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A Metagenomic Approach to Understand Stand Failure in Bromus tectorum

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ABSTACT

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Bromus tectorum (cheatgrass) is an invasive annual grass 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 study, metagenomics was used to better understand and compare the fungal communities between sites that have and have not experienced stand failure. Samples were taken from the soil and surface litter in Winnemucca, Nevada and Skull Valley, Utah.

Results show distinct fungal communities between Winnemucca and Skull Valley, as well as between soil and surface litter. 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 the potential pathogen in the genus *Comoclathris* while the soils had increased abundance of the known cheatgrass pathogen *Epicoccum nigrum*. 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*.

Keyworks: *Bromus tectorum*, metagenomics, stand failure

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INTRODUCTION

Bromus tectorum (cheatgrass) 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 Native grass stands depleted by overgrazing have been replaced by this invader (Mack 1981).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 (Meyer et al. 2007). 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 such that in the following years cheatgrass comes to dominate the community (Meyer et al. 2007). 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 and 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 that were previously occupied by a *B. tectorum* monoculture become largely empty of any visible vegetation.

Many different hypotheses have been put forward to explain the occurrence of stand failures (Klemmedson and Smith 1964; Piemeisel 1938, 1951). Hypotheses have ranged from abiotic factors such as weather, to a number of different fungal agents such as *Microdochium nivale* and *Ustilago bullata* (Klemmedson and Smith 1964; Piemeisel 1938). In recent years, several fungal species have been identified that act as pathogens towards cheatgrass. These pathogenic fungi include *Pyrenophora*

semeniperda, Epicoccum nigrum, an undescribed species of *Fusarium* belonging to the *tricinctum* group (*Fusarium* Link sp. n., FTSG.) and a newly described species named *Clarireedia capillus-albis* responsible for so called bleach blonde syndrome (Meyer et al. 2016).

Pyrenophora semeniperda, *E. nigrum* and *Fusarium* are pathogens that can kill seeds in the seed bank and are therefore potential stand failure causal agents (Beckstead et al. 2007; Meyer et al. 2016; Stewart et al. 2009). Baughman and Meyer (Baughman and Meyer 2013) demonstrated that *P. semeniperda* was not a direct cause of die-off, largely because of its inability to kill rapidly germinating seeds, though it could play a role in rate of post-die-off recovery through its impact on dormant seeds in the carry-over seed bank. Both *Fusarium* sp. n. (FTSG) and *E nigrum*, on the other hand, 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 (Meyer et al. 2014).

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 mat of litter could create an environment that promotes the attack of other pathogenic fungi (Meyer et al. 2016).

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 seeds in the following years (Baughman et al. 2016).

The ability of known fungal pathogens to cause cheatgrass mortality suggests they may play a role in stand failure. However, the fungal community associated with stand failures and with cheatgrass seed beds in general is poorly studied. The objective of the present research is to use a metagenomic

approach to understand the fungal community structure in this system. The goal is 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.

Microbial community composition is often determined by culturing microorganisms on growth medium and identifying them either by morphological or genetic characteristics (Pelis 1997). However, culturing microbes is limited in that many do not readily grow on medium, leaving them out of the identification process and leading to the “great plate count anomaly” (Staley and Konopka 1985). Metagenomics, a relatively new field, can produce data that are not biased towards microorganisms that can be cultured (Gupta and Vakhlu 2011). Metagenomic data are produced by extracting DNA found in an environmental sample and sequencing genomic regions that allow taxonomic identification. Using metagenomics, a great number of the microorganisms found within an environmental sample can be identified (Handelsman 2004). Marker genes, or 'bar-codes', that have enough variability between species are used to discriminate between taxa; however, the marker gene needs to be sufficiently conserved that it is found in the entire target group (Hebert et al. 2003). For fungal taxonomy, both the Internal Transcribed Spacer 1(ITS1) and Internal Transcribed Spacer 2(ITS2) regions are commonly used as taxonomic identifiers. The ITS are regions that flank the 5.8S ribosomal DNA (Toju et al. 2012). While other genes such as β-tubulin, elongation factor 1α and the large subunit of RNA polymerase II have been used to infer taxonomy in fungi, ITS is often considered the most useful due to its widespread adoption, fast rate of evolution and ease in amplification (Raja et al. 2017).

Currently, most marker gene metagenomic studies use short-read sequencing technology such as Illumina. For instance, in bacterial studies, the 16S region is amplified to produce 300 bp forward and reverse paired-end reads, which can be merged to yield a single 600 bp sequence. However, due to the error rate found in many short sequencing reads, it is often necessary to have both the forward and reverse reads overlap, because high accuracy sequencing is essential for correct taxonomic

identification (Kozich et al. 2013; Oulas et al. 2015). Yet, in many cases, a single 300 bp sequence is not sufficient to identify an organism at the species level. In many fungal species, the combined length of the ITS1, and ITS2 regions is longer than 300 bp. This makes it impossible to achieve maximum taxonomic depth in a metagenomics analysis using short-read sequencing technology (Toju et al. 2012).

Long-read sequencing, such as is achieved with PacBio SMRTbell circular adapters, produces accurate sequences that span the entire ITS1 and ITS2 regions (Rhoads and Au 2015). However, such long-read sequencing does not typically produce the same volume of reads when compared to short-read sequencing. While long-read sequencing improves the accuracy of taxonomic identification (Frank et al. 2016), the reduced depth of sequencing limits the ability to detect quantitative differences between individual metagenomic samples (Maas and Hox 2005). With the small number of reads produced by PacBio technology, identifying the differences between treatments becomes difficult because statistical inferences are limited by small sample size. The choice between Illumina and PacBio sequencing presents researchers with a tradeoff. Using PacBio sequencing affords a deep understanding of the exact species that are found in samples but prevents strong statistical inferences from being drawn due to the small sample size. Using Illumina gives a less specific understanding of the exact species found in the samples (perhaps identifying most of the taxa to family or genus) but allowing statistical inferences to be drawn due to the large sample size (Frank et al. 2016; Maas and Hox 2005). To reconcile this trade-off between taxonomic accuracy and depth of coverage, this study adopts a strategy which combines an initial long-read DNA sequencing run of bulked samples. By first performing shallow sequencing with long reads, an accurate reference of expected fungal taxa is established. This reference is then used later, when sequencing at a much greater depth with shorter reads, to improve the confidence in taxonomic identification of the short reads.

Unfortunately stand failures do not occur every year, and the year in which this study began, 2017, experienced no stand failures. To compensate for this lack of stand failure, a remote sensing method

was used to identify sites at two locations in Skull Valley Utah and Winnemucca Nevada where stand failures had occurred in different years since 1990 (Weisberg et al. 2017). A metagenomic approach was used to assess differences in the fungal community within the surface litter and seed bed soil at these sites. To identify fungi that could be implicated as causal agents for stand failures, these data were compared to data from sites that had, according to the remote sensing technology, never experienced a stand failure during the 30-year LANDSAT record. It was hypothesized that whatever had caused the stand failure had persisted in the soil and would be manifested as a difference in fungal community composition between stand failure and non-stand failure sites. Community differences common to both Utah and Nevada study areas, locations separated by hundreds of miles, were hypothesized to more accurately reflect biologically important differences between stand failure and non-stand failure sites.

MATERIALS AND METHODS

Collection of Environmental Samples

As mentioned above, 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. This technique used spectral mixture analysis and machine learning algorithms to predict the probability in each pixel (pulled from the Landsat archive) that a stand failure occurred at a given year (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 1). 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 serving as negative controls where no stand failure has been detected since Landsat data became available. Ten sampling sites were selected from Winnemucca, and 9 sites were selected from Skull Valley (Table 1). At each

site, four 10-meter transects were laid down, and 9 samples of surface litter and soil were collected at randomly selected points along each transect. 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. Surface litter and soil were placed in separate paper sacks for each sample. For both litter and soil, 3 bulks of 3 samples each were created for each transect, yielding a total of 12 soil and 12 litter bulks at each site. Soil and surface litter bulk samples were homogenized separately using a coffee grinder following drying. DNA was extracted from 100g of each homogenized bulk sample 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 bulks (Supplemental Table 1). Four sites from each location (Skull Valley and Winnemucca) had last recorded stand failures in years 2010, 2013, 2014 and 2015. Soil DNA and surface litter DNA bulks for each of the 8 sites were created by combining equal amounts of DNA extracted from the 12 individual bulks described in the previous section. Similar bulks were created by combining DNA extracted from soil or surface litter collected at sites where no stand failure has been detected. For the two sites where no stand failure has been detected, single soil and litter bulks were made from all samples collected at each location. Each of the 20 DNA bulks (Supplemental Table 1) was used to create an individual DNA sequencing library. The libraries were created by using AccuPrime Pfx DNA polymerase to amplify the ITS region using the ITS4 and ITS5 primers (White et al. 1990) modified with 20 unique PacBio barcode tails (Supplemental Table 2) so that the libraries could be combined into a single run for sequencing. PCR was performed using the following conditions: initial denaturation at 95 C for three minutes, followed by 25 cycles consisting of denaturation (95 C for 30 s), annealing (52 C 30 s), and extension (72 C 1

m). Following the 25 cycles a final extension step at 72 C for 5 minutes was used. After PCR amplification clean-up was carried out using the Zymo DNA Clean and Concentrator kit. The 20 libraries were submitted to the BYU DNA Sequencing Center (Provo, UT) for sequencing on the PacBio Sequel machine. There the samples were sequenced using a standard Amplicon protocol with SMRTbell adapters. Demultiplexed sequences were provided by the Sequencing center and read files were imported into a single-end QIIME2 artifact. Using vsearch (Rognes et al. 2016), chimeric sequences were removed, sequences were dereplicated, and operational taxonomic units (OTUs) were generated at 97% similarity. Using the QIIME Naive Bayes classifier (Bokulich et al. 2018), taxonomy was assigned using 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.

Short-Read Sequencing

In preparing the short-read library, all individual samples were used. This differed from the preparation of the long-read library in that all nineteen sample locations were used and the 12 samples per sample type and site were not bulked. With two types of samples per site, 12 replicates in each sample type and 19 sites, there were a total 456 samples

Using a two-step PCR reaction, the ITS1 region of the fungal genome was amplified and samples were multiplexed. PCR was performed using AccuPrime Pfx DNA polymerase. In the first step, the ITS1 region was amplified using primers ITS2-KYO2 and ITS1-F_KYO1. (Toju et al. 2012). The following PCR conditions were used for the first step: initial denaturation at 95 C for three minutes, followed by 25 cycles consisting of denaturation (95 C for 30 s), annealing (52 C 30 s), and extension (72 C 1 m). Following the 25 cycles a final extension step at 72 C for 5 minutes was used. Using a second PCR step, barcodes were added to the amplified region to aid in demultiplexing (see

supplemental tables 3 and 4). The second PCR step had identical conditions to the first with the exception that there were 12 cycles as opposed to 25, and the annealing temperature occurred at 55 C instead of 52 C. In the first PCR reaction, the ITS1 region was amplified. The primers used in this reaction had a five prime overhang that encoded the reverse compliment to the binding site of the primers for the second PCR reaction. The forward and reverse primers in the second reaction had five prime overhangs that included a barcode sequence unique to each reaction for each sample (Cruaud et al. 2017). With 24 unique forward primers that shared sequence with the ITS2-KYO2 primer, and 46 unique reverse primers that shared sequence with the ITS1-F_KYO1 primer, each sample was able to be uniquely barcoded. Barcodes were randomly assigned to each sample. Samples were then pooled and submitted to the BYU sequencing center for 2 x 250 sequencing on an Illumina HiSeq 2500. After sequencing, reads were automatically demultiplexed and returned as paired-end reads.

The returned reads were imported into QIIME 2 where the paired-end reads were joined, chimeric sequences were removed, sequences were dereplicated and OTUs 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 OTU was given a taxonomic identity. Sequences that were not found in at least 12 samples were removed as they were assumed to be the result of either PCR or sequencing error. Samples were rarefied to 10,000 reads (down sampled to 10,000 reads per sample) to allow for standardized comparison between samples. 10,000 reads per sample was chosen to maximize OTUs per sample and minimize sample loss (Supplemental Figure 1). Rarefaction is useful as it allows the comparison of samples that have extreme differences in sequence depth. A sample with 10,000 reads can be compared to a sample with 20,000 reads on a level playing field. 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. It was hypothesized that using PacBio reads, in conjunction with the UNITE database would increase the taxonomic identify of short reads by creating a reference of fungi found in these samples along with their sequences.

Analysis of the Long and Short Read Sequence Data

Using the OTU 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 tests. Weighted distance matrices take into account quantitative information, such as how much of each OTU is in a sample, while unweighted distance matrices are qualitative, calculated from the presence or absence of OTUs. In the PERMANOVA, location, sample type, and stand failure history, along with location by stand failure history were included as terms in the model. PERMANOVA was then applied to each of the four primary sample groups (Winnemucca soil, Winnemucca litter, Skull Valley soil, and Skull Valley litter) separately, with stand failure history (no stand failure vs. stand failure at some point in the past) as the independent variable in each model.

Using Analysis of Composition of Microbiomes (ANCOM) (Mandal et al. 2015), OTU tables from each the four primary sample groups were tested for differences in the composition of microbiomes between sites that had recorded die-offs and sites that did not. ANCOM was also used to identify differences in taxon abundances between Skull Valley and Winnemucca, and between the soil and the surface litter.

