ORIGINAL ARTICLE

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, USA

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

Correspondence
Craig E. Coleman, Department of Plant and Wildlife Sciences, Brigham Young University, Provo, UT, USA.
Email: craig_coleman@byu.edu

Abstract

Cheatgrass (*Bromus tectorum* L.) is an invasive annual grass (Poaceae) that has colonized large portions of the Intermountain West. 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 metabarcoding 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 an increased abundance of a potential pathogen in the genus *Comoclathris*. Skull Valley surface litter stand failure samples had an increased abundance of an undescribed new species in the Rustroemiaceae family which is responsible for the so-called bleach blonde syndrome in cheatgrass, while the soils had an increased abundance of potential pathogens in the genera *Olpidium* and *Monosporascus*.

KEYWORDS
Bromus tectorum, cheatgrass, downy brome, metabarcoding, stand failure

1 | INTRODUCTION

Cheatgrass (*Bromus tectorum* L.) is an invasive annual grass (Poaceae) 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 Native grass stands depleted by overgrazing have been replaced by this invader (Mack, 1981).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 & Vitousek, 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). Since stand failures were first observed in the 1930s, several hypotheses for the occurrence of stand failures have been put forth, ranging from abiotic factors such as weather to some different fungal agents such as *Microdochium nivale* and *Ustilago bullata* (Klemmedson & Smith, 1964; Meyer et al., 2010; Piemeisel, 1938). Several fungal species have been identified that act as pathogens toward cheatgrass, including *Pyrenophora seminiperda*, *Epicoccum nigrum*, an undescribed species of *Fusarium* belonging to the *Tricinctum* group (*Fusarium* Link sp. n., FTSG) and an undescribed new species in the family Rutstroemiaceae which is responsible for so-called bleach blonde syndrome (Meyer et al., 2016). *Pyrenophora seminiperda*, *E. nigrum*, and FTSG are pathogens that kill seeds in the seed bank and are potential stand failure causal agents (Beckstead et al., 2007; Meyer et al., 2016; Stewart et al., 2009). Nevertheless, Baughman and Meyer (Baughman & Meyer, 2013) suggested that *P. seminiperda* 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 carryover 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). The new Rutstroemiaceae species is a crown-infecting pathogen that leaves cheatgrass plants stunted and straw-colored, with inflorescences that fail to mature. When the disease reaches epidemic levels in stands, it can cause the plants to collapse en masse and form a mat of thick dense litter. As the new Rutstroemiaceae species 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 that 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 seedbeds, in general, is poorly understood. The objective of the present research was to use a metabarcoding 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 ITS2 regions for maximizing taxonomic identification capability with high-yield Illumina sequencing of the ITS1 region alone for maximizing depth of coverage.

2 | MATERIALS AND METHODS

2.1 | 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 nine in Skull Valley, based on the year when a stand failure last occurred (Appendix 1, Table A1). 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, nine 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 × 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 before soil sample removal. For both litter and soil, three pools of three 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 2 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).

2.2 | 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 (Appendix 1, Table A1). Soil DNA and surface litter DNA super-pools for each of the eight 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 ITS1 and ITS2 regions as well as the intercalary 5.8S gene using AccuPrime Pfx DNA polymerase (Invitrogen) 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 (Appendix 1, Table A2). The following conditions were used for PCR: initial denaturation at 95°C for 3 min, 25 cycles 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 min. The PCR products were cleaned using a Zymo DNA Clean and Concentrator kit (Zymo Research). The 20 libraries were submitted to the BYU DNA Sequencing Center for sequencing on a PacBio Sequel platform using a standard Amplicon protocol with SMRTbell adapters. Subsequent analysis was done using Qiime2 version 2018.4. Demultiplexed sequences from read files were imported into a single-end QIIME2 artifact. Chimeric sequences were removed, sequences were dereplicated, and ASVs 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 version 8.0 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.

2.3 | 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 min, 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 min. In the second PCR, step barcodes were added to the amplified region (Appendix 1, Table A3 and A4) 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 DNA Sequencing Center for 2 × 250 sequencing on an Illumina HiSeq 2500 platform using custom sequencing primers ITS2-KYO2 and ITS1-F_KYO1 (Toju et al., 2012). After sequencing, reads were automatically demultiplexed and returned as paired-end reads. The Illumina reads are available in the Short Read Archive of GenBank under project PRJNA668186.

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 (Appendix 2, Figure B1). After rarefying the data, the rarefied tables were subsetted individually before performing analyses. The groups were as follows: (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.

2.4 | 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 of 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.

3 | RESULTS

3.1 | 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 well as the intercalary 5.8S gene 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 (Appendix 1, Table A1). Sequencing of the ITS amplicons from these pools yielded 123,664 reads (per pool mean 6182 ± 1440 reads; median 6319 reads) and 614 fungal operational taxonomic units (OTUs). Using the UNITE database, taxonomic assignments were made to the species level for 28% of OTUs (Appendix 1, Table A5). 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 8677 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% (Appendix 1, Table A5). Rarefaction curves suggested adequate saturation of the sampling (Appendix 2, Figure B1). Of all ASVs, 84% were assigned to just 30 taxonomic groups, primarily from the Ascomycota and Basidiomycota (Appendix 1, Table A6; Appendix 2, Figure B2), and just 3 ASVs were 'core', or present in all rarefied samples (Appendix 1, Table A7). Some ASVs were also detected that correspond to the new Rutstroemiaceae species, FTSG, *E. nigrum*, *P. seminiperda*, *U. bullata*, and *Microdochium* sp., all of which are known pathogens of cheatgrass (Klemmedson & Smith, 1964; Meyer et al., 2016; Piemeisel, 1938). 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 (Figure 1). Fungal microbiota composition varied significantly with each 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 the 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 the 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 the 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 (Appendix 1, Table A8), and sample type (Appendix 1, Table A9), respectively. These included FTSG having a greater abundance in soils, and Winnemucca having a greater abundance of the new Rutstroemiaceae species.

3.2 | 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 the year, sample type, location, and history of stand failure (Appendix 2, Figures B3 and B4). 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 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
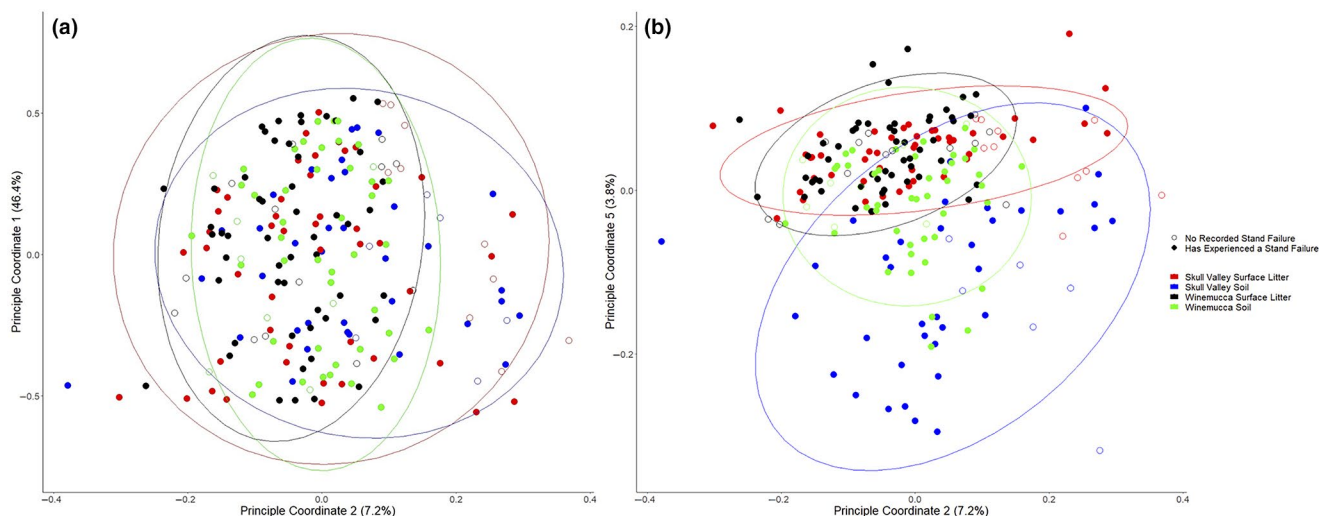
FIGURE 1 Principal coordinate plots of ITS1 ASVs, including (a) the first two principal coordinates and (b) the two principal coordinates, 2 and 5, that best show visual separation of the samples by the two main variables

TABLE 1 PERMANOVA results

	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	

Abbreviations: df, degrees of freedom; F, F statistics; MS, mean of squares; p, p-value; R^2, R^2 value; SS, sum of squares.

