suggest that cheatgrass stand failure has long-term effects on the fungal community of surface litter up to 28 years after a stand failure.

Fungi with Increased Abundances

A shared finding between the two geographic areas is that *Ramimonilia apicalis* and an unidentified fungus belonging to the class *Tremellomycetes* are more abundant at stand failure sites in both study locations. The environmental consequences of *Ramimonilia apicalis* presence are unknown, but it has been identified previously as as a rock inhabiting-fungus in Spain (Egidi *et al.* 2014), in the brain tissue of Alzheimer patients (Alonso *et al.* 2017), and as an endophyte in cheatgrass communities (Ricks *et al.* 2019). Endophytes live within plants, mostly without causing disease; however, with varying environmental conditions, endophytes can change to pathogens (Jia *et al.* 2016; Rai *et al.* 2016), and we cannot rule out that environmental cues could trigger *R. apicalis* to act as a pathogen towards cheatgrass. Conversely, we favor an explanation where the *Tremellomyctes* ASV grows opportunistically under stand failure conditions. There is little evidence of fungi of this class being pathogenic towards any type of plant, although they can be pathogenic towards animals and other fungi (van der Klei *et al.* 2011). Therefore, it seems more likely to us that the fungus belonging to the *Tremellomycetes* interacts with the stand-failure fungal community in a way that allows it to thrive, although the mechanisms for such actions are currently unknown.

Clarireedia capillus-albis is the only known cheatgrass pathogen (Meyer *et al.* 2016) that displayed greater abundance in stand failure versus no-stand failure sites in our study (Fig. 2). These data suggests it may have had a role in stand failure in at least two distinct locations in the Intermountain west. The genus *Clarireedia* contains pathogens responsible for dollar spot disease in turf grasses (Salgado-Salazar *et al.* 2018), as well as *C. capillus-albis*, a known cheatgrass pathogen. While the *Clarireedia* ASV detected in the soil microbiome may not be identical to *C. capillus-albis*, it could be a closely related species that functions pathogenically in a similar manner.

One limitation of our design is that by the time we had sampled each of our post-stand failure soils, cheatgrass was growing abundantly in all locations. Stand failure is temporary, after which cheatgrass communities recover and quickly fill the empty space. This usually rapid re-colonization means that there are few or no areas of sustained cheatgrass stand failure. It may also mean that we should not have expected to find fungal pathogens responsible for the stand failure in these areas, unless the recovery growth of cheatgrass is of pathogen-resistant cheatgrass lineages. An interesting idea for future study would be to collect samples from areas experiencing a stand failure in real time, and test if specific pathogens are common to these areas. Such additional studies could find the use of our PacBio reference, or the description of common fungal organisms across a variety of conditions and soil types, a useful benchmark comparison.

Conclusions

Overall, this study gives a greater understanding of the fungal dynamics within cheatgrass soils and surface litter. Fungi found commonly in these environments have been identified. Our analysis confirmed key differences in the overall community composition, as well as the abundance of individual members of the fungal community, in areas that did or did not experience cheatgrass stand failure. Most differences with stand failure were concentrated in the surface litter and were geography-specific. The increased abundance of *Ramimonilia apicalis* in the surface litter of both Skull Valley and

Winnemucca was a shared difference between locations. Additionally, the abundance of fungal pathogens such as *Olpidium* sp., *Monosporascus* sp.,and *Comoclathris* sp., warrants further investigation to determine if these are causal agents of stand failure. Together, these findings provide insight in the fungal community of a largely unstudied system.

EXPERIMENTAL PROCEDURES

Collection of Environmental Samples

A remote sensing method, with access to the Landsat archive (https://www.usgs.gov/land-resources/nli/landsat), was used to find locations near Winnemucca, Nevada and within Skull Valley, Utah that have experienced stand-failure in the past 30 years. (Weisberg *et al.* 2017). A total of 19 sites were identified, 10 near Winnemucca and 9 in Skull Valley, based on the year when a stand-failure last occurred (Table S1). The year of the most recent stand failure at each of these sites ranged from 1990 to 2015, with two sampling sites at each location where no stand failure has been detected since Landsat data became available. At each site 9 samples of surface litter and soil were collected at randomly selected points along each of four 10-meter transects. Soil samples were collected by pressing a tin can 6 cm diameter x 2.5 cm height into the soil until flush with the surface, then lifting the can and soil out with a small trowel and storing in a small paper sack. The surface litter was removed and placed in a separate paper sack prior to soil sample removal. For both litter and soil, 3 pools of 3 samples each were created for each transect, yielding a total of 12 soil and 12 litter pools at each site. Soil and surface litter pools were dried at room temperature for two weeks and homogenized separately using a coffee grinder. DNA was extracted from 100 g of each homogenized pool using a Quick-DNA Fecal/Soil Microbe Kit (Zymo Research, Irvine, CA).