Because ANCOM has false discovery rate corrections built into its program, many significant abundances differences could have been missed due to the large number of comparisons made. Therefore, the differences in abundance of four fungal pathogens implicated in the die-off phenomenon in previous studies (Meyer et al. 2016) were analyzed separately using standard t-tests. These included

Pyrenophora semeniperda, *Epicoccum. nigrum, Fusarium* sp. n. (FTSG), and *Clarireedia capillus-albis*.

The most abundant OTUs along with OTUs that were found consistently in all samples were calculated in R. These calculations were completed using both the Illumina sequencing data and the PacBio sequencing data. The Faith phylogenetic diversity (Faith 1992) and Shannon diversity (Pielou 1966), were calculated in QIIME2; these diversity measures were run on all the data together, along with each the four primary sample groups. In each test of diversity, one variable was used. The variables tested were location, sample type, stand failure history, and years since stand failure. To assess the impact of the PacBio sequences on the Illumina taxonomic calling, taxonomy of the Illumina data was also calculated without the PacBio data to serve as a reference. The percentage of reads that were called to each taxonomic level were calculated and compared between the two taxonomies.

RESULTS

Sequencing Details and Improvement with PacBio Reads

To create a reference set of fungal OTUs found typically in cheatgrass soils, a subset of all the DNA samples were bulked after DNA extraction. Each bulk consisted of the 12 samples of identical sample type from each sampling site. 20 bulks were created that represented samples from varying locations, years since a die off, and sample types (Supplemental table 2). PacBio sequencing of a fragment spanning the ITS1, 1.5S and ITS2 yielded 123,664 sequences (mean 6182 sequence +- 1,440 reads per sample, median 6,319) and 614 fungal OTUS. 28% of all reads could be assigned to the species level (Table 2). A parallel analysis of fungal composition and diversity in the individual soil and litter samples was conducted by Illumina sequencing of the ITS1 region. A total of 13,000,017 reads

(mean 23,136 +- 67,274 per sample, median 8,677) passed filtered quality and, assigned to a total of 525 OTUs. 38% of all reads were called at the species level (Table 2)

It was hypothesized that the use of the PacBio data would improve the taxonomic level at which all Illumina short reads could be called. To verify this prediction, taxonomic assignments of each OTU derived from the Illumina sequencing were compared with and without the reference PacBio reads. The percent of Illumina reads that were assigned to each taxonomic level was improved after using PacBio reads as a reference (Table 2).

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

When Weighted Unifrac distance was used as a beta diversity metric to determine the variation in the fungal community between sampling sites, variation between the samples as a function of sample type, location, and history of stand failure was readily visualized by PCoA (Figures1-5). When separating samples in the PCoA by location (Figure 1), the Skull Valley samples and Winnemucca samples segregate away from each other. While there is overlap, these results suggest that the two locations have different fungal communities. When separating samples in the PCoA by sample type (Figure 2), similar results are seen. However, all the surface litter samples are encompassed in the overlap between surface litter and soil, while the soil samples have a large amount of non-overlap area in the PCoA. These results could suggest much greater diversity in the soil compared to the surface litter, with the surface litter containing very little that is not seen in the soil. When separating samples in the PCoA by stand failure samples vs. non-stand failures samples (Figures 3-5), very little difference is seen between the two groups (Figure 3). It is not until samples are separated by locations (Figures 4-5) that separation is seen. This separation is much more evident in the Skull Valley samples, where the stand failure, and non-stand failure samples are very distinctly separate from one another.

The visual trends seen in the PCOA plots were statistically supported by using PERMANOVA on the weighted unifrac distance matrix (Table 3) with sample type, location and stand failure history all being significant. Because sample type (soil versus litter) and location (Skull Valley versus Winnemucca) were significant independent variables in the analysis, the variation in the fungal community with history of stand failure was focused on an analysis of each of the four primary sample groups individually (Table 4). These individual analyses showed Skull Valley surface litter from sites that experienced a stand failure to be significantly different from the surface litter of Skull Valley sites that had never experienced a stand failure (p-value 0.023). Similar results were found in Winnemucca surface litter, with a near significant p-value (0.090). In contrast, there was no significant difference between die-off and non-die off sites in fungal composition of soil samples from either location.

To confirm if the differences between communities seen in the PERMANOVA was caused by differences in abundances of fungi, or the complete presence or absence of fungi, both PERMANOVA analyses were rerun using the unweighted distance matrix. These analyses showed that when using the unweighted distance matrix, sample type, location, stand failure history, and the interaction between stand failure history and location were all significant (Table 3). Furthermore, when comparing stand failure groups to non-stand failure groups in each of the four primary sample groups, once again only the Winnemucca surface litter and Skull Valley surface litter had significant differences. (Table 4)

Fungal OTUs Varied in Abundance Between Stand Failure and Non-Stand Failure Sites

After observing statistical differences between groups in PERMANOVA analysis, ANCOM was used to identify OTUs that varied in abundance between sites that had and had not experienced a stand failure. Due to the differences between groups observed in PCoA analysis, and confirmed by PERMANOVA, ANCOM was run on each of the four primary sample groups separately. Each group had OTUs that varied in abundance between sites that had and had not experienced a stand failure.

Some were more abundant in stand failure sites, while others were more abundant in non-stand failure sites (Table 5). From these four analyses, two OTUs were found to have increased abundance in the surface litter of sites that had experienced a stand failure of both Winnemucca and Skull Valley. These OTUs, with the GenBank IDs of MK281810 and MK281667 were identified to the fungal class of *Tremellomycetes* and the fungal species of *Ramimonilia apicalis* respectively.

The four species (*Clarireedia capillus-albis*, *Fusarium, Epiccocum nigrum,* and *Pyrenophora seminiperda)* previously identified as cheatgrass pathogens were not significantly different between samples as mueasured by ANCOM. Standard t tests were run comparing the abundance in stand failure and non-stand failure sites. *Clarireedia capillus-albis* was significantly more abundant in Skull Valley surface litter samples that had a history of stand failure than surface litter samples in Skull Valley that had no history of stand failure. *Epicoccum nigrum* was found to be significantly more abundant in the soil samples from Winnemucca that had a history of stand failure compared to soil samples in Winnemucca that had no history of stand failure (Figure 6). *Pyrenophora semeniperda* and *Fusarium,* while present, were not more abundant in either Skull Valley or Winnemucca samples that had a history of stand failure. It should also be mentioned that other fungal pathogens which target cheatgrass, such as *Ustilago bullata* and *Microdochium* were found in both the litter and soil of Nevada and Utah (Klemmedson and Smith 1964; Piemeisel 1938).

Fungal OTUs Varied in Abundance Between Other Major Groups

ANCOM was also used to assess the differences in abundances of fungal taxa between other treatments. This was done to find differences in fungal composition between the Winnemucca and Skull Valley, and the difference between soil and surface litter. When ANCOM was run between samples taken from Skull Valley and Winnemucca, 103 OTUs were detected as having a difference in abundance between the groups. Of these, 42 were more abundant in Skull Valley sites while 61 were

more abundant in Winnemucca sites (Supplemental table 5). When ANCOM was run between the soil and surface litter samples, 30 OTUs were detected having a difference in abundance between the groups. Of these, 7 were more abundant in the surface litter and 23 were more abundant in the soil (Supplemental table 6).

Increased and Decreased Diversity Found in Treatment Groups

The Faith and Shannon diversity metrics were used to test how diversity varied with stand failure, time from stand failure, sample type, and location. The diversity metrics look at how many distinct OTUs exist in a sample, with the Faith metric using phylogenetic distance to more heavily weight OTUs that are phylogenetically distant. When calculating the Faith phylogenetic diversity index, sample type was found to be significant (p-value 8.37e-10), with increased diversity in soil samples compared to surface litter samples. All other variables, location, stand failure, and time since stand failure were found to be non-significant (Figure 7). When using the Shannon diversity index, Winnemucca samples had a higher diversity than Skull Valley samples (p-value .00242) and soil samples had a higher diversity than surface litter samples (p-value .01932) There was no significant difference between the diversity in stand failure years, and stand failure vs non-stand failure sites (Figure 8).

Year Effects

In one hypothesis for the stand-failures, there could be a loss or decrease in abundance of the causal agent over the years. As time since a stand failure occurred increases, the fungal community could begin to return to normal. If this hypothesis were true, the sites that experienced die-offs in 2015 would be expected to be very different from sites that had not experienced stand failures as well as sites with more distant stand failures. To assess the validity of this hypothesis, distances of each site by year

were plotted against those sites that had not experienced die-offs (Figure 9). Soil samples in both Skull Valley and Winnemucca did not show any difference between years. Surface litter samples in both Skull Valley and Winnemucca did show a difference between years. While in Winnemucca, only the 2015 site differed in distance to the non-stand failure sites, while in Skull Valley, all years that had experienced a stand failure difference from the non-stand failure site. These findings confirm the hypothesis, at least in the case of Winnemucca, that a location that experienced a stand failure recently will differ more from non-stand failure sites than locations that experienced a stand failure many years ago. Also, these findings confirm what was previously established in the PERMANOVA analyses; the differences between stand failure and non-stand failure sites is primarily confined to the surface litter.

Composition of Communities, and Most Abundant OTUs

While the primary objective of this study was to find causal agents for stand failures, a secondary objective was to characterize the fungal communities found in cheatgrass stands. Thus, the thirty most abundant taxa (Supplemental table 7), along with the taxa that were found most consistently between all samples (Supplemental table 8) were calculated. The sequences from the thirty most abundant taxa represented 84% of all sequences

DISCUSSION

Characterization of Typical Cheatgrass Seed Bed Fungal Community

Cheatgrass seed banks contains a wide variety of fungal species. Despite there being a large number of OTUs present, the thirty most abundant taxa comprised 84% of all sequence reads. These 30 OTUs, represent the typical cheatgrass seed bed fungal community. While it is unknown how all these fungi function in the cheatgrass seed bed, research has been done on a number these fungal families

that allows speculation to occur as to their function. For example, many of these, such as *Keissleriella, Preussia, Sparticola* and *Didymosphaeriaceae* most likely act as saprophytes (Cannon and Kirk 2007). Others, such as *Clarireedia capillus-albis* and *Olpidium brassicae* are known plant pathogens (Meyer et al. 2016; Tewari and Bains 1983). There are also a large percentage of OTUs, such as *Vishniacozyma globispora, Cryptococcus, Naganishia,* and *Holtermanniella takashimae* within the *Tremellomycetes* class. Many fungi in this class are yeasts that act as parasites towards other fungi . It is unknown why they are found so prevalently in cheatgrass communities, but it appears that the environment afforded by cheatgrass are conducive towards their growth, indicated by their prevalence.

Geographic Variation and Soil-Surface Litter Differences.

Differing fungal communities in Skull Valley and Winnemucca was expected because the locations differ in many metrics such as climate and geology (Koide et al. 2017; Lekberg et al. 2007). The differences are readily apparent, and can be seen in the PCoA plots that segregate Skull Valley samples away from Winnemucca samples (Figure 1), PERMANOVA results showing a significant p-value (Table 3), diversity (Figure 8) and ANCOM analysis (Supplemental Table 5) that show large differences in the abundance of many taxa. It is not clear why Winnemucca samples have more diversity than Skull valley samples.

Even within a specific location, there can be a large amount of microbial variation when sampling different substrate types (Fierer et al. 2003). This variation is seen in the large difference between soil and surface litter samples which makes sense because these sample types experience different environmental conditions and are composed of differing materials. The differing materials are likely to host different fungal communities. These differences can also be easily seen in PCoA plots (Figure 2), PERMANOVA results (Tables 3), diversity (Figure 7 and 8) and ANCOM results (Supplemental Table 6). Decreased diversity found in litter samples can be explained by the harsher environment of the litter.

It is exposed more to solar radiation and experiences greater drying. These conditions may make it more difficult for organisms to survive, leading to decreased diversity in the surface litter.

Stand Failure History

When the PERMANOVA was run (Table 3), the significant p-value for history of stand failures implies that when controlling for location and sample type there are differences in the fungal communities between the sites that have and have not experienced stand failures. The variation between locations and sample types could mask differences between stand failure and non-stand failure sites; thus, analyses were done on each of the four treatment groups separately. As there is a significant p-value for the interaction term between stand failure history and location, it is concluded that the differences between sites that experienced stand failures and those that did not, differ between locations. In other words, the sites in Skull Valley that have experienced stand failure, have changed from those that have not experienced stand failure in Skull Valley, in a different way from those in Winnemucca. This could potentially imply different causal mechanisms in Skull Valley and Winnemucca. Potentially different mechanisms are be seen in the ANCOM results (Table 5) as the Skull Valley samples that ha e experienced stand failures had increased abundance of many different fungi that were not found to have increased abundance in Winnemucca stand failure sites.

PERMANOVA analyses of each group separately using the weighted distance matrix (Table 4) confined the differences of stand failure and non-stand failure communities to the surface litter samples (with p-values being either significant or near significant). Little difference was seen between the soil samples from stand failure versus non-stand failures sites. 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. PERMANOVA analyses of each group

separately using the unweighted distance matrix (Table 4) also confined the differences to the surface litter in both Winnemucca and Skull Valley. As unweighted distance matrices take into account the presence and absence of OTUs, rather than varying abundances, the significant p-value of the surface litter samples implies that the differences in communities is not only a difference in abundances of the same fungi, but there are qualitative differences in the fungi that are present and absent in stand failure and non-stand failure sites.

