EFFECTS OF FIRE ON SOIL FUNGAL COMMUNITIES AND THE ENVIRONMENTAL DRIVERS OF COMMUNITY VARIATION IN PRAIRIES OF THE PACIFIC NORTHWEST

by

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THESIS ABSTRACT

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Title: Effects of Fire on Soil Fungal Communities and the Environmental Drivers of Community Variation in Prairies of the Pacific Northwest

Prescribed burning is a common management tool used in restoring prairies of the Pacific Northwest, but the effects of fire on soil fungal community composition is unknown. We collected soil from prairies in Washington and Oregon that included a fire chronosequence: burned within one month, within two years, and not burned in approximately 150 years. We analyzed soil fungal community composition differences between regions and among treatments, and we also examined how fire affects fungal functional groups. In addition, we used variation partitioning to determine the relative contribution of abiotic, biotic, spatial, and burning factors to the variation observed in these soil fungal communities. Soil fungal communities were distinct by region, and there were small but significant effects of burning. While we did not observe any significant differences in relative functional group abundances with burning, there were community compositional differences at finer taxonomic scales.

This dissertation includes unpublished coauthored material.

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CHAPTER I

INTRODUCTION

The ideas and questions built upon in this chapter were developed by two primary committee members, including Bitty A. Roy and Sarah T. Hamman, whom contributed substantially to this work. I was the primary contributor to developing these questions and completed all the writing.

Prairies are one of the most endangered ecosystems in the Pacific Northwest (PNW) with more than 90% loss since European settlement in the Willamette Valley (Noss et al. 1995; Veseley and Rosenberg 2010). Prior to conversion for agriculture, prairies were maintained for around 10,000 years by Native American burning (Boyd 1999). Prairie restoration in this region often employs prescribed fires to reduce woodland incursion (DiTomaso and Johnson 2006) and to control invasive species (Finlay 2008). However, there is little information on how fire might affect the soil fungal communities within these C3 perennial bunchgrass prairies. Many studies have focused on soil fungal communities in forests and their response to fire, but relatively few studies have explored fire effects on grassland soil fungal community structure using Next Generation Sequencing (NGS). Restoration of successful plant communities can depend upon belowground fungal communities (van der Heijden et al. 1998). Similarly, the recovery of the soil fungal community could depend upon the recovery of the plant community, given the mutualistic interactions between mycorrhizae and their host plants and the dependency of saprotrophic fungi on litter inputs from the plant community (Dangi et al. 2010).

Identifying the soil fungal communities and their response to prescribed fires is important because fungi regulate rates of essential ecosystem processes such as litter decomposition, especially the breakdown of lignin, which is considered the rate-limiting step in carbon and nutrient cycling within terrestrial ecosystems (Griffith and Roderick 2008). In grassland soils, fungi affect plant community structure through both pathogenic and mutualistic interactions (Johnson and Graham 2013), and contribute to overall soil structure and humus development (Tisdall and Oades 1982; Griffith and Roderick 2008). While grasslands are some of the most fire-adapted ecosystems, fires can alter soil properties affecting soil fungal communities, especially the organic and surface horizons where most aerobic microbial activity takes place (Neary et al. 1999; Docherty et al. 2012).

Prescribed burning has consequences for a variety of soil chemical and physical properties. Fire changes the nutrient levels and organic matter content available in soils (Raison 1979; Wicklow 1973; Smith 2003; Sherman et al. 2005; Azul et al. 2010; Docherty et al. 2011; Augustine et al. 2014; Pereira et al. 2014; Fultz et al. 2016), which can alter the dynamics between mycorrhizae and host plant and the availability of organic matter for decomposition by saprotrophic fungi. Grassland fires affect soil pH (Wicklow 1973; Raison 1979; Sherman et al. 2005) but there is no consensus on the direction of pH change, and it may be context and time dependent. Fire also causes fluctuations in soil nitrate and ammonium concentrations (Augustine et al. 2014; Fultz et al. 2016). Nitrogen is integral to plant growth and one of the most common limiting nutrients in soils. Since saprotrophic fungi are important in decomposing organic nitrogen to produce ammonium, the effects of fire on soil nitrogen may have implications on soil fungal communities.

Fire can fully remove the aboveground community in grasslands, which may indirectly affect belowground communities by altering organic matter inputs and removing host plants with mycorrhizal associations. Removal of host plants and litter may also disrupt fungal pathogen disease cycles (Shearer and Tiffany, 1989; Roy et al. 2014).