Preparation of the Long-Read Reference Library

Of the 19 sites where samples were collected, 12 were chosen to provide DNA sequence information for a taxonomic reference library by producing 20 super-pools (Table S1). Soil DNA and surface litter DNA super-pools for each of the 8 sites were created by combining equal amounts of DNA extracted from the 12 individual pools described in the previous section. For the two sites where no stand failure has been detected, single soil and litter pools were made from all samples collected at each location. Each of the 20 DNA super-pools was used to create an individual DNA sequencing library by PCR amplifying the ITS45 region using AccuPrime Pfx DNA polymerase (Invitrogen, Carlsbad, CA) with ITS4 and ITS5 primers (White *et al.* 1990). For library preparation and sample identification, the primers were modified by adding 20 unique PacBio barcode tails (Table S7). The following conditions were used for PCR: initial denaturation at 95 C for three minutes, 25 cycles of denaturation (95 C for 30 s), annealing (52 C 30 s), and extension (72 C 1 m) and a final extension step at 72 C for 5 minutes. The PCR products were cleaned using a Zymo DNA Clean and Concentrator kit (Zymo Research, Irvine, CA). The 20 libraries were submitted to the BYU DNA Sequencing Center (Provo, UT) for sequencing on a PacBio Sequel platform (Menlo Park, CA) using a standard Amplicon protocol with SMRTbell adapters. Demultiplexed sequences from read files were imported into a single-end QIIME2 artifact. Chimeric sequences were removed, sequences were dereplicated, and OTUs were identified at 97% similarity using vsearch (Rognes *et al.* 2016). Taxonomy was assigned using the QIIME Naive Bayes classifier (Bokulich *et al.* 2018) and the UNITE fungal database (Nilsson *et al.* 2018) as a reference. The sequences and their taxonomic assignments were combined with a downloaded version of the UNITE fungal database to use for the taxonomic assignment of Illumina sequences as described below. The PacBio reads are publicly available in the Short Read Archive of GenBank under project PRJNA68186.

Short-Read Sequencing

All individual samples were used to prepare the Illumina short-read library. With two types of samples per site (surface litter and soil), 12 replicates in each sample type and 19 sites, there were a total of 456 samples. Using a two-step PCR strategy (Cruaud *et al.* 2017), the ITS1 region of the fungal genome was amplified, followed by barcoding and multiplexing. AccuPrime Pfx DNA polymerase was used for all amplifications. In the first step, the ITS1 region was amplified using primers ITS2-KYO2 and ITS1-F_KYO1 (Toju *et al.* 2012) and the following parameters: initial denaturation at 95°C for 3 minutes, followed by 25 cycles consisting of denaturation (95°C for 30 s), annealing (52°C 30 s), and extension (72°C 1 min) and a final extension step at 72°C for 5 minutes. In the second PCR step barcodes were added to the amplified region (Tables S8 and S9) using parameters identical to the first step except that there were 12 cycles rather than 25, and the annealing temperature was 55°C instead of 52°C. Samples were pooled and submitted to the BYU sequencing center for 2 x 250 sequencing on an Illumina HiSeq 2500 platform using custom sequencing primers ITS2-KYO2 and ITS1-F_KYO1 primers (Toju *et al.* 2012). After sequencing, reads were automatically demultiplexed and returned as paired-end reads. The Illumina reads are publicly available in the Short Read Archive of GenBank under project PRJNA68186.