Year Effect

When looking for a year effect (Figure 9), one was seen in the surface litter samples of Winnemucca. The results suggest that after several years, the fungal communities have begun to return to normal, becoming more like non-stand failure communities. The results also show that the biggest difference between stand failure and non-stand failure sites lies in the 2015 stand failure site. This makes sense as the 2015 site had the most recent stand failure in Winnemucca.

Contrasting results were seen in the surface litter of Skull Valley, where no recovery seemed to occur. As all the sites between 1990 and 2015 are grouped away from the non-stand failure sites, it appears that the Skull Valley surface litter does not begin recovering its fungal community. Perhaps an environmental feature of Skull Valley has prevented it from returning to normal.

Fungi with Increased Abundances

When ANCOM was run (Table 5), a number of OTUs were found to have differential abundance between locations that had a stand failure, and locations that had not. OTUs that had increased abundance in stand failure sites could potentially be implicated causal agents. It seems ulikely that tow of these OTUS (*Ramimonilia apicalis* and an unidentified fungus belonging to the class *Tremellomycetes*) were found to have increased abundance in both Winnemucca stand failures sites,

along with Skull Valley stand failure sites. The increased abundance of these two OTUs suggests they are involved in stand failures. Alternatively, stand failure conditions could promote their growth. The other fungi found in ANCOM to have increased abundance in stand failure locations could also be causal agents. While they do not have increased abundance in both Skull Valley and Winnemucca, differing mechanisms leading to stand failure could be at work in the two locations.

Epicoccum nigrum and *C. capillus-albis* are known cheatgrass pathogens. Both had increased abundance in stand failure sites in Winnemucca soils and Skull Valley surface litter respectively (Figure 6). While the difference in abundance between stand failure and non-stand failure sites was not significant after using multiple comparison corrections, if a standard t-test is used they are significant. While ANCOM did not flag them as significant due to multiple comparison corrections, that *E. nigrum* and *C. capillus-albis* were found to be significant under a standard t-test and knowing that they can kill cheatgrass (Meyer et al. 2016)is suggestive that they may be involved in causing stand failure.

Overview of Fungi Found in ANCOM

While *E. nigrum* and *C. capillus-albis* have been shown to kill cheatgrass, the other fungi found in ANCOM (Table 5) have not been directly shown to be pathogenic towards cheatgrass. In the surface litter of Skull Valley, the OTUs identified to *R. apicalis* and *Clarireedia* seem the most likely to act as pathogens towards cheatgrass while *Tremellomyctes* seems an unlikely pathogen.

Ramimonilia. apicalis, which has increased abundance in the surface litter samples of Skull Valley and Winnemucca is highly associated with stand failure. Little work has been done on *R. apicalis*, though it has been shown to live as a rock inhibiting-fungi 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 and Koide 2019). Endophytes live within plants, mostly without causing disease; however, with

varying environmental conditions, endophytes can change to pathogens. Perhaps environmental cues can trigger *R. apicalis* to act as a pathogen towards cheatgrass.

While the OTU identified as *Tremellomyctes* was found in both Skull Valley and Winnemucca surface litter, it seems unlikely that it is pathogenic towards cheatgrass. There is little evidence of fungi of this class being pathogenic towards any type of plant. Many species in this class are yeasts that are non-pathogenic, or pathogenic towards animals and other fungi (van der Klei et al. 2011). It seems possible that stand failures changed the fungal structure and community of cheatgrass stands such that this OTU classified as *Tremellomyctes* can thrive.

It is very likely that the OTU identified to the genus *Clarireedia* can act pathogenically towards cheatgrass. This genus contains pathogens responsible for dollar spot (Salgado-Salazar et al. 2018), as well as *C. capillus-albis*, a known cheatgrass pathogen. While the *Clarireedia* OTU may not be exactly the same as *C. capillus-albis* it could potentially be a closely related species that functions pathogenically in a similar manner.

The other fungi found in Skull Valley surface litter with increased abundance in stand failure sites seem unlikely to be causing stand failures as the genera or species they belong to have not been shown to have pathogenic ability. They are either classified as saprophytes, feeding on dead plant litter, or simply do not feed off plant material at all (Cannon and Kirk 2007; Cheng et al. 2001; Khan et al. 2013; Kohlmeyer et al. 1996; Phukhamsakda et al. 2016; Schmidt et al. 2017).

Within the soil of Skull Valley, there are several fungi, with increased abundance in stand failure sites that could potentially act as pathogens. These fungal OTUs are called to the genera of *Olpidium* and *Monosporascus.* Both have cited instances in which species in their genus act as plant pathogens (Cohen et al. 2000; Teakle 1962). While PERMANOVA analyses did show surface litter samples to have most significant changes, fungi with increased abundances in soil still warrant investigation.

While these increased abundances are not reflected in Winnemucca, it is possible they are location specific.

In Winnemucca, the most likely fungi to be acting as pathogens appear to be *R. apicalis*, which was previously discussed, and potentially an OTU identified to the genus *Comoclathris*. Most of the work on *Comoclathris* has been in the field of phylogenetics where it has been classified in the *Pleosporaceae* family (Ariyawansa et al. 2014). This family has many members such as *Alternaria, Cochliobolus*, *Crivellia* and *Pyrenophora,* all of which can act as plant pathogens (Hosford Jr 1971; Inderbitzin et al. 2006; Nishimura et al. 1978; Pitkin et al. 1996).

Conclusions of Fungi with Increase Abundances

While ANCOM revealed many fungi that were potentially causal agents of stand failure, investigation of the genera and families each OTU belonged to disqualified them as potential causal agents. The majority of these fungi are saprotrophs or yeast fungi that have little or no pathogenic ability. However, in Skull Valley, the fungal OTUs identified to *R. apicalis, C. capillus-albis, Clarireedia* were found to be potentially pathogenic in the surface litter, while the fungal OTUs identified to *Olpidium* and *Monosporascus* could be pathogenic in the soil. In Winnemucca, the OTUs identified to *R. apicalis* and *Comoclathris* were found to be potentially pathogenic in the surface litter while *R. apicalis* and *E. nigrum* could be pathogenic in the soil. While *E. nigrum* and *C. capillus-albis* have been shown to act as pathogens towards cheatgrass, this has not been demonstrated in the other fungi with increased abundance in stand failure sites. In future studies, these fungi should be isolated, and tested for their ability to act as pathogens towards cheatgrass and induce stand failure.

CONCLUSIONS

Overall, this study gives a greater understanding to the fungal dynamics within cheatgrass soils and surface litter. Fungi found commonly in these environments have been identified. It was confirmed that previously identified cheatgrass pathogens *C. capillus-albis* and *E. nigrum* have increased abundance in Skull Valley surface litter and Winnemucca soil respectively, indicating they could be linked to stand failure. Furthermore, it has been confirmed that there are differences in the fungal communities between the sites that have experienced die-offs and those that have not; specifically, these differences appear to be concentrated in the surface litter. Many of these differences appear to vary by location. While many of the differences are likely due to chance, the increased abundance of *Ramimonilia apicalis* in the surface litter of both Skull Valley and Winnemucca, along with the other potential fungal pathogens such as *Olpidium, Monosporascus,*and *Comoclathris,* warrant further investigation. Whether the increased abundance of these fungi is due to random chance or is biologically meaningful is unknown. Future studies will need to be performed to verify if these fungi have pathogenic effects on cheatgrass. It was confirmed that previously identified cheatgrass pathogens *C. capillus-albis* and *E. nigrum* have increased abundance in Skull Valley surface litter and Winnemucca soil respectively, indicating they could be linked to stand failure.

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. Journal of Alzheimer's Disease **58**(1): 55-67.

Ariyawansa, H.A., Phookamsak, R., Tibpromma, S., Kang, J.-C., and Hyde, K.D. 2014. A Molecular and Morphological Reassessment of *Diademaceae*. The Scientific World Journal **2014**.

Baughman, O.W., and Meyer, S.E. 2013. Is *Pyrenophora semeniperda* the cause of Downy Brome (*Bromus tectorum*) Die-Offs? Invasive Plant Science and Management **6**(1): 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? Journal of Arid Environments **124**(Supplement C): 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? Annals of Botany **99**(5): 907-914.

Bokulich, N.A., Kaehler, B.D., Rideout, J.R., Dillon, M., Bolyen, E., Knight, R., Huttley, G.A., and Caporaso, J.G. 2018. Optimizing Taxonomic Classification of Marker-Gene Amplicon Sequences with QIIME 2's q2-feature-classifier luPgin. Microbiome **6**(1): 90.

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. Nature Methods **13**(7): 581.

Cannon, P.F., and Kirk, P.M. 2007. Fungal families of the world. Cabi. pp. 192, 287, 447, 454

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, 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., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., and Knight, R. 2010. QIIME Allows Analysis of High-Throughput Community Sequencing data. Nature Methods **7**(5): 335-336.

Cheng, M.-F., Chiou, C.C., Liu, Y.-C., Wang, H.-Z., and Hsieh, K.-S. 2001. *Cryptococcus laurentii* Fungemia in a Premature Neonate. Journal of Clinical Microbiology **39**(4): 1608-1611.

Cohen, R., Pivonia, S., Burger, Y., Edelstein, M., Gamliel, A., and Katan, J. 2000. Toward Integrated Management of *Monosporascus* Wilt of Melons in Israel. Plant Disease **84**(5): 496-505.

Cruaud, P., Rasplus, J.-Y., Rodriguez, L.J., and Cruaud, A. 2017. High-throughput Sequencing of Multiple Amplicons for Barcoding and Integrative taxonomy. Scientific reports **7**: 41948.

D'Antonio, C.M., and Vitousek, P.M. 1992. Biological Invasions by Exotic Grasses, the Grass/Fire Cycle, and Global Change. Annual Review of Ecology and Systematics **23**(1): 63-87.

Egidi, E., De Hoog, G., Isola, D., Onofri, S., Quaedvlieg, W., De Vries, M., Verkley, G., Stielow, J.B., Zucconi, L., and Selbmann, L. 2014. Phylogeny and Taxonomy of Meristematic Rock-Inhabiting Black Fungi in the *Dothideomycetes* based on Multi-locus Phylogenies. Fungal Diversity **65**(1): 127-165.

Faith, D.P. 1992. Conservation Evaluation and Phylogenetic Diversity. Biological Conservation **61**(1): 1-10.

Fierer, N., Schimel, J.P., and Holden, P.A. 2003. Variations in Microbial Community Composition through twoSoil Depth Profiles. Soil Biology and Biochemistry **35**(1): 167-176.

Frank, J.A., Pan, Y., Tooming-Klunderud, A., Eijsink, V.G., McHardy, A.C., Nederbragt, A.J., and Pope, P.B. 2016. Improved Metagenome Assemblies and Taxonomic Binning using Long-read Circular Consensus Sequence Data. Scientific Reports **6**: 25373.

Gupta, P., and Vakhlu, J. 2011. Metagenomics: a Quantum Jump from Bacterial Genomics. Indian Journal of Microbiology **51**(4): 539.

Handelsman, J. 2004. Metagenomics: Application of Genomics to Uncultured Microorganisms. Microbiology and Molecular Biology Reviews **68**(4): 669-685.

Hebert, P.D., Cywinska, A., Ball, S.L., and deWaard, J.R. 2003. Biological Identifications Through DNA Barcodes. Proceedings. Biological Sciences **270**(1512): 313-321.

Hosford Jr, R.M. 1971. A Form of *Pyrenophora trichostoma* Pathogenic to Wheat and other Grasses. Phytopathology **61**(1): 28-32.

Inderbitzin, P., Shoemaker, R.A., O'neill, N.R., Turgeon, B.G., and Berbee, M.L. 2006. Systematics and Mating systems of two Fungal Pathogens of Opium Poppy: the Heterothallic *Crivellia papaveracea* with a *Brachycladium penicillatum*Asexual State and a Homothallic Species with a *Brachycladium papaveris* Asexual state. Botany **84**(8): 1304-1326.

Khan, Z., Gené, J., Ahmad, S., Cano, J., Al-Sweih, N., Joseph, L., Chandy, R., and Guarro, J. 2013. *Coniochaeta polymorpha*, a New Species from Endotracheal Aspirate of a Preterm Neonate, and Transfer of *Lecythophora* species to *Coniochaeta*. Antonie Van Leeuwenhoek **104**(2): 243-252.

Klemmedson, J.O., and Smith, J.G. 1964. Cheatgrass (*Bromus tectorum* L.). The Botanical Review **30**(2): 226-262.

Kohlmeyer, J., Volkmann-Kohlmeyer, B., and Eriksson, O.E. 1996. Fungi on *Juncus roemerianus*. New Marine and Terrestrial Ascomycetes. Mycological Research **100**(4): 393-404.

Koide, R.T., Ricks, K.D., and Davis, E.R. 2017. Climate and Dispersal Influence the Structure of Leaf Fungal Endophyte Communities of *Quercus gambelii* in the Eastern Great Basin, USA. Fungal Ecology **30**: 19-28.

Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., and Schloss, P.D. 2013. Development of a Dual-index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform. Applied Environmental Microbiology **79**(17): 5112-5120.

Lekberg, Y., Koide, R.T., Rohr, J.R., ALDRICH-WOLFE, L., and Morton, J.B. 2007. Role of Niche Restrictions and Dispersal in the Composition of Arbuscular Mycorrhizal Fungal Communities. Journal of Ecology **95**(1): 95-105.

Maas, C.J., and Hox, J.J. 2005. Sufficient Sample Sizes for Multilevel Modeling. Methodology **1**(3): 86-92.

Mack, R.N. 1981. Invasion of *Bromus tectorum* L. into Western North America: An Ecological Chronicle. Agro-Ecosystems **7**(2): 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. Microbial Ecology in Health and Disease **26**(1): 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. *Edited by* M.J. Germino and J.C. Chambers and C.S. Brown. Springer International Publishing, Cham. pp. 193-223.

Meyer, S.E., Quinney, D., Nelson, D.L., and Weaver, J. 2007. Impact of the Pathogen *Pyrenophora semeniperda* on *Bromus tectorum* Seedbank Dynamics in North American Cold Deserts. Weed Research **47**(1): 54-62.

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 Research **54**(5): 511-519.

Nilsson, R.H., Larsson, K.-H., Taylor, A.F., Bengtsson-Palme, J., Jeppesen, T.S., Schigel, D., Kennedy, P., Picard, K., Glöckner, F.O., and Tedersoo, L. 2018. The UNITE Database for Molecular Identification of Fungi: Handling Dark Taxa and Parallel Taxonomic Classifications. Nucleic Acids Research **47**(D1).

Nishimura, S., Sugihara, M., Kohmoto, K., and Otani, H. 1978. Two Different Phases in Pathogenicity of the *Alternaria* Pathogen Causing Black Spot Disease of Japanese Pear. Journal of the Faculty of Agriculture, Tottori University **13**: 1-10.

Oulas, A., Pavloudi, C., Polymenakou, P., Pavlopoulos, G.A., Papanikolaou, N., Kotoulas, G., Arvanitidis, C., and Iliopoulos, I. 2015. Metagenomics: Tools and Insights for Analyzing Next-Generation Sequencing Data Derived from Biodiversity Studies. Bioinformatics and Biology Insights **9**: 75-88.

Pelis, K. 1997. Species and Specificity: an Interpretation of the History of Immunology. Medical History **41**(2): 251-253.

Phukhamsakda, C., Ariyawansa, H.A., Phillips, A.J., Wanasinghe, D.N., Bhat, D.J., McKenzie, E.H., Singtripop, C., Camporesi, E., and Hyde, K.D. 2016. Additions to *Sporormiaceae*: Introducing two novel genera, *Sparticola* and *Forliomyces*, from *Spartium*. Cryptogamie, Mycologie **37**(1): 75-98.

Pielou, E.C. 1966. Shannon's Formula as a Measure of Specific Diversity: its Use and Misuse. The American Naturalist **100**(914): 463-465.

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

Piemeisel, R.L. 1951. Causes affecting change and rate of change in a vegetation of annuals in Idaho. Ecology **32**(1): 53-72.

Pitkin, J.W., Panaccione, D.G., and Walton, J.D. 1996. A putative Cyclic Peptide Efflux Pump Encoded by the TOXA Gene of the Plant-pathogenic Fungus *Cochliobolus carbonum*. Microbiology **142**(6): 1557-1565.

Raja, H.A., Miller, A.N., Pearce, C.J., and Oberlies, N.H. 2017. Fungal Identification Using Molecular Tools: a Primer for the Natural Products Research Community. Journal of Natural Products **80**(3): 756-770.

Rhoads, A., and Au, K.F. 2015. PacBio Sequencing and its Applications. Genomics, Proteomics & Bioinformatics **13**(5): 278-289.

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., Tredway, L.P., Clarke, B.B., and Crouch, J.A. 2018. Clarireedia: A New Fungal Genus Comprising four Pathogenic Species Responsible for Dollar Spot Disease of Turfgrass. Fungal Biology **122**(8): 761-773.

Schmidt, S.K., Vimercati, L., Darcy, J.L., Arán, P., Gendron, E.M.S., Solon, A.J., Porazinska, D., and Dorador, C. 2017. A *Naganishia* in High Places: Functioning Populations or Dormant Cells from the Atmosphere? Mycology **8**(3): 153-163.

Staley, J.T., and Konopka, A. 1985. Measurement of in situ Activities of Nonphotosynthetic Microorganisms in Aquatic and Terrestrial Habitats. Annual Reviews in Microbiology **39**(1): 321-346.

Stewart, T., Allen, P., and Meyer, S.E. 2009. First Report of *Pyrenophora seminiperda* in Turkey and Greece. Plant Disease **93**(12): 1351-1351.

Teakle, D. 1962. Transmission of Tobacco Necrosis Virus by a Fungus, *Olpidium brassicae*. Virology **18**(2): 224-231.

Tewari, J., and Bains, P. 1983. Fungi Associated with the Roots of Clover in Alberta. 1. *Olpidium brassicae* and Ligniera sp. Canadian Plant Disease Survey **63**(2): 35-37.

Toju, H., Tanabe, A.S., Yamamoto, S., and Sato, H. 2012. High-Coverage ITS Primers for the DNA-Based Identification of Ascomycetes and Basidiomycetes in Environmental Samples. PLoS One **7**(7): e40863.

van der Klei, I., Veenhuis, M., Brul, S., Klis, F.M., De Groot, P.W., Müller, W.H., van Driel, K.G., and Boekhout, T. 2011. Cytology, Cell alls and Septa: a Summary of Yeast Cell Biology from a Phylogenetic Perspective. The Yeasts. Elsevier. pp. 111-128.

Weisberg, P.J., Dilts, T.E., Baughman, O.W., Meyer, S.E., Leger, E.A., Van Gunst, K.J., and 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**(Supplement C): 173-181.

Whisenant, S.G. 1990. Changing Fire Frequencies on Idaho's Snake River Plains: Ecological and Management Implications. *Edited by* F.S. US Department of Agriculture, Intermountain Research Station, Ogden Utah. pp. 5-7.

White, T.J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and Direct Sequencing of Fungal Ribosomal RNA genes for Phylogenetics. PCR Protocols: a Guide to Methods and Applications. *Edited by* M.A. Innis and D.H. Gelfand and J.J. Sninsky and T.J. White. pp. 315-322.

Ziska, L.H., Reeves, J.B., and Blank, B. 2005. The Impact of Recent Increases in Atmospheric CO2 on Biomass Production and Vegetative Retention of Cheatgrass (*Bromus tectorum*): Implications for Fire Disturbance. Global Change Biology **11**(8): 1325-1332..

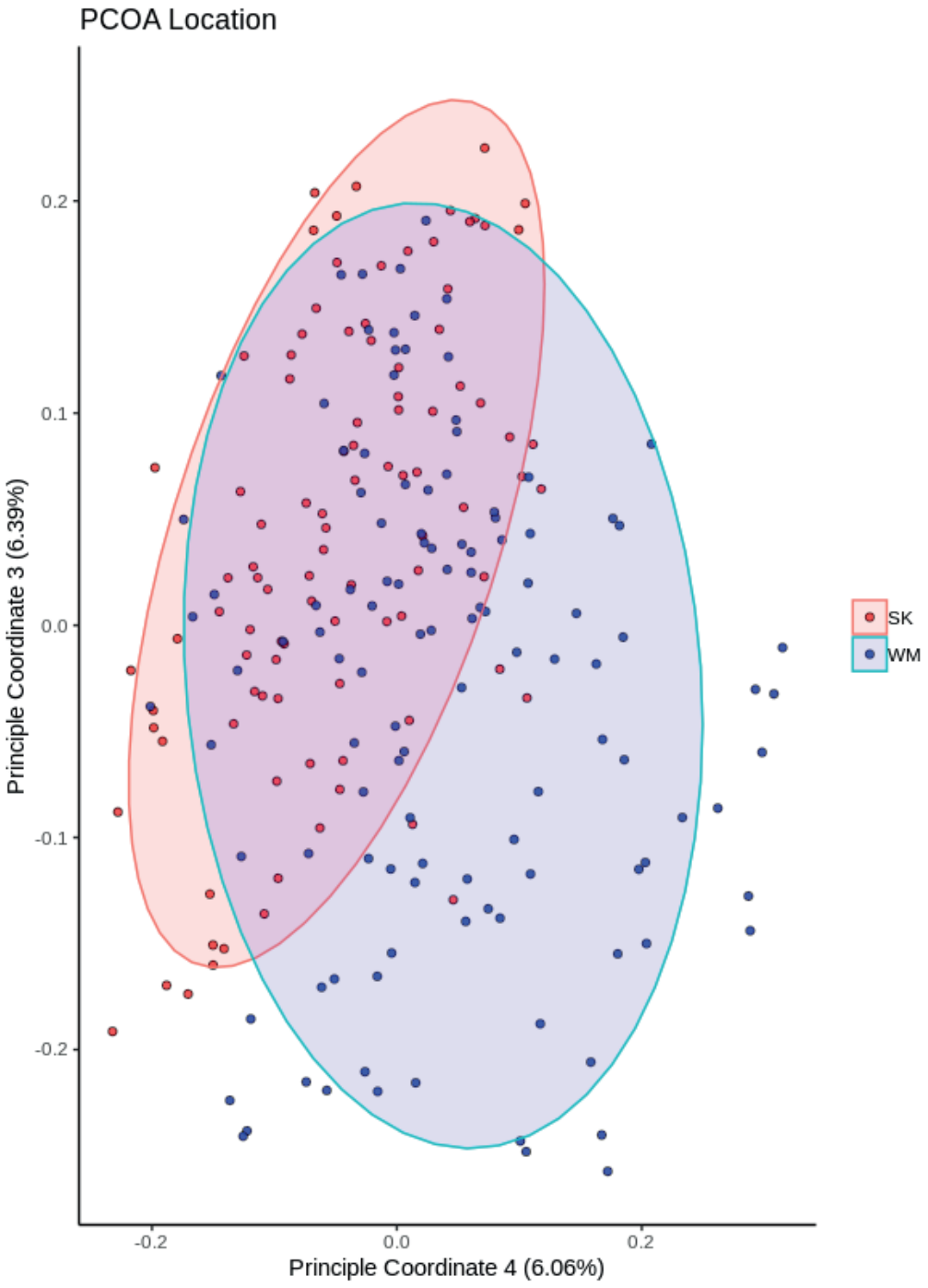


Figure 1. Illumina Principal Coordinate plot of Ilumina data by Location. Skull Valley (SK) and Winnemucca (WM) are plotted on Principle Coordinate 3 and 4 as these two coordinates are what most divided the samples by location. The shaded ovals represent 95% confidence ellipses.

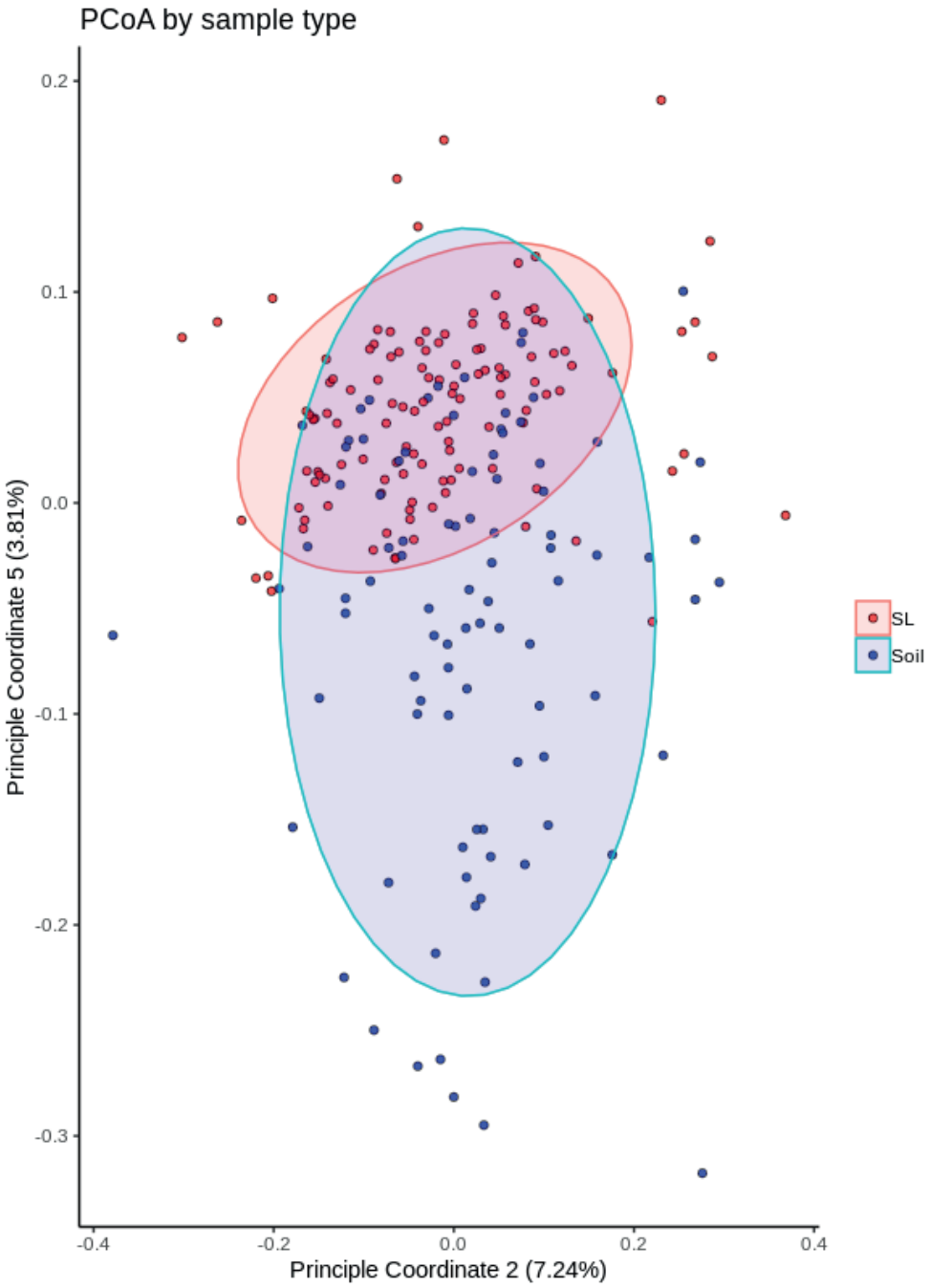


Figure 2. Principal Coordinate plot of Illumina data by Sample Type. Surface litter (SL) and soil are plotted on Principle Coordinate 2 and 5 as these two coordinates are what most divided the samples by location. The shaded ovals represent 95% confidence ellipses.