Little is known about the species diversity of soil fungi in PNW prairies and the consequences of prescribed fires on these soil fungal communities. Determining which soil fungal species are present and their relative abundances is the initial step for determining the complex interactions between members of the soil fungal community and the associated plant community. Given that mycorrhizal communities can improve host plant fitness as well as influence plant community structure (Smith et. al 1999), understanding how mycorrhizal communities in these prairies respond to prescribed burns is important for restoration efforts in these upland prairies. A better understanding of the response of soil fungal communities to fire, including plant pathogens, saprotrophs, and mycorrhizal groups, and determining whether these responses differ across spatial scales may help inform fire management practices following burns, such as reintroducing plant and fungal species.

To examine the consequences of prairie fire on soil fungi, we used a chronosequence of one month, two years, and approximately 150 years since burning in prairies from two regions, the Puget Trough in Washington State and the Willamette Valley in Oregon. We characterized the soil fungal communities using Next Generation Sequencing (NGS), and collected data about the plant communities present, land use history, the abiotic environment (e.g., soil nutrients, climate), and spatial relationships

among plots. This data set allowed us to determine how much of the differences among soil fungal communities among sites and between regions were determined by fire, other abiotic factors, spatial distance, and the vegetation present. We assessed how fire affects species diversity of the total soil fungal community and whether burning influences community composition. We also investigated how fire affects the relative abundances of fungal functional groups, which are fungi grouped by the function they serve in the ecosystem (e.g. saprotrophs, ectomycorrhizae, etc.). We focused on how fire affects species diversity and community composition of four key functional groups: arbuscular mycorrhizae, ectomycorrhizae, plant pathogens, and saprotrophs.

CHAPTER II

EFFECTS OF FIRE ON SOIL FUNGAL COMMUNITIES AND THE ENVIRONMENTAL DRIVERS OF COMMUNITY VARIATION IN PRAIRIES OF THE PACIFIC NORTHWEST

The methods, results, and discussion in this chapter were developed in part by two primary committee members, including Bitty A. Roy and Sarah T. Hamman, whom contributed substantially to this work. I was the primary contributor to developing these methods, results, and discussion and completed all the writing.

Methods

Study System

The study includes six remnant and restored PNW prairies. Three prairies were chosen from the Washington Puget Trough south of Olympia – Glacial Heritage, West Rocky, and Wolf Haven – and three from the Oregon Willamette Valley west of Eugene – Willow Creek, North Eaton and Shore. *Festuca roemeri* is the dominant native grass in each prairie.

Time since restoration and historic land use varied among sites. Portions of all sampled prairies in Washington and Oregon have experienced various management regimens, including herbicide applications, mowing, rotational partial burns, native planting and native seeding. The prairies in Washington are mostly remnant prairies managed by the Center for Natural Land Management (CNLM) and Washington Department of Fish and Wildlife (WDFW). Restoration of Glacial Heritage began in 1996 and is the only Washington prairie in this study that has experienced mycorrhizal inoculations. Wolf Haven restoration began in 2005 and is managed by CNLM.

Management at this site includes regularly scheduled rotational partial burns and manual removal and herbicide applications to control invasive plant species. West Rocky restoration began in 2007 and is managed by WDFW. Management at this site includes rotational partial burns and herbicide applications and mowing to control invasive plant species.

The prairies in the Willamette Valley were more degraded than those in Washington and more of the *F. roemeri* was planted in Oregon versus the remnants in Washington. The Oregon prairies were largely restored from agriculture and pastureland. Invasive plant species were more abundant in the Oregon prairies we sampled versus the Washington prairies. The Willow Creek restoration began in 2008 by the Nature Conservancy; it regularly receives rotational partial burns, herbicide applications, and is mown every year except the plot burned that year. Restoration treatments of North Eaton began in 2010 when the U.S. Army Corps of Engineers (USACE) began experimental treatment rotations, including herbicide applications and mowing to control for invasive species. The North Eaton plot that was burned the year of sampling was mowed and sprayed to control invasives. Shore prairie restoration began in 2011 with experimentally rotated treatments including mowing and herbicide application. The Shore plot that was burned the year of sampling was also mowed and had herbicide applied. North Eaton and Shore prairies had not been maintained by fire for approximately 150 years, so the year we sampled was the first year USACE had included fire in their restoration program for these two prairies.