The sequenced reads were imported into QIIME2 where the paired-end reads were joined, chimeric sequences were removed, sequences were dereplicated and ASVs were called using the DADA2 pipeline (Callahan *et al.* 2016). Using the QIIME2 Naive Bayes classifier (Bokulich *et al.* 2018), a combined database of the previous PacBio runs and the UNITE database (Nilsson *et al.* 2018), each ASV was assigned a taxonomic identity. Sequences that were not found in at least 12 samples were removed. Samples were rarefied to 10,000 reads per sample, to maximize reads per sample and minimize sample loss (Fig. S1). After rarefying the data, the rarefied tables were subsetted individually before performing analyses. The groups were: 1) all samples; 2) soil samples from Skull Valley; 3)

surface litter samples from Skull Valley; 4) soil samples from Winnemucca; and 5) surface litter samples from Winnemucca.

Analysis of the Long and Short Read Sequence Data

Using the ASV table created from the Illumina sequencing, weighted and unweighted Unifrac distance matrices were calculated in QIIME2 (Caporaso *et al.* 2010), and used in Principal Coordinate Analysis (PCoA) plots and for PERMANOVA. PERMANOVA was performed using the R vegan package (Oksanen *et al.* 2019). Using Analysis of Composition of Microbiomes (ANCOM) (Mandal *et al.* 2015), ASV tables from each the four primary sample groups were tested for differences in the composition of microbiomes between sample treatments. To find differences in fungal abundances of previously hypothesized causes of stand failure (Meyer *et al.* 2016) that may have been missed due to ANCOM's multiple comparison correction we performed Wilcoxon signed rank tests in R. The Faith phylogenetic diversity (Faith 1992) and Shannon diversity (Pielou 1966), were calculated in QIIME2.

ACKNOWLEDGMENTS

We are grateful for the assistance of the following individuals involved in collecting soil samples for this study: Joseph Brehm, Alexa Jensen, Cassady Harris and Kody Rominger. Funding for this research was through the USDA Forest Service Rocky Mountain Research Station agreement no. 2016-JV-11221632-123.

LITERATURE CITED

Alonso, R., Pisa, D., Aguado, B., and Carrasco, L. (2017) Identification of fungal species in brain tissue from alzheimer's disease by next-generation sequencing. J Alzheimers Dis 58:55-67.

Baughman, O.W., and Meyer, S.E. (2013) Is *Pyrenophora semeniperda* the cause of downy brome (*Bromus tectorum*) die-offs? Invasive Palnt Sci Manag 6:105-111.

Baughman, O.W., Meyer, S.E., Aanderud, Z.T., and Leger, E.A. (2016) Cheatgrass die-offs as an opportunity for restoration in the Great Basin, USA: Will local or commercial native plants succeed where exotic invaders fail? J Arid Environ 124:193-204.

Beckstead, J., Meyer, S.E., Molder, C.J., and Smith, C. (2007) A Race for survival:Can *Bromus tectorum* seeds escape *Pyrenophora semeniperda*-caused mortality by germinating quickly? Ann Bot 99:907-914.

Beckstead, J., Street, L.E., Meyer, S.E. and Allen, P.S. (2011) Fire effects on the cheatgrass seed bank pathogen *Pyrenophora semeniperda*. Rangel Ecol Manag 64:148-157.

Bokulich, N.A., Kaehler, B.D., Rideout, J.R., Dillon, M., Bolyen, E., Knight, R., *et al.* (2018) Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome 6:90.

Brooks, M.L., D'antonio, C.M., Richardson, D.M., Grace, J.B., Keeley, J.E., DiTomaso, *et al.* (2004) Effects of invasive alien plants on fire regimes. BioScience 54:677-688.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., and Holmes, S.P. (2016) DADA2:High-resolution sample inference from Illumina amplicon data. Nat Methods 13:581.

Cannon, P.F., and Kirk, P.M. (2007) Fungal families of the world. Oxfordshire, UK: CAB International.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., *et al.* (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335-336.

Cruaud, P., Rasplus, J.-Y., Rodriguez, L.J., and Cruaud, A. (2017) High-throughput sequencing of multiple amplicons for barcoding and integrative taxonomy. Sci Rep 7:41948.

D'Antonio, C.M., and Vitousek, P.M. (1992) Biological invasions by exotic grasses, the grass/fire cycle, and global change. Annu Rev Ecol Evol Syst 23:63-87.