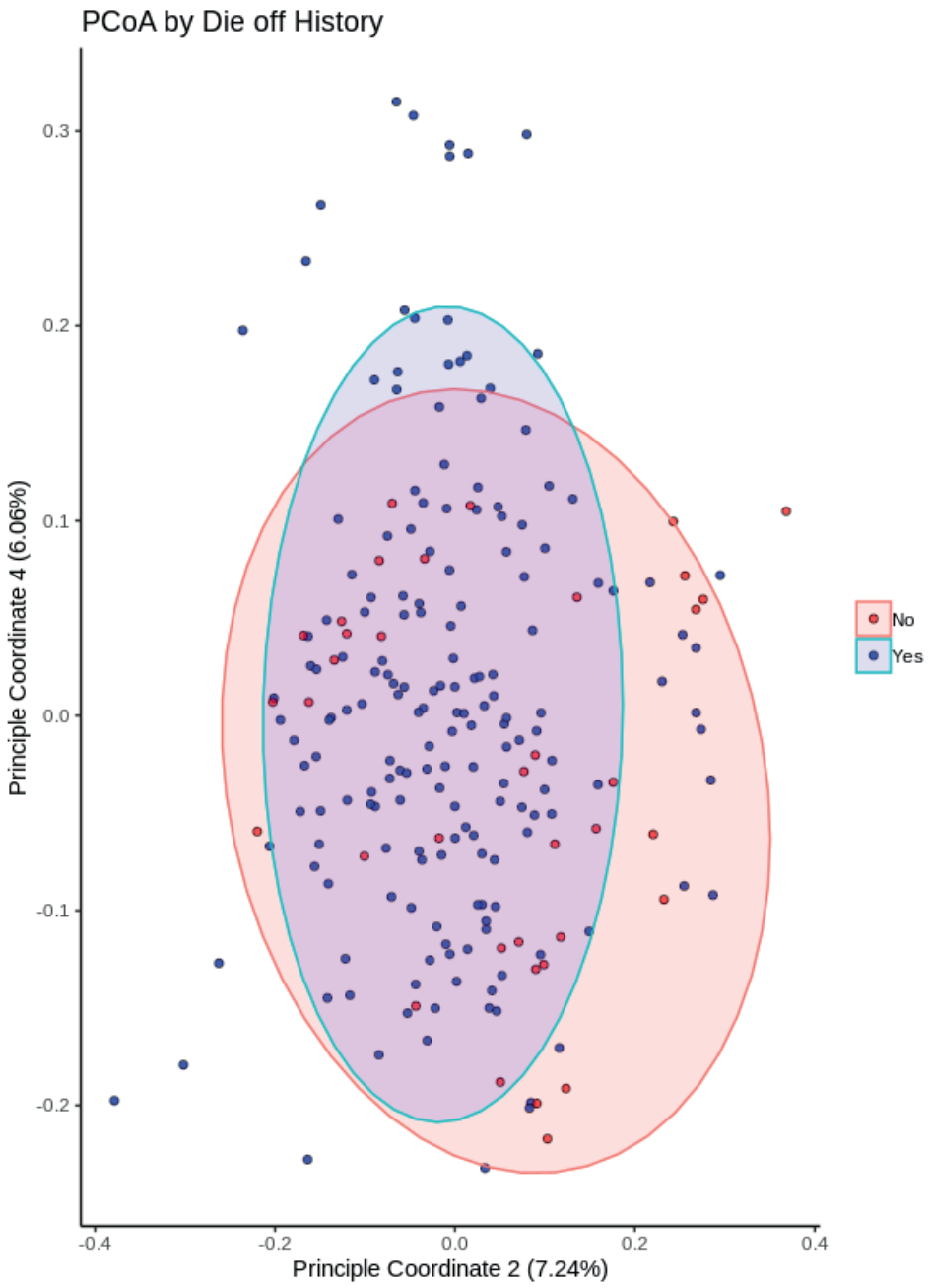


Figure 3. Principal Coordinate plot of Illumina data by stand failure history. Sites that have had a stand failure (label yes) and sites that have not had a stand failure (labeled no) are plotted on Principle Coordinate 2 and 4 as these two coordinates are what most divided the samples by stand failure history. The shaded ovals represent 95% confidence ellipses.

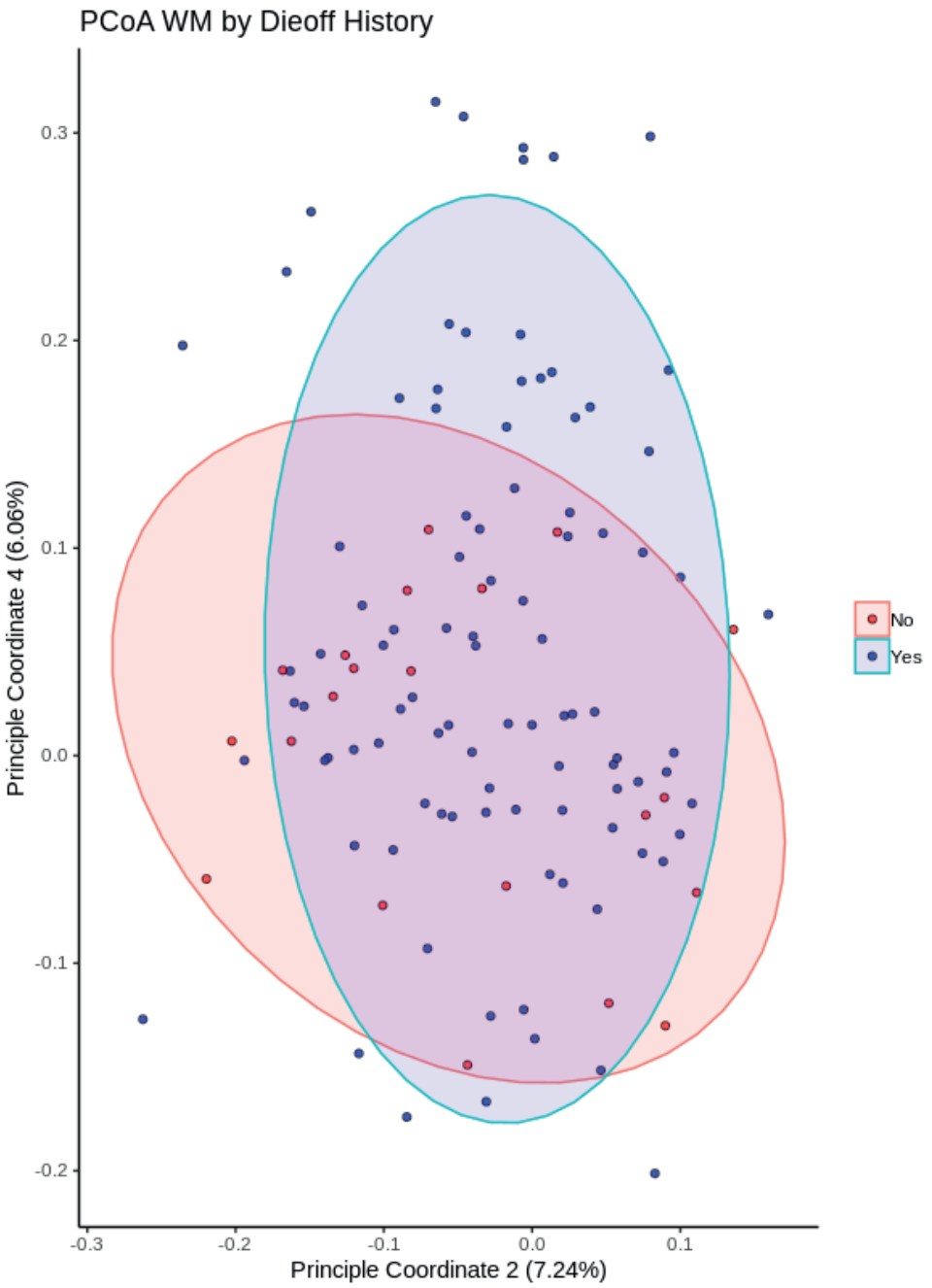


Figure 4. Principal Coordinate plot of Illumina data by stand failure history in Winnemucca. Sites that have had a stand failure (label yes) and sites that have not had a stand failure (labeled no) are plotted on Principle Coordinate 2 and 4 as these two coordinates are what most divided the samples by stand failure history. The shaded ovals represent 95% confidence ellipses.

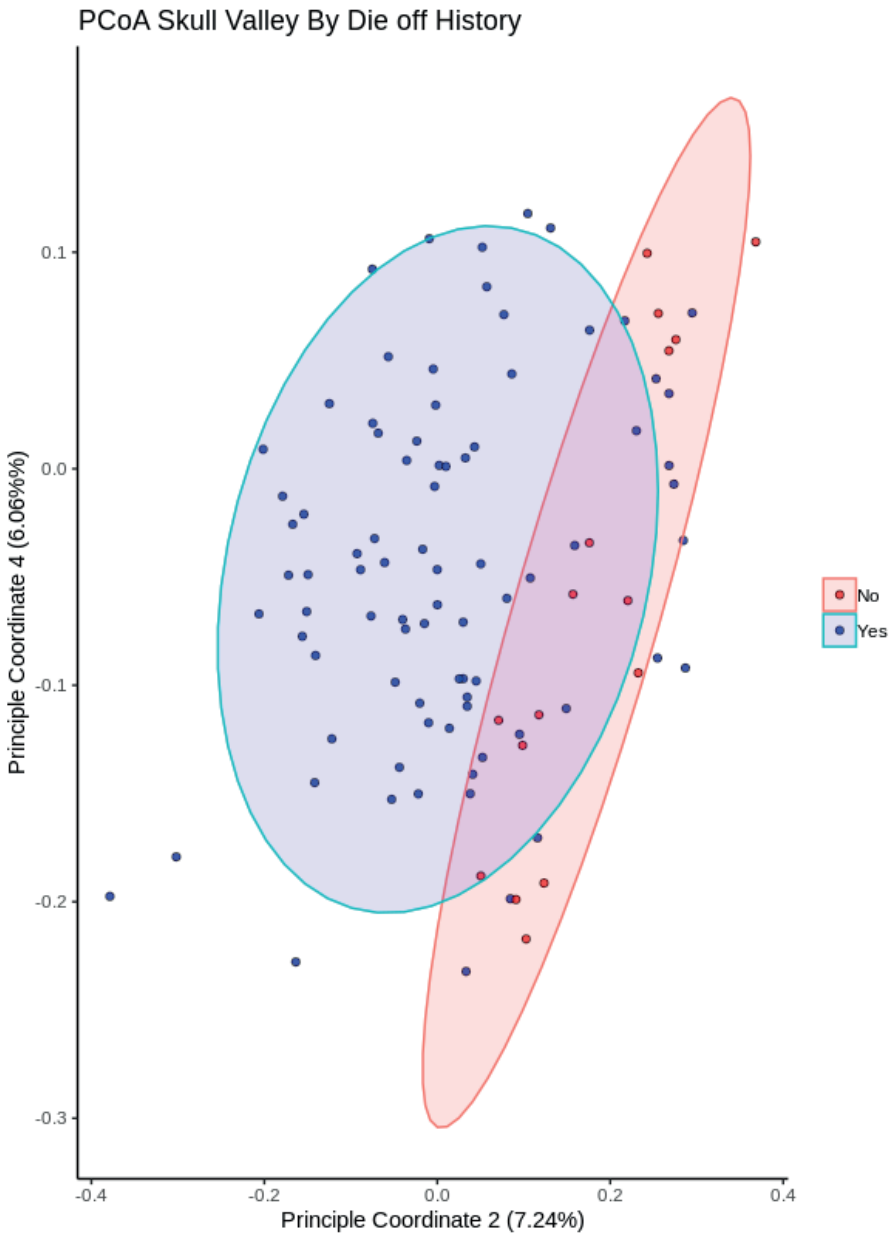


Figure 5. Principal Coordinate plot of Illumina data by stand failure history in Skull Valley. Sites that have had a stand failure (label yes) and sites that have not had a stand failure (labeled no) are plotted on Principle Coordinate 2 and 4 as these two coordinates are what most divided the samples by stand failure history. The shaded ovals represent 95% confidence ellipses.