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	

taxonomic diversity in soil versus surface litter samples and greater diversity in the Winnemucca samples than Skull Valley.

3.3 | 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 (Appendix 2, Figure B5). 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

TABLE 3 ASVs identified by ANCOM. Shows the taxonomic identity and the relative abundance of ASV that differed between locations with stand failure

	GenBank ID	+	−	Taxonomy Phylum	Class	Order	Family	Genus	Species	FUNGuild description Trophic mode
Winnemucca SL	MK281667.1	506.2	32.7	A	D	Botryosphaeriales	Planistromellaceae	Ramimonilia		No data
	MK281810.1	343.5	18.2	B	T					No data
	MK281714.1	228.9	9.1	A	D	Pleosporales	Pleosporaceae	Comoclathris		Saprotroph
Winnemucca soil	MK281810.1	112.3	11.3	B	T					No data
	MK281667.1	108.2	15	A	D	Botryosphaeriales	Planistromellaceae	Ramimonilia	Apicalis	No data
Skull valley SL	MK281916.1	1503.6	117.2	A	D	Pleosporales	Lentitheciaceae	Keissleriella		Saprotroph
	MK281802.1	518.5	70.4	A	D	Pleosporales	Sporormiaceae	Sparticola		Saprotropoh
	MK281737.1	513.3	6.8	A	S	Coniochaetales	Coniochaetaceae	Coniochaeta	Polymorpha	Pathotroph – saprotroph – symbiotroph
	MK281662.1	165	14.4	B	T	Filobasidiales	Filobasidiaceae	Naganishia	Friedmannii	No data
	MK281822.1	67.6	3.7	A	L	Helotiales	Rutstroemiaceae			Saprotroph
	MK281810.1	45.4	5	B	T					No data
	MK281667.1	44.9	7.7	A	D	Botryosphaeriales	Planistromellaceae	Ramimonilia	Apicalis	No data
	MK281941.1	44.6	5.1	B	T	Tremellales	Tremellaceae	Cryptococcus		Pathotroph – saprotroph – symbiotroph
	MK281736.1	14.2	424.6	B	T	Filobasidiales	Filobasidiaceae	Naganishia	Albida	No data
	MK281670.1	9.2	0.7	A	D	Pleosporales	Lentitheciaceae	Keissleriella		Saprotroph
	MK281899.1	7.1	28.7	A	D	Pleosporales				No data
	MK281660.1	2.6	176.1	B	T	Tremellales	Bulleribasidiaceae	Vishniacozyma	Globispora	Pathotroph – saprotroph – symbiotroph
	MK281900.1	2.1	59.5	A	D	Pleosporales	pleosporaceae	Neocamarosporium		Pathogen Saprotroph
	MK281809.1	1.4	37.9	B	T	Filobasidiales	Filobasidiaceae	Filobasidium	Magnum	Saprotroph

(Continues)

TABLE 3 (Continued)

	GenBank ID	+	−	Taxonomy						FUNGuild description
				Phylum	Class	Order	Family	Genus	Species	Trophic mode
Skull valley soil	MK281916.1	1015.5	58.3	A	D	Pleosporales	Lentitheciaceae	Keissleriella		Saprotroph
	MK281699.1	332.8	48.8	O	Ol	Olpidiales	Olpidiaceae	Olpidium		Pathotroph
	MK281802.1	169.9	18	A	D	Pleosporales	Sporormiaceae	Sparticola		Saprotroph
	MK281736.1	104.8	538.2	B	T	Filobasidiales	Filobasidiaceae	Naganishia	Albida	No data
	MK281941.1	42.9	9.5	B	T	Tremellales	Tremellaceae	Cryptococcus		Pathotroph – saprotroph – symbiotroph
	MK281743.1	21.1	4.5	A	S	Xylariales	Diatrypaceae	Monosporascus		Pathotroph
	MK281899.1	14.8	201.7	A	D	Pleosporales				No data
	MK281743.1	4.5	21.1	A	S	Xylariales	Diatrypaceae	Monosporascus		Pathotroph
	MK281660.1	2.3	21.5	B	T	Tremellales	Bulleribasidiaceae	Vishniacozyma	Globispora	Pathotroph – saprotroph – symbiotroph
	MK281711.1	0.3	69.7	A	D	Pleosporales				No data

Note: (+) abundance in locations with stand failure, (−) abundance in locations with no-stand failure, (A) Ascomycota, (B) Basidiomycota, (D) Dothideomycetes, (L) Leotiomycetes, (O) Olpidiomycota, (OI) Olpidiomycetes, (S) Sordariomycetes, (SL) surface litter, (T) Tremellomycetes. The final column gives the FUNGuild description of the ASV's trophic mode.

all other samples, binned into stand failure or non-stand failure groups (Appendix 2, Figure B6). Unweighted Unifrac distances in Skull Valley, but not Winnemucca, 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.

4 | 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 seedbed 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 & Kirk, 2007). Others, such as the new Rutstroemiaceae species and *Olpidium brassicae*, are known plant pathogens (Meyer et al., 2016; Tewari & Bains, 1983). There is also a large percentage of ASVs, such as *Vishniacozyma globispora*, *Cryptococcus*, *Naganishia*, and *Holtermanniella takashimae* within the class Tremellomycetes of the Basidiomycota. Many fungi in this class are yeasts that act as parasites toward 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.

4.1 | 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, the PERMANOVA results do 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.

4.2 | 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 toward the non-die-off community (Winnemucca, NV), and the other displaying long-term
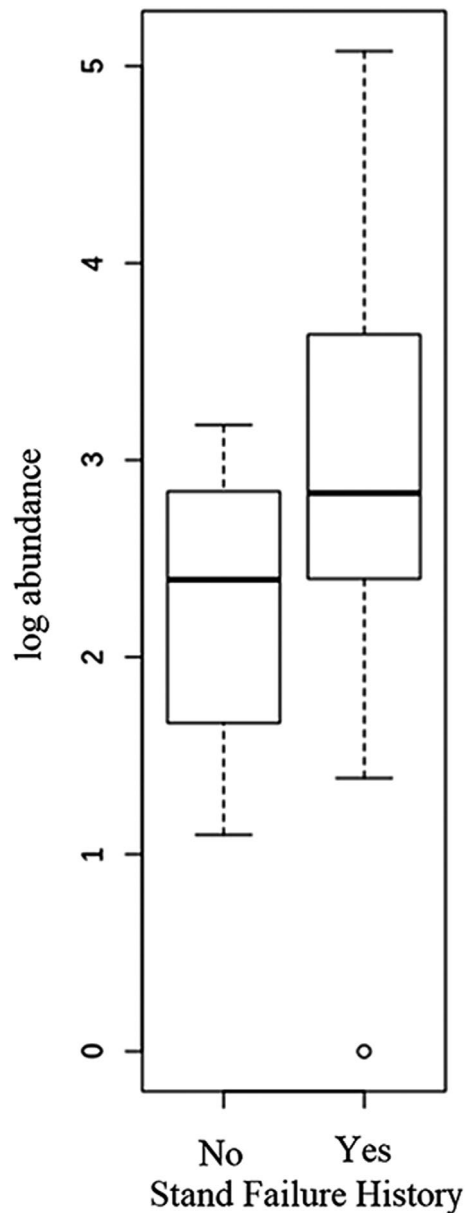
FIGURE 2 The abundance of the undescribed Rutstroemiaceae species. Log abundance in samples that have (yes) and have not (no) had a stand failure in the past

divergence from samples collected in areas that never experienced a die-off (Skull Valley, UT). Because this effect was detected using weighted, but not unweighted 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 (Appendix 2, Figure B4). 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 showed changes that persist for at least 28 years (Appendix 2, Figure B5). 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; 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.

4.3 | Fungi with increased abundances

A shared finding between the two geographic areas is that *R. apicalis* (GenBankID MK281667.1) and an unidentified fungus belonging to the class Tremellomycetes (GenBankID MK281810.1) are more abundant at stand failure sites in both study locations. The environmental consequences of *R. apicalis* presence are unknown, but it has been identified previously 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 & Koide, 2019). Endophytes live within plants, mostly without causing disease; however, with varying environmental conditions, endophytes can change to pathogens (Jia et al., 2016; Rai & Agarkar, 2016), and we cannot rule out that environmental cues could trigger *R. apicalis* to act as a pathogen toward 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 toward any type of plant, although they can be pathogenic toward 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.