Egidi, E., De Hoog, G., Isola, D., Onofri, S., Quaedvlieg, W., De Vries, M., *et al.* (2014) Phylogeny and taxonomy of meristematic rock inhabiting black fungi in the *Dothideomycetes* based on multi-locus phylogenies. Fungal Divers. 65:127-165.

Faith, D.P. (1992) Conservation evaluation and phylogenetic diversity. Biol Conserv 61:1-10.

Jia, M., Chen, L., Xin, H.-L., Zheng, C.-J., Rahman, K., Han, T., and Qin, L.-P. (2016) A friendly relationship between endophytic fungi and medicinal plants:a systematic review. Front Microbiol 7:906.

Klemmedson, J.O., and Smith, J.G. (1964) Cheatgrass (*Bromus tectorum* L.). Bot Rev 30:226-262.

Mack, R.N. (1981) Invasion of *Bromus tectorum* L. into western North America:An ecological chronicle. Agro-Ecosystems 7:145-165.

Mandal, S., Van Treuren, W., White, R.A., Eggesbø, M., Knight, R., and Peddada, S.D. (2015) Analysis of composition of microbiomes:a novel method for studying microbial composition. Microb Ecol Health Dis 26:27663.

Meyer, S.E., Beckstead, J., and Pearce, J. (2016) Community ecology of fungal pathogens on *Bromus tectorum*. In Exotic Brome-Grasses in Arid and Semiarid Ecosystems of the Western US: Causes, Consequences, and Management Implications. Germino, M.J., Chambers, J.C. and Brown, C.S. (eds). New York City: Springer International Publishing, pp. 193-223.

Meyer, S.E., Franke, J.L., Baughman, O.W., Beckstead, J., and Geary, B. (2014) Does *Fusarium*-caused seed mortality contribute to *Bromus tectorum* stand failure in the Great Basin? Weed Res 54:511-519.

Meyer, S.E., Nelson, D.L., Clement, S. and Ramakrishnan, A. (2010) Ecological genetics of the *Bromus tectorum* (Poaceae)–*Ustilago bullata* (Ustilaginaceae) pathosystem: A role for frequency−dependent selection? Am J Bot 97:1304-1312.

Nilsson, R.H., Larsson, K.-H., Taylor, A.F., Bengtsson-Palme, J., Jeppesen, T.S., Schigel, D., *et al.* (2018) The UNITE database for molecular identification of fungi:handling dark taxa and parallel taxonomic classifications. Nucleic Acids Res 47.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., *et al.* (2019) Vegan: Community Ecology Package. R package version 2.5-5.

Pielou, E.C. (1966) Shannon's formula as a measure of specific diversity:its use and misuse. Am Nat 100:463-465.

Piemeisel, R.L. (1938) Changes in weedy plant cover on cleared sagebrush land and their probable causes. US Department of Agriculture Technical Bulletin No. 654.

Rai, M., and Agarkar, G. (2016) Plant–fungal interactions:what triggers the fungi to switch among lifestyles? Crit Rev Microbiol 42:428-438.

Ricks, K.D., and Koide, R.T. (2019) Biotic filtering of endophytic fungal communities in *Bromus tectorum*. Oecologia:1-11.

Rognes, T., Flouri, T., Nichols, B., Quince, C., and Mahé, F. (2016) VSEARCH: A versatile open source tool for metagenomics. PeerJ 4:e2584.

Salgado-Salazar, C., Beirn, L.A., Ismaiel, A., Boehm, M.J., Carbone, I., Putman, A.I., *et al.* (2018) *Clarireedia*: A new fungal genus comprising four pathogenic species responsible for dollar spot disease of turfgrass. Fungal Biol 122:761-773.