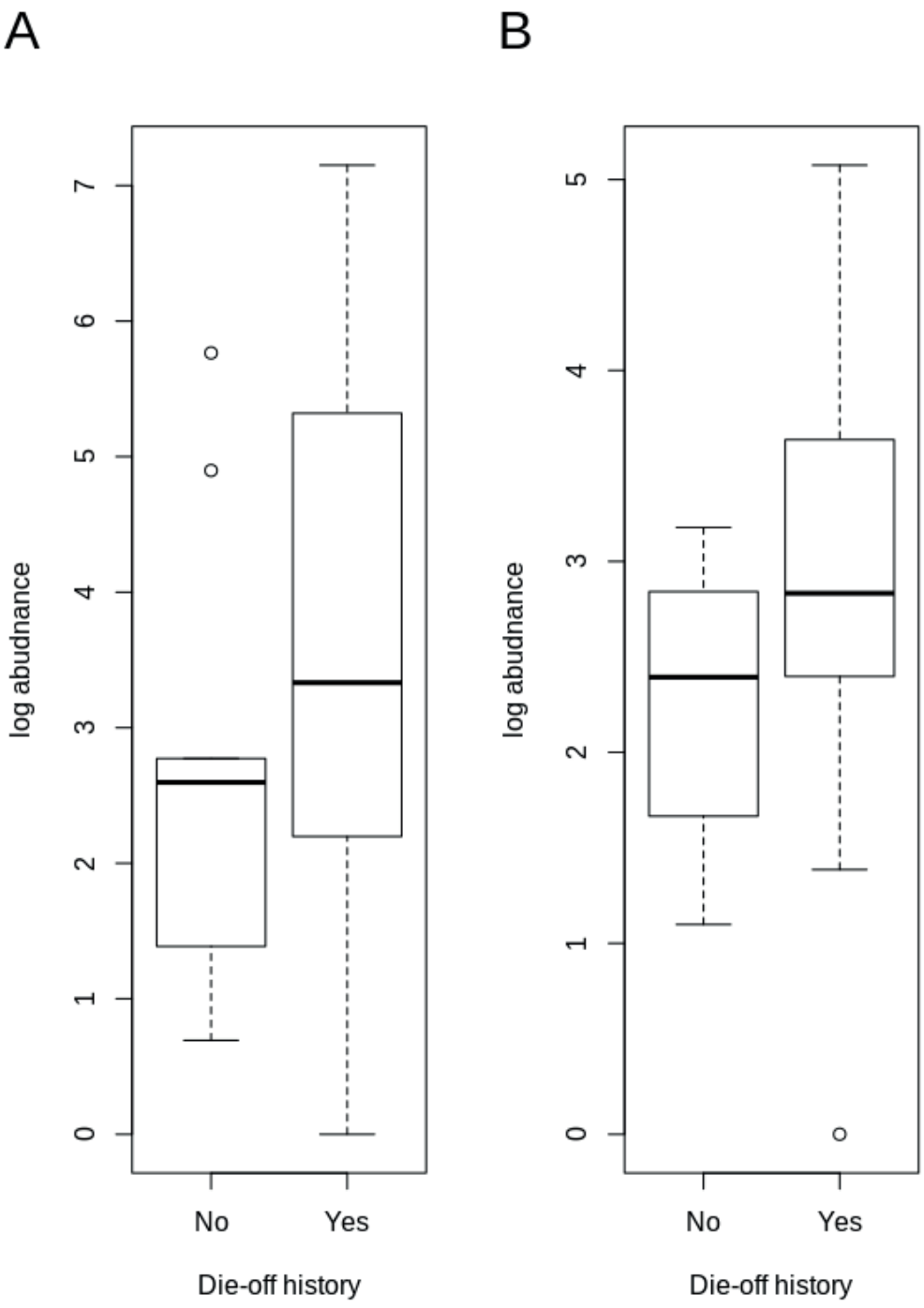


Figure 6. Abundances of Epicoccum nigrum and Clarireedia capillus-albis. Log abundance of specific Fungi in samples that have (yes) and have not (no) had a stand failure in the past. **A** shows that abundance of Epicoccum nigrum in Nevada Soils while **B** shows the abundance of Clarireedia capillus-albis in Utah surface litter.

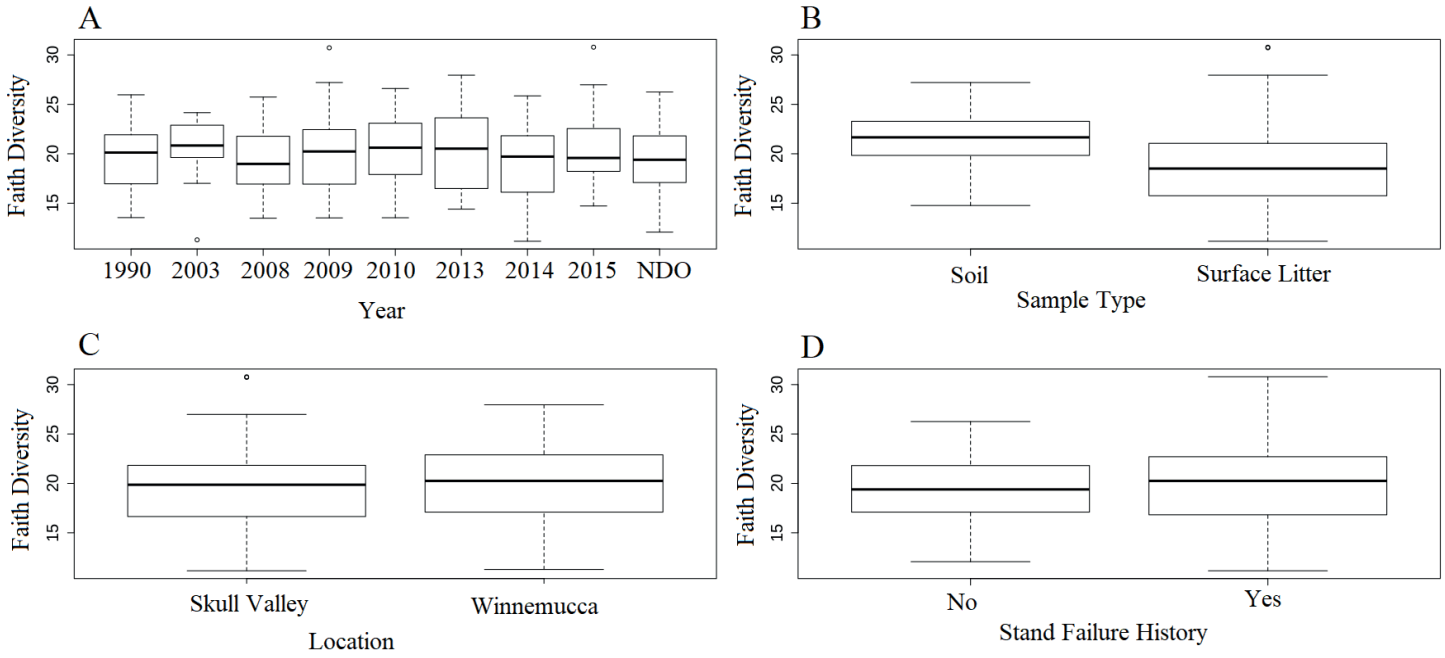


Figure 7. Comparison of Faith Diversity between groups. **A.** Comparing diversity of differing years in which a stand failure occurred. **B.** Comparing the diversity of both sample types. **C.** Comparing the diversity between both locations, Skull Valley Utah and Winnemucca Nevada. D. Comparing the diversity between samples that have experienced a stand failure in the past (Yes) and those that have not (No).

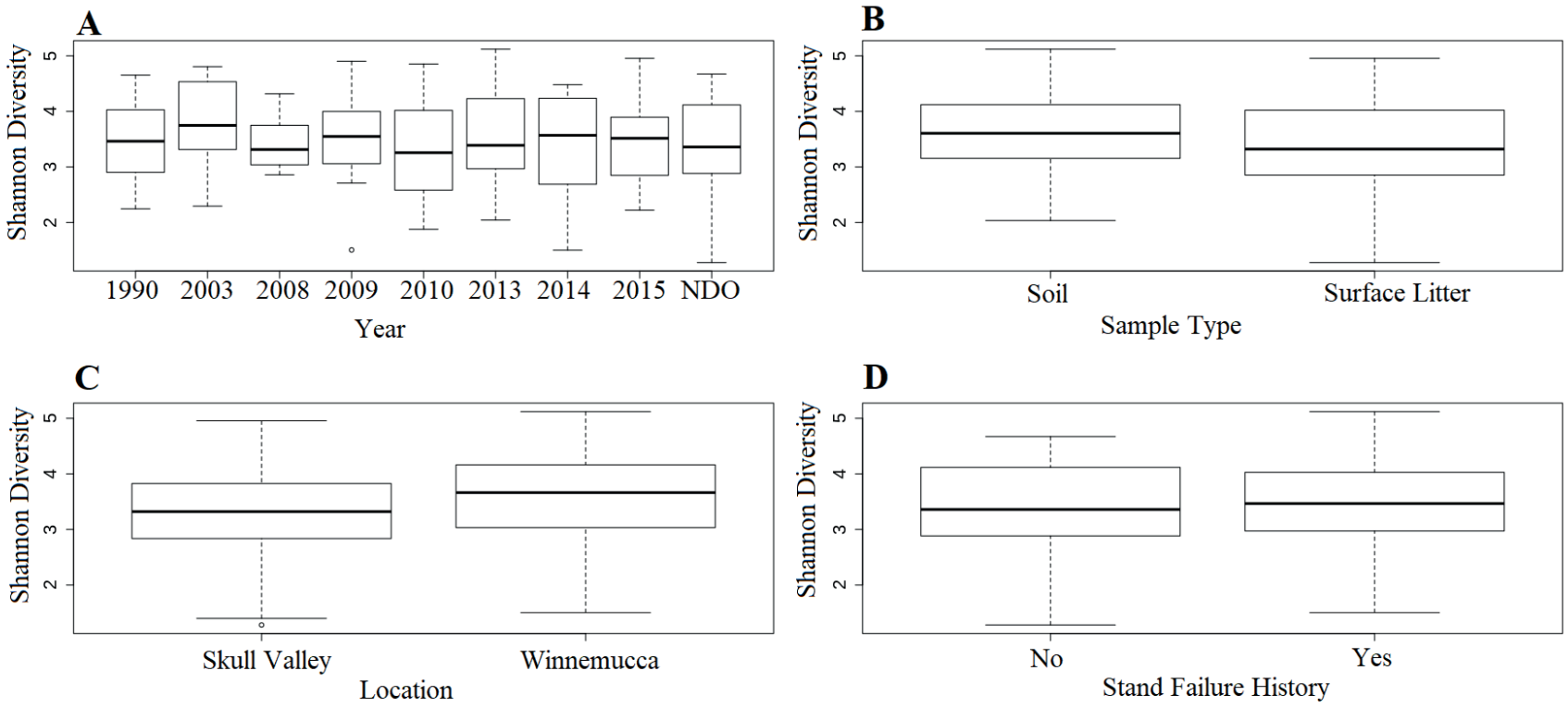


Figure 8. Comparison of Shannon Diversity between groups. A. Comparing diversity of differing years in which a stand failure occurred. B. Comparing the diversity of both sample types. C. Comparing the diversity between both locations, Skull Valley Utah and Winnemucca Nevada. D. Comparing the diversity between samples that have experienced a stand failure in the past (Yes) and those that have not (No).