The new Rutstroemiaceae species 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 (Figure 2). These data suggest it may have had a role in stand failure in at least two distinct locations in the Intermountain West.

5 | 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 *R. 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 whether these are causal agents of stand failure. Together, these findings provide insight into the fungal community of a largely unstudied system.

There has been considerable debate over the use of ITS1 or ITS2 as a marker for taxonomic identification in fungal metabarcoding, including potential biases introduced by the selection of primers used to amplify these regions (Bellemain et al., 2010; Blaalid et al., 2013; Ihrmark et al., 2012; Li et al., 2020; Monard et al., 2013; Toju et al., 2012; Yang et al., 2018). Our choice to sequence the entire ITS region using the PacBio platform was motivated by the inherent limitations of taxonomic identification using ITS1 or ITS2 alone. Nevertheless, we recognize that the choice of ITS1 rather than ITS2 in our second sequencing step on the Illumina platform may have introduced biases that caused us to miss important species that are causal to stand failure.

Another 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 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 whether 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.

CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTION

Nathan J. Ricks: Data curation (lead); Formal analysis (lead); Investigation (equal); Methodology (equal); Visualization (equal); Writing-original draft (lead); Writing-review & editing (lead). **Taryn Williamson:** Investigation (equal); Methodology (equal). **Susan E. Meyer:** Conceptualization (lead); Funding acquisition (lead); Methodology (supporting); Project administration (supporting); Supervision (equal); Validation (equal); Writing-review & editing (supporting). **John M. Chaston:** Formal analysis (supporting); Methodology (supporting); Project administration (supporting); Supervision (equal); Validation (equal); Visualization (equal); Writing-review & editing (equal). **Craig E. Coleman:** Conceptualization (supporting); Funding acquisition (supporting); Methodology (supporting); Project administration (lead); Resources (lead); Supervision (equal); Validation (equal); Visualization (equal); Writing-review & editing (equal).

ETHICS STATEMENT

None required.

DATA AVAILABILITY STATEMENT

The PacBio and Illumina sequence reads are available from GenBank as BioProject accession PRJNA668186: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA668186.

ORCID

John M. Chaston https://orcid.org/0000-0002-2625-6413
Craig E. Coleman https://orcid.org/0000-0002-7244-2532

REFERENCES

Alonso, R., Pisa, D., Aguado, B., & Carrasco, L. (2017). Identification of fungal species in brain tissue from alzheimer's disease by next-generation sequencing. *Journal of Alzheimer's Disease*, *58*, 55–67.

Baughman, O. W., & Meyer, S. E. (2013). Is *Pyrenophora seminiperda* the cause of downy brome (*Bromus tectorum*) die-offs? *Invasive Plant Science and Management*, *6*, 105–111.

Baughman, O. W., Meyer, S. E., Aanderud, Z. T., & 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? *Journal of Arid Environments*, *124*, 193–204.

Beckstead, J., Meyer, S. E., Molder, C. J., & Smith, C. (2007). A race for survival: Can *Bromus tectorum* seeds escape *Pyrenophora seminiperda*-caused mortality by germinating quickly? *Annals of Botany, 99*, 907–914.

Beckstead, J., Street, L. E., Meyer, S. E., & Allen, P. S. (2011). Fire effects on the cheatgrass seed bank pathogen *Pyrenophora seminiperda*. *Rangeland Ecology & Management*, *64*, 148–157.

Bellemain, E., Carlsen, T., Brochmann, C., Coissac, E., Taberlet, P., & Kauserud, H. (2010). ITS as an environmental DNA barcode for fungi: An *in silico* approach reveals potential PCR biases. *BMC Microbiology*, *10*, 189.

Blaalid, R., Kumar, S., Nilsson, R. H., Abarenkov, K., Kirk, P. M., & Kauserud, H. (2013). ITS1 versus ITS2 as DNA metabarcodes for fungi. *Molecular Ecology Resources*, *13*, 218–224.

Bokulich, N. A., Kaehler, B. D., Rideout, J. R., Dillon, M., Bolyen, E., Knight, R., Huttley, G. A., & Gregory Caporaso, J. (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, J. M., Hobbs, R. J., Pellant, M., & Pyke, D. (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., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*, 581.

Cannon, P. F., & Kirk, P. M. (2007). *Fungal families of the world*. CAB International.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Peña, A. G., Goodrich, J. K., Gordon,

J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., … Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, *7*, 335–336.

Cruaud, P., Rasplus, J.-Y., Rodriguez, L. J., & Cruaud, A. (2017). High-throughput sequencing of multiple amplicons for barcoding and integrative taxonomy. *Scientific Reports*, *7*, 41948.

D'Antonio, C. M., & Vitousek, P. M. (1992). Biological invasions by exotic grasses, the grass/fire cycle, and global change. *Annual Review of Ecology Evolution and Systematics*, *23*, 63–87.

Egidi, E., De Hoog, G. S., Isola, D., Onofri, S., Quaedvlieg, W., De Vries, M., Verkley, G. J. M., Stielow, J. B., Zucconi, L., & Selbmann, L. (2014). Phylogeny and taxonomy of meristematic rock inhabiting black fungi in the Dothideomycetes based on multi-locus phylogenies. *Fungal Diversity*, *65*, 127–165.

Faith, D. P. (1992). Conservation evaluation and phylogenetic diversity. *Biological Conservation*, *61*, 1–10.

Ihrmark, K., Bödeker, I. T. M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., Strid, Y., Stenlid, J., Brandström-Durling, M., Clemmensen, K. E., & Lindahl, B. D. (2012). New primers to amplify the fungal ITS2 region – Evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiology Ecology*, *82*, 666–667.

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

Klemmedson, J. O., & Smith, J. G. (1964). Cheatgrass (*Bromus tectorum* L.). *Botanical Review*, *30*, 226–262.

Li, S., Deng, Y. E., Wang, Z., Zhang, Z., Kong, X., Zhou, W., Yi, Y., & Qu, Y. (2020). Exploring the accuracy of amplicon-based internal transcribed spacer markers for a fungal community. *Molecular Ecology Resources*, *20*, 170–184.

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., & Peddada, S. D. (2015). Analysis of composition of microbiomes: A novel method for studying microbial composition. *Microbial Ecology in Health and Disease*, *26*, 27663.

Meyer, S. E., Beckstead, J., & Pearce, J. (2016). Community ecology of fungal pathogens on *Bromus tectorum*. In M. J. Germino, J. C. Chambers, & C. S. Brown (Eds.), *Exotic brome-grasses in arid and semiarid ecosystems of the Western US: Causes, consequences, and management implications* (pp. 193–223). Springer International Publishing.

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

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

Monard, C., Gantner, S., & Stenlid, J. (2013). Utilizing ITS1 and ITS2 to study environmental fungal diversity using pyrosequencing. *FEMS Microbiology Ecology*, *84*, 165–175.

Nilsson, R. H., Larsson, K.-H., Taylor, A. F., Bengtsson-Palme, J., Jeppesen, T. S., Schigel, D., Kennedy, P., Picard, K., Glöckner, F. O., Tedersoo, L., Saar, I., Kõljalg, U., & Abarenkov, K. (2018). The UNITE database for molecular identification of fungi: Handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Research*, *47*, D259–D264.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., Ohara, R. B., Simpson, G. L., Solymos, P., Henry, M., Stevens, H., Szoecs, E., & Wagner, H. (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. *The American Naturalist*, *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., & Agarkar, G. (2016). Plant–fungal interactions: What triggers the fungi to switch among lifestyles? *Critical Reviews in Microbiology*, *42*, 428–438.

Ricks, K. D., & Koide, R. T. (2019). Biotic filtering of endophytic fungal communities in *Bromus tectorum*. *Oecologia*, *189*, 993–1003.

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

Stewart, T., Allen, P., & Meyer, S. E. (2009). First report of *Pyrenophora seminiperda* in Turkey and Greece. *Plant Disease*, *93*, 1351.

Tewari, J., & Bains, P. (1983). Fungi associated with the roots of clover in Alberta. 1. *Olpidium brassicae* and *Ligniera* sp. *Canadian Plant Disease Survey*, *63*, 35–37.