411 Stewart, T., Allen, P., and Meyer, S.E. (2009) First report of *Pyrenophora seminiperda* in Turkey and
412 Greece. Plant Disease 93:1351-1351.
413 Tewari, J., and Bains, P. (1983) Fungi associated with the roots of clover in Alberta. 1. *Olpidium*
414 *brassicae* and *Ligniera* sp. Canadian Plant Disease Survey 63:35-37.
415 Toju, H., Tanabe, A.S., Yamamoto, S., and Sato, H. (2012) High-Coverage ITS primers for the DNA-
416 based identification of Ascomycetes and Basidiomycetes in environmental samples. PLoS One
417 7:e40863.
418 van der Klei, I., Veenhuis, M., Brul, S., Klis, F.M., De Groot, P.W., Müller, W.H., *et al.* (2011)
419 Cytology, cell walls and septa: A summary of yeast cell biology from a phylogenetic
420 perspective. In The Yeasts, 5th edn. Kurtzman, C.P., Fell, J.W., and Boekhout, T. (eds).
421 Amsterdam: Elsevier. pp. 111-128.
422 Weisberg, P.J., Dilts, T.E., Baughman, O.W., Meyer, S.E., Leger, E.A., Van Gunst, K.J., and Cleeves, L.
423 (2017) Development of remote sensing indicators for mapping episodic die-off of an invasive
424 annual grass (*Bromus tectorum*) from the Landsat Archive. Ecol Indic 79:173-181.
425 Whisenant, S.G. (1990) Changing fire frequencies on Idaho's Snake River plains: Ecological and
426 management implications. In Proceedings - Symposium on Cheatgrass Invasion, Shrub Die-off,
427 and Other Aspects of Shrub Biology and Management. General Technical Report INT-GTR-
428 276. McArthur, E.D., Romney, E.M., Smith, S.D., and Tueller, P.D. (eds). Ogden:USDA Forest
429 Service, pp. 4-10.
430 White, T.J., Bruns, T., Lee, S., and Taylor, J. (1990) Amplification and direct sequencing of fungal
431 ribosomal RNA genes for phylogenetics. In PCR Protocols: A Guide to Methods and
432 Applications. Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (eds). Cambridge: Academic
433 Press, pp. 315-322.

434 Ziska, L.H., Reeves, J.B., and Blank, B. (2005) The impact of recent increases in atmospheric CO2 on
435 biomass production and vegetative retention of cheatgrass (*Bromus tectorum*): Implications for
436 fire disturbance. Glob Chang Biol 11:1325-1332.

437

FIGURES

Figure 1. Principal coordinate plots of ITS1 ASVs, including A) the first two principal coordinates and B) the two principal coordinates, 2 and 5, that best show visual separation of the samples by the two main variables.

Figure 2. Abundance of Clarireedia capillus-albis. Log abundance in samples that have (Yes) and have not (No) had a stand failure in the past.

438

TABLES

Table 1. PERMANOVA results. Df = degrees of freedom, SS = sum of squares, MS = mean of squares, F = F statistics, R^2 = R^2 value. P = p-value.

	Weighted						Unweighted					
	df	**SS**	**MS**	**R**	**R^2**	**p**	**df**	**SS**	**MS**	**F**	**R^2**	**p**
Sample Type	1	1.07	1.07	6.66	0.03	0.005	1	1	1	10.14	0.04	0.001
Location	1	1.33	1.33	8.3	0.04	0.001	1	1.35	1.35	13.73	0.06	0.001
Stand Failure History	1	0.38	0.38	2.36	0.01	0.054	1	0.3	0.3	3.02	0.01	0.001
Location*Stand Failure Histroy	1	0.55	0.55	3.45	0.01	0.014	1	0.27	0.27	2.76	0.01	0.001
Residuals	211	33.9	0.16		0.91		211	20.71	0.1		0.88	
Total	215	37.232			1		215	23.62			1	

446 *Table 2. Subsampled PERMANOVA results.*

	Weighted						Unweighted					
	df	**SS**	**MS**	**F**	**R^2**	**p**	**df**	**SS**	**MS**	**F**	**R^2**	**p**
	Winnemucca Soil											
Stand Failure History	1	0.19	1.88	1.16	0.02	0.28	1	0.14	0.14	1.54	0.03	0.052
Residuals	49	7.92	0.16		0.98		49	4.58	0.09		0.97	
Total	50	8.11			1		50	4.72			1	
	Winnemucca Surface Litter											
Stand Failure History	1	0.30	0.30	1.87	0.03	0.09	1	0.16	0.16	1.54	0.02	0.04
Residuals	64	10.36	0.16		0.97		64	6.51	0.10		0.98	
Total	65	10.66			1		65	6.67			1	
	Skull Valley Soil											
Stand Failure History	1	0.17	0.17	1.16	0.03	0.3	1	0.22	0.22	2.30	0.05	0.002
Residuals	40	5.74	0.14		0.97		40	3.86	0.10		0.95	
Total	41	5.91			1		41	4.09			1	
	Skull Valley Surface Litter											
Stand Failure History	1	0.63	0.63	3.74	0.06	0.018	1	0.24	0.24	2.52	0.04	0.001
Residuals	5	9.20	0.17		0.94		55	5.27	0.10		0.96	
Total	56	9.82			1		56	5.51			1	