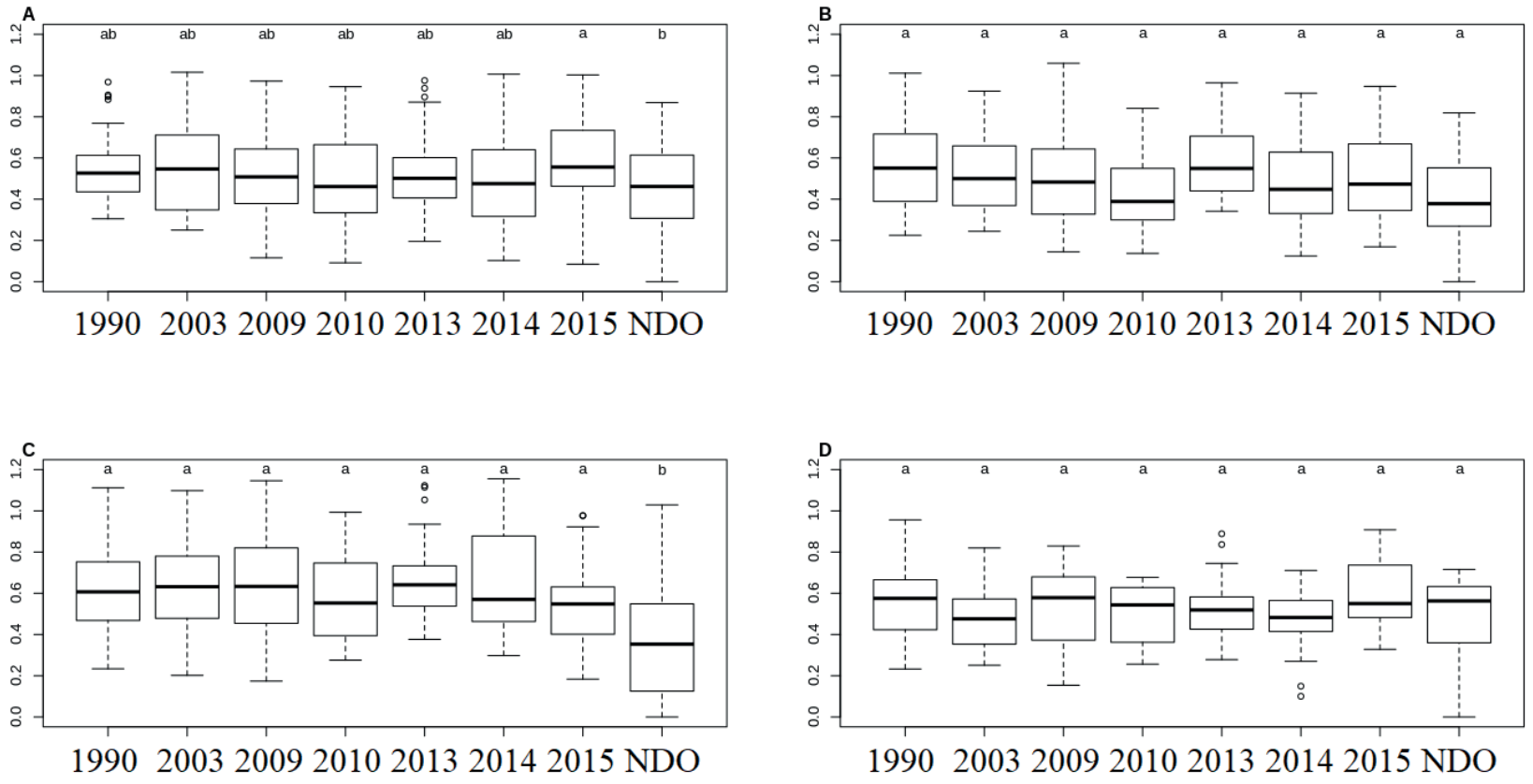


Figure 9. Searching for an effect by year. The weighted unifrac distance of each year to sites that had never had a stand failure (NDO). Above each box shows the groupings by multicomp analysis. (A) Surface litter from Winnemucca, Nevada., B shows the soil from Winnemucca Nevada, C shows the surface litter from Skull Valley Utah and D shows the soil from Skull Valley Utah.

TABLES

Table 1. Sample Locations.

Year	GPS	
Utah		
None	40.1419	112.668
None	40.13996	-112.641
1990	40.1388	-112.711
2008	40.17711	-112.728
2009	40.39453	-112.948
2010	40.2752	-112.631
2013	40.32838	-112.777
2014	40.34031	-112.686
2015	40.29299	-112.77
Nevada		
None	40.69066	-117.894
None	40.6989	-117.899
1990	40.69205	-117.938
2003	40.68962	-117.964
2009	40.69183	-117.959
2009 Site 2	40.69305	-117.923
2010	40.69839	118.044
2013	40.69445	-117.938
2014	40.68664	-117.983
2015	40.68791	-117.966

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

Table 2. Reads called to each taxonomic level.

Taxonomic Level	PacBio reads	Ilumina With PacBio Reference	Ilumina Without PacBio 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

Second column shows the percentage of PacBio reads called to each taxonomic level. The third column shows the percentage of Ilumina reads called to each taxonomic level using the PacBio reads as the references, while the fourth column shows the Ilumina reads without using the PacBio reference.

Table 3. PERMANOVA results.

Variable	P-value -weighted	P-value -unweighted
Sample type	.002	.001
Location	.001	.001
Stand failure History	.042	.001
Location * Stand failure history	.023	.001

P-values of each variable in the regression model built from the Illumina weighted and unweighted unifrac distance matrix. The term stand failure history refers to whether or not a stand failure had occurred in that site.

Table 4. Subsampled PERMANOVA results.

Group being compared	p-value from weighted Distance matrix– Presence or Absence of Stand failure	p-value from unweighted distance matrix– Presence or Absence of Stand failure
Nevada Soil	.274	.274
Nevada Surface Litter	.090	.032
Utah Soil	.30	.286
Utah Surface Litter	.020	.02

PERMANOVA tests were run separately on each of the treatment groups, using the weighted and unweighted distance matrix. P-values reported reflect the significance of the stand failure variable, measuring the difference between sites that had and had not experienced a stand failure.

Table 5. OTUS identified by ANCOM.

		Taxonomy						
	GenBank ID	**Kingdom**	**Phyllum**	**Class**	**Order**	**Family**	**Genus**	**Species**
More Abundant in Winnemucca Surface litter Samples with a History of Stand-failure	**MK281667.1**	Fungi	Ascomycota	Dothideomycetes	Botryosphaeriales	Planistromellaceae	Ramimonilia	
	MK281714.1	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Comoclathris	
	MK281810.1	Fungi	Basidiomycota	Tremellomycetes				
More Abundant in Samples with a History of Stand-failure	**MK281667.1**	Fungi	Ascomycota	Dothideomycetes	Botryosphaeriales	Planistromellaceae	Ramimonilia	Ramimonilia apicalis
	MK281810.1	Fungi	Basidiomycota	Tremellomycetes				
More Abundant in Samples with a History of Stand-failure	**MK281667.1**	Fungi	Ascomycota	Dothideomycetes	Botryosphaeriales	Planistromellaceae	Ramimonilia	Ramimonilia apicalis
	MK281662.1	Fungi	Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	Naganishia	Naganishia friedmannii
	MK281737.1	Fungi	Ascomycota	Sordariomycetes	Coniochaetales	Coniochaetaceae	Coniochaeta	Coniochaeta polymorpha
	MK281802.1	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	Sparticola	
	MK281822.1	Fungi	Ascomycota	Leotiomycetes	Helotiales	Rutstroemiaceae	Clarireedia	
	MK281941.1	Fungi	Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	Cryptococcus	
	MK281916.1	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Lentitheciaceae	Keissleriella	
	MK281670.1	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Lentitheciaceae	Keissleriella	
	MK281810.1	Fungi	Basidiomycota	Tremellomycetes				
More Abundant in samples without a history of Stand Failure	**MK281660.1**	Fungi	Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae	Vishniacozyma	Vishniacozyma globispora
	MK281736.1	Fungi	Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	Naganishia	Naganishia albida
	MK281899.1	Fungi	Ascomycota	Dothideomycetes	Pleosporales			
	MK281809.1	Fungi	Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	Filobasidium	Filobasidium magnum
	MK281900.1	Fungi	Ascomycota	Dothideomycetes	Pleosporales	pleosporaceae	Neocamarosporium	
More Abundant in Samples with a History of Stand-failure	**MK281916.1**	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Lentitheciaceae	Keissleriella	
	MK281699.1	Fungi	Olpidiomycota	Olpidiomycetes	Olpidiales	Olpidiaceae	Olpidium	
	MK281743.1	Fungi	Ascomycota	Sordariomycetes	Xylariales	Diatrypaceae	Monosporascus	
	MK281802.1	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	Sparticola	
	MK281941.1	Fungi	Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	Cryptococcus	
More Abundant in Samples without a History of Stand-failure	**MK281660.1**	Fungi	Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae	Vishniacozyma	Vishniacozyma globispora
	MK281711.1	Fungi	Ascomycota	Dothideomycetes	Pleosporales			

	MK281736.1	Fungi	Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	Naganishia	Naganishia albida
	MK281899.1	Fungi	Ascomycota	Dothideomycetes	Pleosporales			
	MK281743.1	Fungi	Ascomycota	Sordariomycetes	Xylariales	Diatrypaceae	Monosporascus	

OTUs, their taxonomy, and their GenBank ID numbers detected to be significant by ANCOM in the four different treatment groups.

SUPPLEMENTAL MATERIAL

Supplemental Tables

Supplemental Table 1. Experimental design of the Long-Read Reference Library

Sample Number	Location	Sample Type	Stand Failure Year
1	Utah	Soil	2015
2	Utah	Soil	2014
3	Utah	Soil	2013
4	Utah	Soil	2010
5	Utah	Soil	Never
6	Utah	Surface Litter	2015
7	Utah	Surface Litter	2014
8	Utah	Surface Litter	2013
9	Utah	Surface Litter	2010
10	Utah	Surface Litter	Never
11	Nevada	Soil	2015
12	Nevada	Soil	2014
13	Nevada	Soil	2013
14	Nevada	Soil	2010
15	Nevada	Soil	Never
16	Nevada	Surface Litter	2015
17	Nevada	Surface Litter	2014
18	Nevada	Surface Litter	2013
19	Nevada	Surface Litter	2010
20	Nevada	Surface Litter	Never

Supplemental Table 2. 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

Supplemental Table 3. 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

Supplemental Table 4**.** 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				

Supplemental Table 5. Differential abundance between sites. These were the OTUs that varied in their abundance between Utah and Nevada. The first column shows their GenBank Accession number, while the second column specifies if they were more abundant in Nevada or Utah

		Taxonomy					
GenBank ID		Kingdom	Phyllum	Class	Order	Family	Genus
MK281724	Nevada	Fungi	Ascomycota	Arthoniomycetes	Lichenostigmatales	Phaeococcomycetaceae	Phaeococcomyces
MK281667	Nevada	Fungi	Ascomycota	Dothideomycetes	Botryosphaeriales	Planistromellaceae	Ramimonilia
MK281744	Nevada	Fungi	Ascomycota	Dothideomycetes	Capnodiales		
MK281741	Nevada	Fungi	Ascomycota	Dothideomycetes	Capnodiales		
MK281918	Nevada	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Didymosphaeriaceae	
MK281772	Nevada	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	Sclerostagonospora
MK281828	Nevada	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria
MK281912	Nevada	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria
MK281843	Nevada	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria
MK281714	Nevada	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Comoclathris
MK281812	Nevada	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Epicoccum
MK281796	Nevada	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Pyrenophora
MK281730	Nevada	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	Preussia
MK281723	Nevada	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	Preussia
MK282099	Nevada	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	
MK281932	Nevada	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	
MK282093	Nevada	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	
MK281909	Nevada	Fungi	Ascomycota	Dothideomycetes	Tubeufiales		
MK282113	Nevada	Fungi	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	Chlorociboria
MK281767	Nevada	Fungi	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	Crocicreas
MK281770	Nevada	Fungi	Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae	Cistella
MK281870	Nevada	Fungi	Ascomycota	Leotiomycetes	Helotiales	Rutstroemiaceae	Clarireedia
MK281758	Nevada	Fungi	Ascomycota	Leotiomycetes	Helotiales	Rutstroemiaceae	Clarireedia
MK281694	Nevada	Fungi	Ascomycota	Leotiomycetes	Helotiales	Rutstroemiaceae	Clarireedia
MK281674	Nevada	Fungi	Ascomycota	Leotiomycetes	Helotiales	Rutstroemiaceae	Clarireedia
MK281728	Nevada	Fungi	Ascomycota	leotiomycetes	Helotiales		
MK281863	Nevada	Fungi	Ascomycota	leotiomycetes	Helotiales		

MK281834	Nevada	Fungi	Ascomycota	Sordariomycetes	Coniochaetales		
MK281807	Nevada	Fungi	Ascomycota	Sordariomycetes	Coniochaetales		
MK281879	Nevada	Fungi	Ascomycota	Sordariomycetes	Coniochaetales		
MK281867	Nevada	Fungi	Ascomycota	Sordariomycetes	Coniochaetales		
MK281664	Nevada	Fungi	Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae	Podospora
MK281942	Nevada	Fungi	Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae	Podospora
MK281727	Nevada	Fungi	Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae	
MK281820	Nevada	Fungi	Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae	
MK281769	Nevada	Fungi	Ascomycota	Sordariomycetes	Sordariales		
MK281935	Nevada	Fungi	Ascomycota	Sordariomycetes	Sordariales		
MK281712	Nevada	Fungi	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	Anthostomella
MK281782	Nevada	Fungi	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	
MK281805	Nevada	Fungi	Ascomycota	Taphrinomycetes	Taphrinales	Protomycetaceae	Protomyces
MK281817	Nevada	Fungi	Ascomycota				
MK281814	Nevada	Fungi	Ascomycota				
MK282069	Nevada	Fungi	Ascomycota				
MK281689	Nevada	Fungi	Basidiomycota	Agaricomycetes	Auriculariales	Auriculariales_fam_Incertae_sedis	Oliveonia
MK281734	Nevada	Fungi	Basidiomycota	Agaricomycetes			
MK281685	Nevada	Fungi	Basidiomycota	Cystobasidiomycetes	Erythrobasidiales		
MK281752	Nevada	Fungi	Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	Naganishia
MK281948	Nevada	Fungi	Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	Naganishia
MK281671	Nevada	Fungi	Basidiomycota	Tremellomycetes	Filobasidiales	Piskurozymaceae	Solicoccozyma
MK281891	Nevada	Fungi	Basidiomycota	Tremellomycetes	Holtermanniales	Holtermanniales_fam_Incertae_sedis	Holtermanniella
MK282104	Nevada	Fungi	Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae	Dioszegia
MK281889	Nevada	Fungi	Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae	Vishniacozyma
MK282054	Nevada	Fungi	Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae	Vishniacozyma
MK281985	Nevada	Fungi	Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae	Vishniacozyma
MK281881	Nevada	Fungi	Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	Cryptococcus
MK281840	Nevada	Fungi	Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	Cryptococcus
MK282109	Nevada	Fungi	Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	Cryptococcus
MK281810	Nevada	Fungi	Basidiomycota	Tremellomycetes			
MK281993	Nevada	Fungi	Chytridiomycota				