Toju, H., Tanabe, A. S., Yamamoto, S., & Sato, H. (2012). High-Coverage ITS primers for the DNA-based identification of Ascomycetes and Basidiomycetes in environmental samples. *PLoS One*, *7*, e40863.

van der Klei, I., Veenhuis, M., Brul, S., Klis, F. M., De Groot, P. W., Müller, W. H., Kenneth, G. A., Van Driel, K. G. A., & Boekhout, T. (2011). Cytology, cell walls and septa: A summary of yeast cell biology from a phylogenetic perspective. In C. P. Kurtzman, J. W. Fell, & T. Boekhout (Eds.), *The yeasts* (5th ed., pp. 111–128). Elsevier.

Weisberg, P. J., Dilts, T. E., Baughman, O. W., Meyer, S. E., Leger, E. A., Van Gunst, K. J., & Cleeves, L. (2017). Development of remote sensing indicators for mapping episodic die-off of an invasive annual grass (*Bromus tectorum*) from the Landsat Archive. *Ecological Indicators*, *79*, 173–181.

Whisenant, S. G. (1990). Changing fire frequencies on Idaho's Snake River plains: Ecological and management implications. In E. D. McArthur, E. M. Romney, S. D. Smith, & P. D. Tueller (Eds.), *Proceedings - Symposium on cheatgrass invasion, shrub die-off, and other aspects of shrub biology and management. General technical report INT-GTR-276* (pp. 4–10). USDA Forest Service.

White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), *PCR protocols: A guide to methods and applications* (pp. 315–322). Academic Press.

Yang, R.-H., Su, J.-H., Shang, J.-J., Wu, Y.-Y., Li, Y., Bao, D.-P., & Yao, Y.-J. (2018). Evaluation of the ribosomal DNA internal transcribed spacer (ITS), specifically ITS1 and ITS2, for the analysis of fungal diversity by deep sequencing. *PLoS One*, *13*, e0206428.

Ziska, L. H., Reeves, J. B., & Blank, B. (2005). The impact of recent increases in atmospheric CO_2 on biomass production and vegetative retention of cheatgrass (*Bromus tectorum*): Implications for fire disturbance. *Global Change Biology*, *11*, 1325–1332.

APPENDIX A

TABLE A1 GPS coordinates of sampling locations along with years in which stand-failures were detected

Year	GPS		Reference[a]
Skull Valley, Utah			
None	40.1419	112.668	X[b]
None	40.13996	−112.641	X[b]
1990	40.1388	−112.711	
2008	40.17711	−112.728	
2009	40.39453	−112.948	
2010	40.2752	−112.631	X
2013	40.32838	−112.777	X
2014	40.34031	−112.686	X
2015	40.29299	−112.77	X
Winnemucca, Nevada			
None	40.69066	−117.894	X[c]
None	40.6989	−117.899	X[c]
1990	40.69205	−117.938	
2003	40.68962	−117.964	
2009	40.69183	−117.959	
2009	40.69305	−117.923	
2010	40.69839	118.044	X
2013	40.69445	−117.938	X
2014	40.68664	−117.983	X
2015	40.68791	−117.966	X

[a]An X indicates locations where samples were used to generate the long-read reference library.

[b]Samples from these two sites were combined to generate a single pooled sample.

[c]Samples from these two sites were combined to generate a single pooled sample.

TABLE A2 PacBio Barcodes used for surface litter and soil samples

Years of detected stand failure	Soil	Litter
Utah		
None	GTGTGAGATATATATC	TCAGACGATGCGTCAT
2010	ACACACAGACTGTGAG	TCAGACGATGCGTCAT
2013	GCAGACTCTCACACGC	TCACACTCTAGAGCGA
2014	ATGCTCACTACTACAT	GTACACGCTGTGACTA
2015	CGCATCTGTGCATGCA	TGCTCGCAGTATCACA
Nevada		
None	GCTCGTCGCGCGCACA	TATCTCTGTAGAGTCT
2010	GCGCGATACGATGACT	TCTATGTCTCAGTAGT
2013	ACTCTCGCTCTGTAGA	TGCGAGCGACTCTATC
2014	CTGCGCAGTACGTGCA	GACAGCATCTGCGCTC
2015	GAGATACGCTGCAGTC	CAGTGAGAGCGCGATA

TABLE A3 Forward primers used in Illumina sequencing

Forward primers			
GGCCATAT	TTCGATGG	GTGTCACA	ACGTGATC
AGAGCAGT	CTCTAGAG	AACCGGTT	TGGTCAAC
ACCTGTTC	CAGACTCA	AGTGTCTG	CTTGGTAG
TATAGCGC	GTAGAGGT	CAGTCTCT	ATCGGCAT
GTACGATC	AGTGGTGA	GTGTTCTC	TGAGGACA
CACTTCTG	ATGGCCTA	AGTCTGTG	AACCTTCC

TABLE A4 Reverse primers used in Illumina sequencing

Reverse primers				
CCGCTTAT	GAAGCAAC	TCGTACCT	GAGAGAGA	TGTCGACA
CTACAGCA	GTGTCTCT	AAGGATGC	GTAGACCT	TCTCACTG
AACGTTGC	AGGAACCA	GGTTGCAT	GTTGCTAG	CAGATGTC
AGGAGTTG	GAGTCAGA	GTGTAGTC	AGAGCACA	CACAACAC
GGATCCAT	GTGAGTGA	TTCGTTCG	CAAGCAAG	ATCGTTCC
ACTCTGTC	CCTAGGAT	TGTGAGAG	CTTGGTAG	ACCAGTAC
CATGTGCA	TGACTGTG	GTACCTAG	AACCAACC	AGAGACAC
ACCTTGCT	TTGCTACC	CATCACCT	GAGTACAG	TTCCATGC
AACGAACG	GTACCAAC	CGTTCCTA	TGTGTGAC	GAGTAGAC
CAACCTAG				

TABLE A5 The percentage of reads assigned to each taxonomic level using data from PacBio sequencing and the UNITE database to create a reference library and Illumina sequencing data and the UNITE database with (+) or without (−) the reference library

Taxonomic level	PacBio	Ilumina +reference	Ilumina −reference
Species	28.49	43.82	37.99
Genus	71.48	78.50	41.88
Family	87.75	83.23	43.01
Order	93.40	88.35	45.94
Class	94.84	91.98	46.58
Phylum	96.17	92.37	74.74
Kingdom	98.52	99.92	99.99

TABLE A6 Thirty most abundant ASVs in all samples

GenBank ID	Kingdom	Phylum	Class	Order	Family	Genus	Species	Average read number
MK281756.1	Fungi	Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae	Vishniacozyma	V. globispora	2310 ± 1828.3
MK281916.1	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Lentitheciaceae	Keissleriella		1066.9 ± 1150.5
MK281946.1	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	Preussia		707.7 ± 737.3
MK281840.1	Fungi	Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	Cryptococcus		536 ± 950.7
MK281836.1	Fungi	Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae	Vishniacozyma	V. globispora	525.1 ± 809.8
MK281982.1	Fungi							420.7 ± 866
MK281760.1	Fungi	Ascomycota	Sordariomycetes	Sordariales				379.4 ± 1138.1
MK281802.1	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	Sparticola		292.1 ± 501.9
MK281889.1	Fungi	Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae	Vishniacozyma	V. victoriae	184.1 ± 349.6
MK281667.1	Fungi	Ascomycota	Dothideomycetes	Botryosphaeriales	Planistromellaceae	Ramimonilia	R. apicalis	161.6 ± 414.2
MK281834.1	Fungi	Ascomycota	Sordariomycetes	Coniochaetales	Coniochaetaceae	Coniochaeta	C. polymorpha	156.6 ± 409.9
MK281737.1	Fungi	Ascomycota	Sordariomycetes	Coniochaetales	Coniochaetaceae	Coniochaeta	C. polymorpha	152.5 ± 562.9
MK281662.1	Fungi	Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	Naganishia	N. friedmannii	133.1 ± 274
MK281726.1	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria		132.2 ± 228.9
MK281841.1	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria		124.9 ± 432.2
MK281810.1	Fungi	Basidiomycota	Tremellomycetes	Pleosporales				120.6 ± 217.7
MK281932.1	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae			110.6 ± 227.5
MK281699.1	Fungi	Olpidiomycota	Olpidiomycetes	Olpidiales	Olpidiaceae	Olpidium		91.7 ± 201.4
MK281665.1	Fungi	Ascomycota	Dothideomycetes	Capnodiales	Davidiellaceae	Cladosporium		90.0 ± 284.9
MK281878.1	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Didymellaceae			85.4 ± 334.8
MK281714.1	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Comoclathris	C. spartii	65.6 ± 201.2
MK281804.1	Fungi	Olpidiomycota	Olpidiomycetes	Olpidiales	Olpidiaceae	Olpidium	O. brassicae	64.5 ± 224.6
MK281736.1	Fungi	Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	Naganishia	N. albida	64.1 ± 199.7
MK281674.1	Fungi	Ascomycota	Leotiomycetes	Helotiales	Rutstroemiaceae	Rutstroemia		59 ± 193.0
MK281837.1	Fungi	Ascomycota	Dothideomycetes	Dothideales	Aureobasidiaceae	Aureobasidium	A. pullulans	56.1 ± 146.2
MK281772.1	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	Sclerostagonospora		54.4 ± 228.0
MK281855.1	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Lophiostomataceae	Lophiostoma	L. multiseptatum	50.9 ± 137.1
MK281816.1	Fungi	Basidiomycota	Tremellomycetes					48.2 ± 135.5
MK281695.1	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Didymosphaeriaceae			42.9 ± 145.7
MK281891.1	Fungi	Basidiomycota	Tremellomycetes	Holtermanniales	Holtermanniales fam Incertae sedis	Holtermanniella	H. takashimae	41.4 ± 150.6