447

448

Table 3. ASVs identified by ANCOM. Shows the taxonomic identity and the relative abundance of ASV that differed between locations with stand failure. (+) abundance in locations with stand failure, (-) abundance in locations with no stand failure, (A) Ascomycota, (B) Basidiomycota, (D) Dothideomycetes, (L) Leotiomycetes, (O) Olpidiomycota, (Ol) Olpidiomycetes, (T) Tremellomycetes, (S) Sordariomycetes, (SL) surface litter.

				Taxonomy					
	GenBank ID	**+**	**-**	**Phylum**	**Class**	**Order**	**Family**	**Genus**	**Species**
Winnemucca SL	**MK281667.1**	506.2	32.7	A	D	Botryosphaeriales	Planistromellaceae	Ramimonilia	
	MK281810.1	343.5	18.2	B	T				
	MK281714.1	228.9	9.1	A	D	Pleosporales	Pleosporaceae	Comoclathris	
Winnemucca Soil	**MK281810.1**	112.3	11.3	B	T				
	MK281667.1	108.2	15	A	D	Botryosphaeriales	Planistromellaceae	Ramimonilia	apicalis
Skull Valley SL	**MK281916.1**	1503.6	117.2	A	D	Pleosporales	Lentitheciaceae	Keissleriella	
	MK281802.1	518.5	70.4	A	D	Pleosporales	Sporormiaceae	Sparticola	
	MK281737.1	513.3	6.8	A	S	Coniochaetales	Coniochaetaceae	Coniochaeta	polymorpha
	MK281662.1	165	14.4	B	T	Filobasidiales	Filobasidiaceae	Naganishia	friedmannii
	MK281822.1	67.6	3.7	A	L	Helotiales	Rutstroemiaceae	Clarireedia	
	MK281810.1	45.4	5	B	T				
	MK281667.1	44.9	7.7	A	D	Botryosphaeriales	Planistromellaceae	Ramimonilia	apicalis
	MK281941.1	44.6	5.1	B	T	Tremellales	Tremellaceae	Cryptococcus	
	MK281736.1	14.2	424.6	B	T	Filobasidiales	Filobasidiaceae	Naganishia	albida
	MK281670.1	9.2	0.7	A	D	Pleosporales	Lentitheciaceae	Keissleriella	
	MK281899.1	7.1	28.7	A	D	Pleosporales			
	MK281660.1	2.6	176.1	B	T	Tremellales	Bulleribasidiaceae	Vishniacozyma	globispora
	MK281900.1	2.1	59.5	A	D	Pleosporales	pleosporaceae	Neocamarosporium	
	MK281809.1	1.4	37.9	B	T	Filobasidiales	Filobasidiaceae	Filobasidium	magnum
Skull Valley Soil	**MK281916.1**	1015.5	58.3	A	D	Pleosporales	Lentitheciaceae	Keissleriella	
	MK281699.1	332.8	48.8	O	Ol	Olpidiales	Olpidiaceae	Olpidium	
	MK281802.1	169.9	18	A	D	Pleosporales	Sporormiaceae	Sparticola	
	MK281736.1	104.8	538.2	B	T	Filobasidiales	Filobasidiaceae	Naganishia	albida
	MK281941.1	42.9	9.5	B	T	Tremellales	Tremellaceae	Cryptococcus	
	MK281743.1	21.1	4.5	A	S	Xylariales	Diatrypaceae	Monosporascus	
	MK281899.1	14.8	201.7	A	D	Pleosporales			
	MK281743.1	4.5	21.1	A	S	Xylariales	Diatrypaceae	Monosporascus	
	MK281660.1	2.3	21.5	B	T	Tremellales	Bulleribasidiaceae	Vishniacozyma	globispora
	MK281711.1	0.3	69.7	A	D	Pleosporales			

454