MK281982	Nevada	Fungi					
MK282120	Nevada	Fungi					
MK281878	Utah	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Didymellaceae	
MK281864	Utah	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Didymosphaeriaceae	
MK281695	Utah	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Didymosphaeriaceae	
MK282102	Utah	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria
MK281785	Utah	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria
MK282100	Utah	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria
MK281947	Utah	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria
MK281841	Utah	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria
MK281928	Utah	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria
MK281873	Utah	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria
MK281726	Utah	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria
MK281818	Utah	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Comoclathris
MK281866	Utah	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Comoclathris
MK281900	Utah	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Neocamarosporium
MK281754	Utah	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Pyrenophora
MK281907	Utah	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	Preussia
MK281766	Utah	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	Preussia
MK281745	Utah	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	
MK281832	Utah	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	
MK281793	Utah	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	
MK281899	Utah	Fungi	Ascomycota	Dothideomycetes	Pleosporales		
MK281886	Utah	Fungi	Ascomycota	Dothideomycetes	Pleosporales		
MK281826	Utah	Fungi	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	Tetracladium
MK281757	Utah	Fungi	Ascomycota	Leotiomycetes	Helotiales	Rutstroemiaceae	Clarireedia
MK282085	Utah	Fungi	Ascomycota	Pezizomycetes	Pezizales	Ascobolaceae	Ascobolus
MK281759	Utah	Fungi	Ascomycota	Sordariomycetes	Coniochaetales	Coniochaetaceae	Coniochaeta
MK281737	Utah	Fungi	Ascomycota	Sordariomycetes	Coniochaetales	Coniochaetaceae	Coniochaeta
MK281938	Utah	Fungi	Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae	
MK281760	Utah	Fungi	Ascomycota	Sordariomycetes	Sordariales		
MK281743	Utah	Fungi	Ascomycota	Sordariomycetes	Xylariales	Diatrypaceae	Monosporascus

MK281786	Utah	Fungi	Ascomycota				
MK282059	Utah	Fungi	Ascomycota				
MK281949	Utah	Fungi	Ascomycota				
MK281762	Utah	Fungi	Ascomycota				
MK281777	Utah	Fungi	Basidiomycota	Agaricomycetes			
MK281736	Utah	Fungi	Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	Naganishia
MK281794	Utah	Fungi	Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	Naganishia
MK281753	Utah	Fungi	Basidiomycota	Tremellomycetes	Tremellales	Rhynchogastremataceae	Papiliotrema
MK282066	Utah	Fungi	Basidiomycota	Tremellomycetes	Tremellales		
MK282035	Utah	Fungi	Basidiomycota				
MK281906	Utah	Fungi	Chytridiomycota				
MK281656	Utah	Fungi	Olpidiomycota	Olpidiomycetes	Olpidiales	Olpidiaceae	Olpidium

Supplemental Table 6. Differential abundance between sample types. These were the OTUs that varied in their abundance between soil and surface litter. 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

		Taxonomy						
Gene Bank Accession	**Sample type in which it was more Abundant**	**Kingdom**	**Phyllum**	**Class**	**Order**	**Family**	**Genus**	**Species**
MK281707	Soil	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Lophiostomataceae	Lophiostoma	Lophiostoma multiseptatum
MK281726	Soil	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria	
MK281793	Soil	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae		
MK281886	Soil	Fungi	Ascomycota	Dothideomycetes	Pleosporales			
MK281874	Soil	Fungi	Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae	Cistella	
MK282085	Soil	Fungi	Ascomycota	Pezizomycetes	Pezizales	Ascobolaceae	Ascobolus	
MK281923	Soil	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Fusarium	
MK281760	Soil	Fungi	Ascomycota	Sordariomycetes	Sordariales			
MK281846	Soil	Fungi	Ascomycota	Sordariomycetes	Sordariales			
MK281672	Soil	Fungi	Ascomycota	Sordariomycetes	Xylariales	Diatrypaceae	Monosporascus	
MK281743	Soil	Fungi	Ascomycota	Sordariomycetes	Xylariales	Diatrypaceae	Monosporascus	
MK281657	Soil	Fungi	Ascomycota	Sordariomycetes	Xylariales	Microdochiaceae	Microdochium	
MK281949	Soil	Fungi	Ascomycota					
MK282001	Soil	Fungi	Basidiomycota	Agaricomycetes				
MK281736	Soil	Fungi	Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	Naganishia	Naganishia albida
MK281892	Soil	Fungi	Basidiomycota					
MK281738	Soil	Fungi	Chytridiomycota					
MK281906	Soil	Fungi	Chytridiomycota					
MK282079	Soil	Fungi	Olpidiomycota	Olpidiomycetes	Olpidiales	Olpidiaceae	Olpidium	Olpidium brassicae
MK281656	Soil	Fungi	Olpidiomycota	Olpidiomycetes	Olpidiales	Olpidiaceae	Olpidium	Olpidium brassicae
MK281804	Soil	Fungi	Olpidiomycota	Olpidiomycetes	Olpidiales	Olpidiaceae	Olpidium	Olpidium brassicae
MK281699	Soil	Fungi	Olpidiomycota	Olpidiomycetes	Olpidiales	Olpidiaceae	Olpidium	
MK282077	Soil	Fungi						
MK281936	Surface litter	Fungi	Ascomycota	Arthoniomycetes	Lichenostigmatales	Phaeococcomycetaceae	Phaeococcomyces	

MK281724	Surface litter	Fungi	Ascomycota	Arthoniomycetes	Lichenostigmatales	Phaeococcomycetaceae	Phaeococcomyces	
MK281667	Surface litter	Fungi	Ascomycota	Dothideomycetes	Botryosphaeriales	Planistromellaceae	Ramimonilia	Ramimonilia apicalis
MK281851	Surface litter	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Comoclathris	
MK281802	Surface litter	Fungi	Ascomycota	Sordariomycetes	Sordariales			
MK281889	Surface litter	Fungi	Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae	Vishniacozyma	Vishniacozyma victoriae
MK281810	Surface litter	Fungi	Basidiomycota	Tremellomycetes				

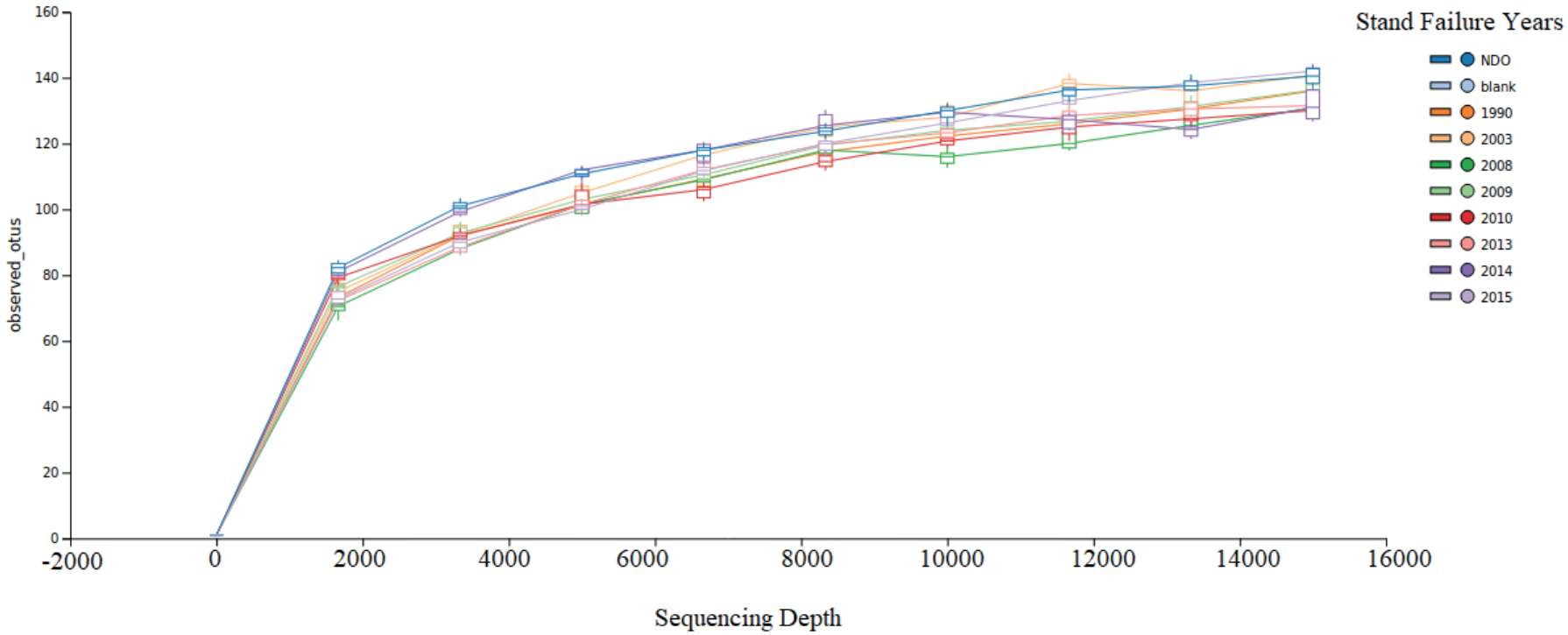
Supplemental Table 7. Thirty most abundant OTUs in all samples

Gene Bank ID	Kingdom	Phyllum	Class	Order	Family	Genus	Species
MK281756.1	Fungi	Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae	Vishniacozyma	Vishniacozyma globispora
MK281916.1	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Lentitheciaceae	Keissleriella	
MK281946.1	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	Preussia	
MK281840.1	Fungi	Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	Cryptococcus	
MK281836.1	Fungi	Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae	Vishniacozyma	Vishniacozyma globispora
MK281982.1	Fungi						
MK281760.1	Fungi	Ascomycota	Sordariomycetes	Sordariales			
MK281802.1	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	Sparticola	
MK281889.1	Fungi	Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae	Vishniacozyma	Vishniacozyma victoriae
MK281667.1	Fungi	Ascomycota	Dothideomycetes	Botryosphaeriales	Planistromellaceae	Ramimonilia	Ramimonilia apicalis
MK281834.1	Fungi	Ascomycota	Sordariomycetes	Coniochaetales	Coniochaetaceae	Coniochaeta	Coniochaeta polymorpha
MK281737.1	Fungi	Ascomycota	Sordariomycetes	Coniochaetales	Coniochaetaceae	Coniochaeta	Coniochaeta polymorpha
MK281662.1	Fungi	Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	Naganishia	Naganishia friedmannii
MK281726.1	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria	
MK281841.1	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria	
MK281810.1	Fungi	Basidiomycota	Tremellomycetes				
MK281932.1	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae		
MK281699.1	Fungi	Olpidiomycota	Olpidiomycetes	Olpidiales	Olpidiaceae	Olpidium	
MK281665.1	Fungi	Ascomycota	Dothideomycetes	Capnodiales	Davidiellaceae	Cladosporium	
MK281878.1	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Didymellaceae		
MK281714.1	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Comoclathris	Comoclathris spartii
MK281804.1	Fungi	Olpidiomycota	Olpidiomycetes	Olpidiales	Olpidiaceae	Olpidium	Olpidium brassicae
MK281736.1	Fungi	Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	Naganishia	Naganishia albida
MK281674.1	Fungi	Ascomycota	Leotiomycetes	Helotiales	Rutstroemiaceae	Clarireedia	Clarireedia capillus albis
MK281837.1	Fungi	Ascomycota	Dothideomycetes	Dothideales	Aureobasidiaceae	Aureobasidium	Aureobasidium pullulans
MK281772.1	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	Sclerostagonospora	
MK281855.1	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Lophiostomataceae	Lophiostoma	Lophiostoma multiseptatum
MK281816.1	Fungi	Basidiomycota	Tremellomycetes				
MK281695.1	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Didymosphaeriaceae		
MK281891.1	Fungi	Basidiomycota	Tremellomycetes	Holtermanniales	Holtermanniales fam Incertae sedis	Holtermanniella	Holtermanniella takashimae

Supplemental Table 8. Illumina OTUs found in every sample

Taxonomy					
Phyllum	Class	Order	Family	Genus	Species
Ascomycota	Dothideomycetes	Pleosporales	Lentitheciaceae	Keissleriella	
Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	Preussia	
Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae	Vishniacozyma	Vishniacozyma globispora

Supplemental Figure



Supplemental Figure 1. Rarefaction curve, representing the number of OTUs found at each rarefaction level.