TABLE A7 Illumina ASVs found in every sample

Taxonomy					
Phylum	**Class**	**Order**	**Family**	**Genus**	**Species**
Ascomycota	Dothideomycetes	Pleosporales	Lentitheciaceae	*Keissleriella*	
Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	*Preussia*	
Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae	*Vishniacozyma*	*V. globispora*

TABLE A8 Differential abundance of ASVs that varied in their abundance between Utah and Nevada

GenBank ID		Phylum	Class	Order	Family	Genus	Nevada mean abundance	Utah mean abundance
MK281724	Nevada	Ascomycota	Arthoniomycetes	Lichenostigmatales	Phaeococcomycetaceae	Phaeococcomyces	38.81 ± 83.5	4.66 ± 14.92
MK281667	Nevada	Ascomycota	Dothideomycetes	Botryosphaeriales	Planistromellaceae	Ramimonilia	273.7 ± 535.5	29.1 ± 64.7
MK281744	Nevada	Ascomycota	Dothideomycetes	Capnodiales			4.4 ± 10.9	0.1 ± .38
MK281741	Nevada	Ascomycota	Dothideomycetes	Capnodiales			2.27 ± 5.0	0.4 ± .7
MK281918	Nevada	Ascomycota	Dothideomycetes	Pleosporales	Didymosphaeriaceae		40.7 ± 236.0	3.1 ± 22.2
MK281772	Nevada	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	Sclerostagonospora	95.0 ± 303.9	6.4 ± 20.6
MK281828	Nevada	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria	31.4 ± 71.6	4.0 ± 6.6
MK281912	Nevada	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria	6.2 ± 23.5	0.2 ± 0.7
MK281843	Nevada	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria	11.0 +/ 32.3	3.0 ± 7.2
MK281714	Nevada	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Comoclathris	118.2 ± 262.5	3.5 ± 8.2
MK281812	Nevada	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Epicoccum	29.1 ± 40.3	6.3 ± 14.4
MK281796	Nevada	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Pyrenophora	5.9 ± 24.5	0.3 ± 6.1
MK281730	Nevada	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	Preussia	17.1 ± 51.3	2.7 ± 14.4
MK281723	Nevada	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	Preussia	20.0 ± 82.2	0.6 ± 1.5
MK282099	Nevada	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae		4.3 ± 8.0	0.1 ± 0.2
MK281932	Nevada	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae		200.1 ± 353.5	4.8 ± 9.1
MK282093	Nevada	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae		3.9 ± 7.6	0.06 ± 0.3
MK281909	Nevada	Ascomycota	Dothideomycetes	Tubeufiales			4.7 ± 12.3	6.1 ± 35.0
MK282113	Nevada	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	Chlorociboria	4.8 ± 22.53	0.14 ± 0.47
MK281767	Nevada	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	Crocicreas	46.4 ± 210.0	1.7 ± 3.1
MK281770	Nevada	Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae	Cistella	46.4 ± 101.3	4.6 ± 6.0
MK281870	Nevada	Ascomycota	Leotiomycetes	Helotiales	Rutstroemiaceae	Rutstroemia	35.6 ± 104.5	5.6 ± 26.2
MK281758	Nevada	Ascomycota	Leotiomycetes	Helotiales	Rutstroemiaceae	Rutstroemia	4.1 ± 14.2	0.2 ± 0.9
MK281694	Nevada	Ascomycota	Leotiomycetes	Helotiales	Rutstroemiaceae	Rutstroemia	14.1 ± 47.2	0.4 ± 0.7
MK281674	Nevada	Ascomycota	Leotiomycetes	Helotiales	Rutstroemiaceae	Rutstroemia	105.9 ± 253.6	5.5 ± 10.9
MK281728	Nevada	Ascomycota	leotiomycetes	Helotiales			3.2 ± 5.5	0.3 ± 1.1
MK281863	Nevada	Ascomycota	leotiomycetes	Helotiales			3.1 ± 9.9	0.1 ± 0.4
MK281834	Nevada	Ascomycota	Sordariomycetes	Coniochaetales			282.8 ± 525.6	7.6 ± 15.3
MK281807	Nevada	Ascomycota	Sordariomycetes	Coniochaetales			4.1 ± 9.5	0.1 ± 0.4
MK281879	Nevada	Ascomycota	Sordariomycetes	Coniochaetales			0.7 ± 1.7	0.0 ± 0.0
MK281867	Nevada	Ascomycota	Sordariomycetes	Coniochaetales			13.5 ± 63.4	0.4 ± 1.3
MK281664	Nevada	Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae	Podospora	15.8 ± 145.9	0.1 ± .3

(Continues)

TABLE A8 (Continued)

GenBank ID		Phylum	Class	Order	Family	Genus	Nevada mean abundance	Utah mean abundance
MK281942	Nevada	Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae	*Podospora*	6.1 ± 25.2	0.2 ± .9
MK281727	Nevada	Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae		37.3 ± 84.3	15.2 ± 55.4
MK281820	Nevada	Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae		17.7 ± 51.8	0.9 ± 3.1
MK281769	Nevada	Ascomycota	Sordariomycetes	Sordariales			24.7 ± 79.1	1.0 ± 2.2
MK281935	Nevada	Ascomycota	Sordariomycetes	Sordariales			7.7 ± 37.2	0.1 ± .5
MK281712	Nevada	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	*Anthostomella*	48.8 ± 139.2	0.8 ± 1.9
MK281782	Nevada	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae		41.1 ± 183.9	0.7 ± 1.7
MK281805	Nevada	Ascomycota	Taphrinomycetes	Taphrinales	Protomycetaceae	*Protomyces*	2.2 ± 4.4	0.3 ± 1.1
MK281817	Nevada	Ascomycota					7.8 ± 19.3	4.6 ± 17.2
MK281814	Nevada	Ascomycota					6.9 ± 18.7	0.3 ± 1.3
MK282069	Nevada	Ascomycota					4.5 ± 9.5	0.2 ± .6
MK281689	Nevada	Basidiomycota	Agaricomycetes	Auriculariales	Auriculariales_fam_Incertae_sedis	*Oliveonia*	1.8 ± 5.6	0.2 ± 1.3
MK281734	Nevada	Basidiomycota	Agaricomycetes				20.2 ± 66.7	3.7 ± 19.8
MK281685	Nevada	Basidiomycota	Cystobasidiomycetes	Erythrobasidiales			5.7 ± 7.8	1.4 ± 3.3
MK281752	Nevada	Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	*Naganishia*	19.5 ± 113.2	1.1 ± 2.3
MK281948	Nevada	Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	*Naganishia*	9.2 ± 31.5	0.7 ± 1.7
MK281671	Nevada	Basidiomycota	Tremellomycetes	Filobasidiales	Piskurozymaceae	*Solicoccozyma*	2.2 ± 5.4	0.2 ± 0.5
MK281891	Nevada	Basidiomycota	Tremellomycetes	Holtermanniales	Holtermanniales_fam_Incertae_sedis	*Holtermanniella*	72.9 ± 199.4	4.2 ± 10.1
MK282104	Nevada	Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae	*Dioszegia*	16.9 ± 36.4	2.2 ± 8.8
MK281889	Nevada	Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae	*Vishniacozyma*	285.3 ± 422.7	64.3 ± 173.2
MK282054	Nevada	Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae	*Vishniacozyma*	1.6 ± 2.8	0.2 ± 0.6
MK281985	Nevada	Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae	*Vishniacozyma*	1.1 ± 3.7	0.1 ± 0.3
MK281881	Nevada	Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	*Cryptococcus*	43.6 ± 108.4	4.4 ± 12.4
MK281840	Nevada	Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	*Cryptococcus*	776.4 ± 1135.4	252.3 ± 553.6
MK282109	Nevada	Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	*Cryptococcus*	27.3 ± 80.2	5.2 ± 13.1
MK281810	Nevada	Basidiomycota	Tremellomycetes				199.6 ± 267.3	27.2 ± 56.2
MK281993	Nevada	Chytridiomycota					15.2 ± 109.8	0.3 ± 0.9
MK281982	Nevada						685.9 ± 1089	107.2 ± 244.3
MK282120	Utah						9.2 ± 53.4	58.2 ± 187.4
MK281878	Utah	Ascomycota	Dothideomycetes	Pleosporales	Didymellaceae		8.8 ± 18.8	175.8 ± 479.8

(Continues)

TABLE A8 (Continued)

GenBank ID		Phylum	Class	Order	Family	Genus	Nevada mean abundance	Utah mean abundance
MK281864	Utah	Ascomycota	Dothideomycetes	Pleosporales	Didymosphaeriaceae		4.9 ± 16.8	43.5 ± 266.5
MK281695	Utah	Ascomycota	Dothideomycetes	Pleosporales	Didymosphaeriaceae		16.9 ± 84.1	73.7 ± 190.9
MK282102	Utah	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria	1.5 ± 5.4	21.7 ± 64.5
MK281785	Utah	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria	2.5 ± 7.5	8.8 ± 17.4
MK282100	Utah	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria	0.2 ± 0.5	12.2 ± 30.5
MK281947	Utah	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria	0.2 ± 0.7	9.3 ± 24.4
MK281841	Utah	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria	4.7 ± 0.7	266.9 ± 610.1
MK281928	Utah	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria	1.6 ± 2.9	52.7 ± 118.7
MK281873	Utah	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria	2.3 ± 7.3	8.0 ± 34.3
MK281726	Utah	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria	69.4 ± 161.3	206.5 ± 22.7
MK281818	Utah	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Comoclathris	0.0 ± 0.1	3.8 ± 9.1
MK281866	Utah	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Comoclathris	1.0 ± 1.5	47.6 ± 111.7
MK281900	Utah	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Neocamarosporium	0.1 ± 0.4	7.6 ± 54.7
MK281754	Utah	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Pyrenophora	1.3 ± 4.8	2.7 ± 5.1
MK281907	Utah	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	Preussia	0.3 ± 0.8	12.4 ± 68.1
MK281766	Utah	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	Preussia	0.1 ± 0.3	20.7 ± 187.1
MK281745	Utah	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae		1.8 ± 7.8	5.2 ± 13.1
MK281832	Utah	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae		0.9 ± 3.7	3.8 ± 11.9
MK281793	Utah	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae		5.0 ± 13.1	24.6 ± 76.7
MK281899	Utah	Ascomycota	Dothideomycetes	Pleosporales			1.6 ± 6.8	23.9 ± 75.2
MK281886	Utah	Ascomycota	Dothideomycetes	Pleosporales			1.3 ± 2.7	20.8 ± 60.0
MK281826	Utah	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	Tetracladium	0.2 ± 0.4	4.4 ± 17.7
MK281757	Utah	Ascomycota	Leotiomycetes	Helotiales	Rutstroemiaceae	Rutstroemia	1.9 ± 3.9	58.5 ± 160.6
MK282085	Utah	Ascomycota	Pezizomycetes	Pezizales	Ascobolaceae	Ascobolus	0.2 ± 0.9	5.4 ± 20.6
MK281759	Utah	Ascomycota	Sordariomycetes	Coniochaetales	Coniochaetaceae	Coniochaeta	0.1 ± 0.2	1.4 ± 4.1
MK281737	Utah	Ascomycota	Sordariomycetes	Coniochaetales	Coniochaetaceae	Coniochaeta	11.2 ± 18.6	319.6 ± 801.6
MK281938	Utah	Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae		1.6 ± 2.9	37.4 ± 119.8
MK281760	Utah	Ascomycota	Sordariomycetes	Sordariales			25.6 ± 36.8	797.7 ± 1585
MK281743	Utah	Ascomycota	Sordariomycetes	Xylariales	Diatrypaceae	Monosporascus	5.2 ± 18.0	12.5 ± 23.6
MK281786	Utah	Ascomycota					0.5 ± 18.0	3.8 ± 9.3
MK282059	Utah	Ascomycota					0.4 ± 1.0	12.3 ± 44.9

(Continues)

TABLE A8 (Continued)

GenBank ID		Phylum	Class	Order	Family	Genus	Nevada mean abundance	Utah mean abundance
MK281949	Utah	Ascomycota					0.9 ± 4.0	2.3 ± 5.0
MK281762	Utah	Ascomycota					0.2 ± 4.0	2.3 ± 5.0
MK281777	Utah	Basidiomycota	Agaricomycetes				0.2 ± .8	2.2 ± 5.5
MK281736	Utah	Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	*Naganishia*	1.3 ± 5.0	15.4 ± 60.9
MK281794	Utah	Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	*Naganishia*	16.5 ± 44.4	120.3 ± 281.5
MK281753	Utah	Basidiomycota	Tremellomycetes	Tremellales	Rhynchogastremataceae	*Papiliotrema*	1.8 ± 3.9	7.1 ± 15.5
MK282066	Utah	Basidiomycota	Tremellomycetes	Tremellales			0.4 ± 0.9	14.2 ± 42.5
MK282035	Utah	Basidiomycota					2.2 ± 4.8	18.1 ± 59.9
MK281906	Utah	Chytridiomycota					13.1 ± 35.8	14.9 ± 29.9
MK281656	Utah	Olpidiomycota	Olpidiomycetes	Olpidiales	Olpidiaceae	*Olpidium*	2.3 ± 4.2	54.1 ± 185.2

Note: The first column shows their GenBank Accession number, while the second column specifies if they were more abundant in Nevada or Utah.

TABLE A9 Differential abundance between sample types. These were the ASVs that varied in their abundance between soil and surface litter

GenBank accession	Sample type in which it was more abundant	Phylum	Class	Order	Family	Genus	Species	Soil mean abundance	Surface litter mean abundance
MK281657	Soil	Ascomycota	Sordariomycetes	Xylariales	Microdochiaceae	Microdochium		61.6 ± 119.8	4.9 ± 9.3
MK281671	Soil	Basidiomycota	Tremellomycetes	Filobasidiales	Piskurozymaceae	Solicoccozyma		2.7 ± 6.0	0.2 ± 0.5
MK281699	Soil	Olpidomycota	Olpidomycetes	Olpidiales	Olpidiaceae	Olpidium		198.2 ± 272.6	11.2 ± 17.2
MK281703	Soil	Ascomycota	Arthoniomycetes					0.7 ± 1.2	0.1 ± 0.3
MK281738	Soil	Chytridiomycota						4.0 ± 8.5	0.4 ± 0.9
MK281760	Soil	Ascomycota	Sordariomycetes	Sordariales				783.9 ± 1617.7	73.7 ± 300.2
MK281804	Soil	Olpidomycota	Olpidomycetes	Olpidiales	Olpidiaceae	Olpidium		144.1 ± 328.5	4.2 ± 6.8
MK281932	Soil	Ascomycota	Dothidiomycetes	Pleosporales	Sporormiaceae			202.8 ± 383.6	40.8 ± 115.6
MK281949	Soil	Ascomycota						3.3 ± 6.5	0.2 ± 0.5
MK282079	Soil	Olpidomycota	Olpidomycetes	Olpidiales	Olpidiaceae	Olpidium	O. brassicae	11.9 ± 22.8	0.4 ± 0.8
MK281802	Surface litter	Ascomycota	Sordariomycetes	Sordariales				137.9 ± 220.8	408.7 ± 612.7
MK281810	Surface litter	Basidiomycota	Tremellomycetes					58.5 ± 92.1	167.7 ± 268.2
MK281837	Surface litter	Ascomycota	Dothideomycetes	Dothideales	Aureobasidiaceae	Aureobasidium	A. pullulans	16.4 ± 49.4	86.1 ± 183.6
MK281928	Surface litter	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria		3.2 ± 8.6	41.5 ± 108.5
MK281936	Surface litter	Ascomycota	Arthoniomycetes	Lichenostigmatales	Phaeococcomycetaceae	Phaeococcomyces		0.9 ± 1.9	6.8 ± 14.3

Note: The first column shows their GenBank Accession number, while the second column specifies if they were found more abundantly in the soil or the surface litter.
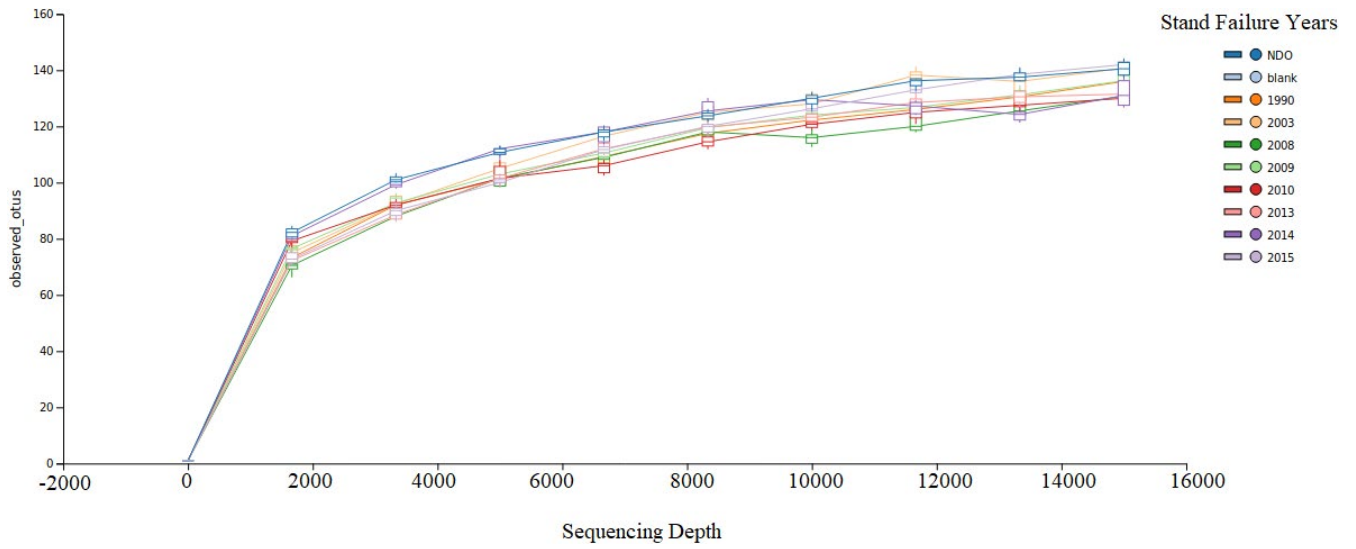
APPENDIX B

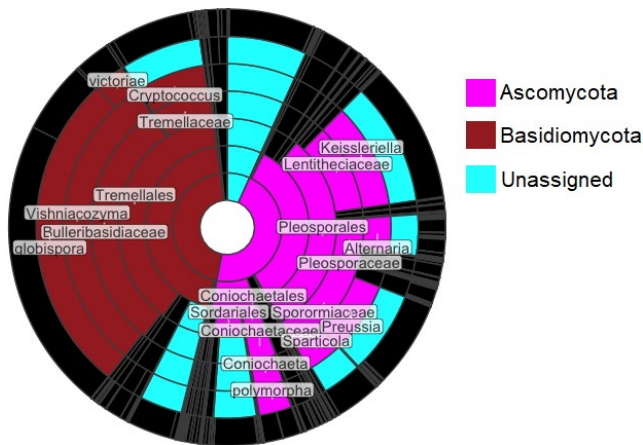**FIGURE B1** Rarefaction curve, representing the number of ASVs found at each rarefaction level
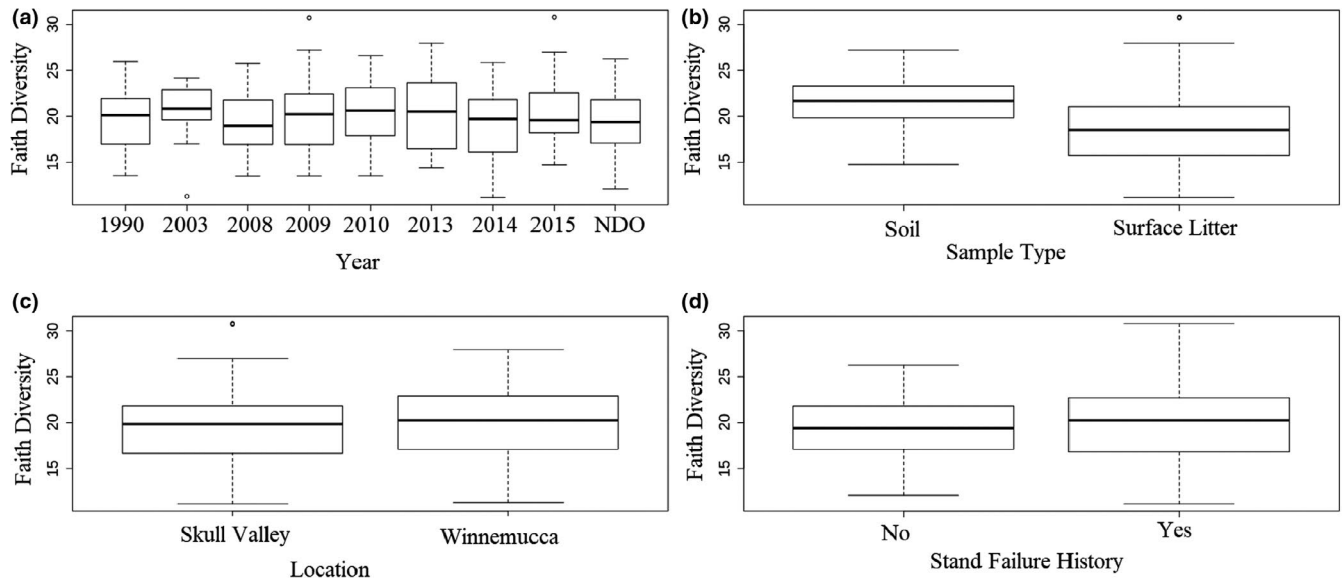

FIGURE B2 Krona Chart. Visualization of the taxonomic assignment of sequencing reads. The inner ring is phylum; then class, order, family, genus, species. The outer ring represents ASVs

FIGURE B3 Comparison of Faith Diversity between groups. (a) Comparing diversity of differing years in which a stand failure occurred. (b) Comparing the diversity of both sample types. (c) Comparing the diversity between both locations, Skull Valley Utah and Winnemucca Nevada. (d) Comparing the diversity between samples that have experienced a stand failure in the past (Yes) and those that have not (No)
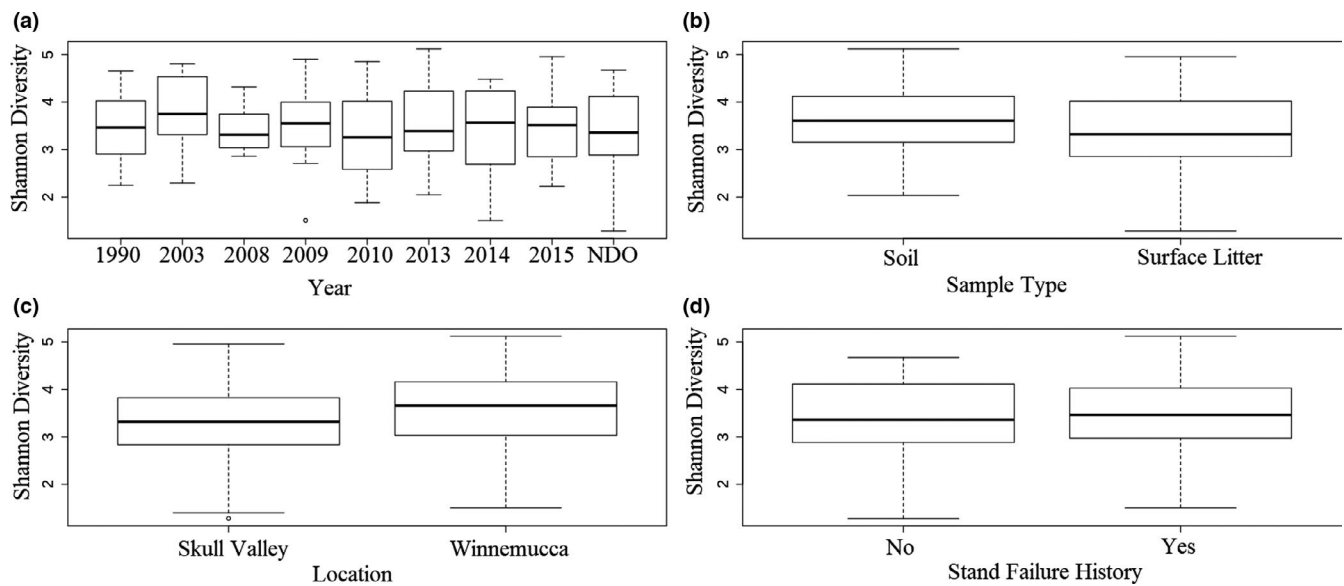
FIGURE B4 Comparison of Shannon Diversity between groups. (a) Comparing diversity of differing years in which a stand failure occurred. (b) Comparing the diversity of both sample types. (c) Comparing the diversity between both locations, Skull Valley Utah and Winnemucca Nevada. (d) Comparing the diversity between samples that have experienced a stand failure in the past (Yes) and those that have not (No)

(a)

(b)

(c)

(d)
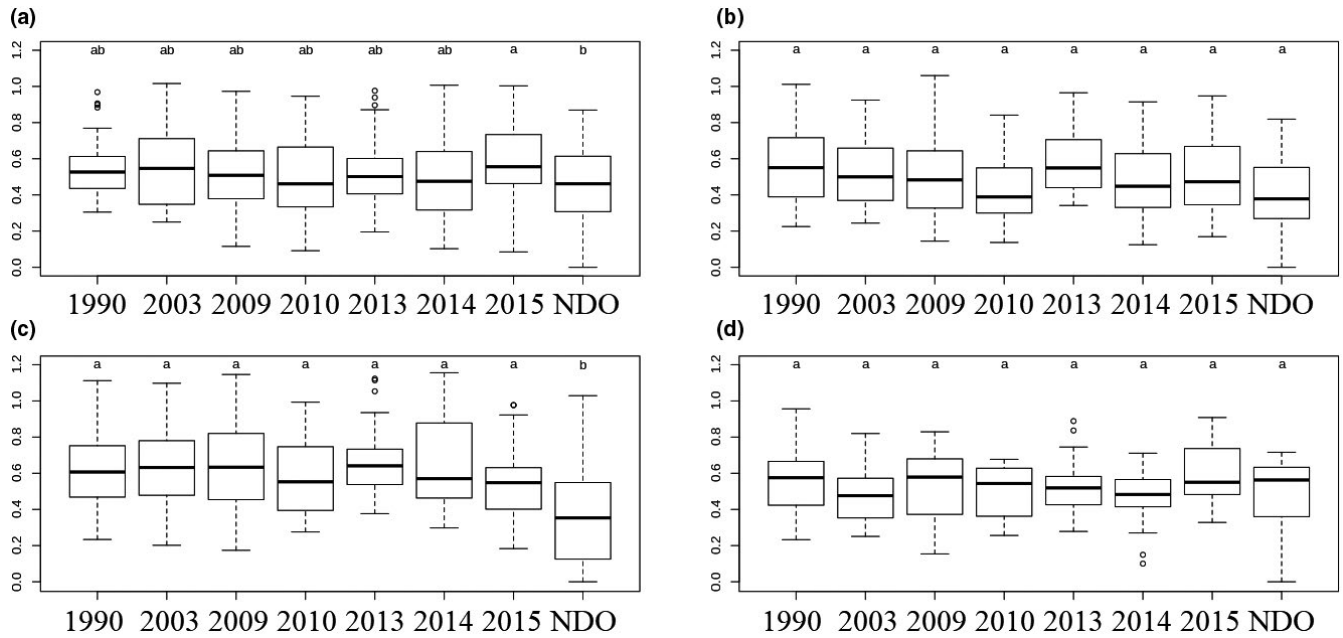
FIGURE B5 Unifrac distances to non-stand failure. The weighted unifrac distance of each year to sites that had never had a stand failure (NDO). Above each box shows the groupings by multicomp analysis. (a) Surface litter from Winnemucca, Nevada., (b) shows the soil from Winnemucca Nevada, (c) shows the surface litter from Skull Valley Utah and (d) shows the soil from Skull Valley Utah
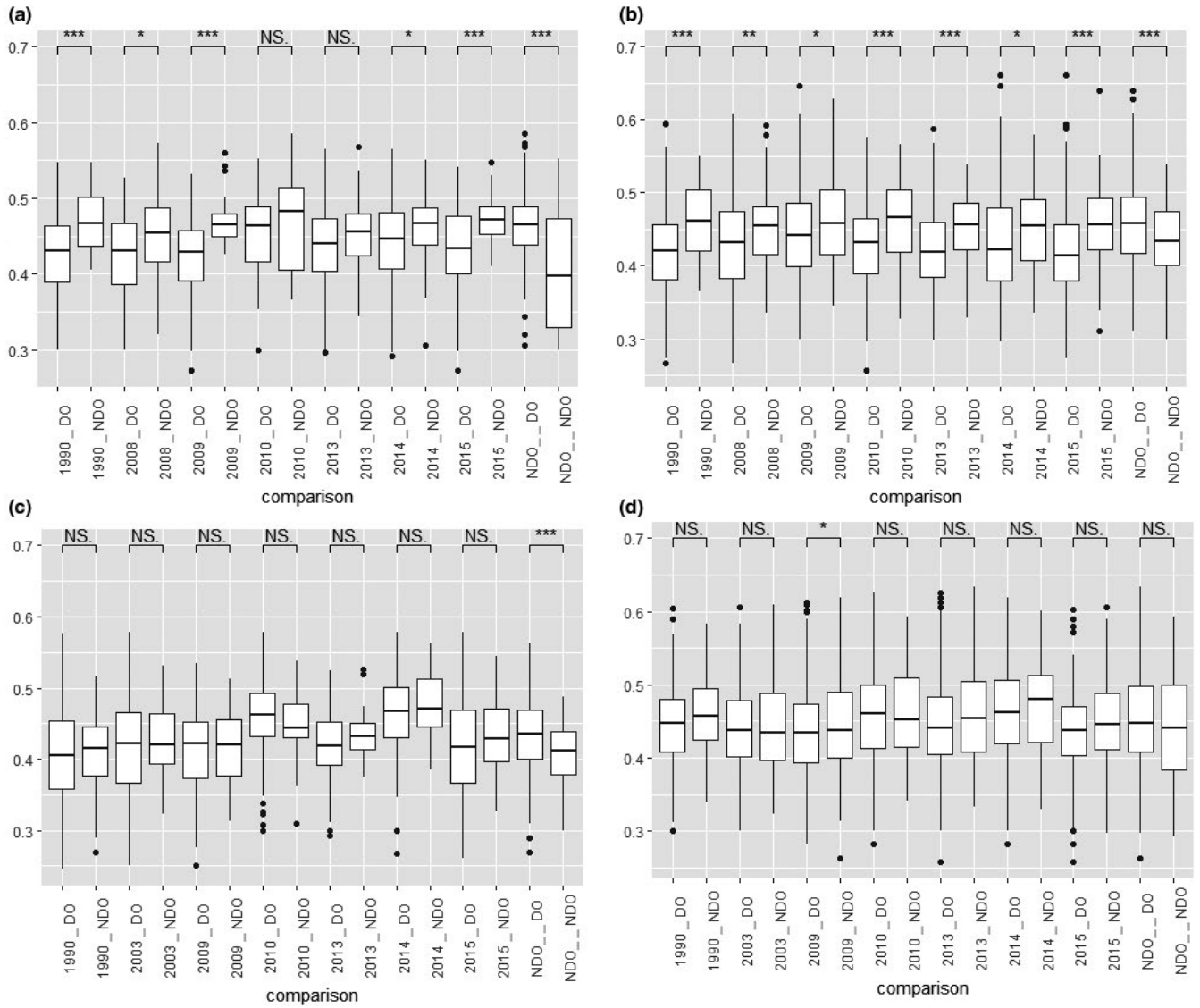
FIGURE B6 Distances of samples from each year to samples all stand failure sites (Ex. 1990 _ DO) compared with samples of all non-stand failure sites (1990_NDO). (a) Skull Valley Soil (b) Skull Valley Surface Litter (c) Winnemucca Soil (d) Winnemucca Surface Litter