Tables I-III outline the site characteristics in terms of location, climate, vegetation, and soil. The Willamette Valley (Eugene, OR) and Puget Trough (Olympia,

WA) differ in many of these characteristics. The mean annual precipitation for the Willamette Valley prairies is approximately 1120 mm versus 1260 mm for the Puget Trough. The mean annual temperature for the West Eugene prairies is 11.4 °C versus 10.5 °C for Olympia (PRISM Climate Group). Elevations for the Oregon prairies ranged from 115 to 139 meters, while the prairies in Washington ranged from 42 to 83 meters above sea level. The soil types differed between the regions (Table III); the Puget Trough soils contained higher fractions of gravel than the Willamette Valley soils. To examine vegetative cover prior to restoration, land use data was acquired from the Oregon Spatial Data Library (1990) and the Washington Department of Ecology (1992). Land use data is given in Table II.

Sample Collection

In early fall 2016, we had three treatments that varied from site to site. Each prairie had a portion burned that fall and the rest left unburned. The portion left unburned included sites that had been burned within the last two years and sites that had not been burned in approximately 150 years. Table IV outlines our sampling setup.

In November 2016 approximately one-month post-burn, we took six soil samples from each treatment plot within the six prairies for a total of 84 samples. We chose sample locations based on a random number table for direction degrees and step number. A plastic PVC meter quadrat was centered over the *F. roemeri* plant nearest the sampling location. Soil cores were taken over an individual *F. roemeri* plant to include soil, roots, and shoots to a depth of 15 cm using a 2.54 cm diameter PVC pipe. PVC corers were sterilized with 95% ethanol between samples. Within the quadrat, digital photographs were taken and later used to digitally measure percent vegetative cover in SamplePoint

Table I. Site locations, elevation in meters, and mean annual precipitation (MAP) and temperature (MAT).

Site	Region	Latitude	Longitude	Elevation (m)	MAP (mm)	MAT (°C)
Willow Creek	Oregon	44.029	-123.174	133.31	1135.82	11.4
North Eaton	Oregon	44.102	-123.258	116	1114.43	11.4
Shore	Oregon	44.1	-123.27	119.23	1101.23	11.4
Glacial Heritage	Washington	46.868	-123.047	46.2	1270.69	10.6
Wolf Haven	Washington	46.902	-122.848	79.35	1254.91	10.5
West Rocky	Washington	46.895	-122.874	71.92	1251.32	10.5

Table II. Site characteristics for vegetative cover in percentages, average burn severity across site, and land use prior to restoration.

Site	Litter Cover (%)	Vegetative Cover (%)	Bare Soil Cover (%)	Burn Severity	Land Use
Willow Creek	24.4	55.6	17.6	4.22	Pasture; Developed; Grassland
North Eaton	15.2	64.7	20	4.33	Tree Plantation; Shrubland; Developed
Shore	26.7	37.7	27.8	3.96	Tree Plantation; Agriculture
Glacial Heritage	35.4	30	21.8	4.55	Grassland
Wolf Haven	41.5	28.8	22.1	4.33	Grassland; Shrubland
West Rocky	24.1	33.2	40.3	4.02	Grassland; Wetland

Table III. Site characteristics for edaphic properties, including soil pH, C:N ratios, nitrates and ammonium concentrations, and soil types.

Site	pH	C:N Ratio	Nitrate (mmol/g)	Ammonium (mmol/g)	Soil Type
Willow Creek	5.03	9.86	3.82×10^{-5}	0.0004	Clay loam; Silt loam; Silty clay loam
North Eaton	4.93	11.09	1.46×10^{-5}	0.00075	Silty Clay Loam
Shore	5.17	11.44	1.13×10^{-5}	0.00105	Silty Clay Loam; Loam
Glacial Heritage	5.45	13.32	1.35×10^{-5}	0.00124	Gravelly sandy loam
Wolf Haven	5.46	14.04	4.33×10^{-5}	0.00228	Gravelly sandy loam
West Rocky	5.63	12.28	6.97×10^{-5}	0.00135	Gravelly sandy loam

Table IV. Sampling setup for each prairie. Check marks indicate we had this treatment plot for that site.

		Time Since Burning		
Region	**Site**	**~150 years**	**2 years**	**1 month**
Washington	Glacial Heritage	✓	✓	✓
	Wolf Haven		✓	✓
	West Rocky		✓	✓
Oregon	Willow Creek	✓	✓	✓
	North Eaton	✓		✓
	Shore	✓		✓

(Booth et al. 2006). Wolf Haven burned sample point 6 did not have a digital photograph, so we excluded this sample for variation partitioning. This was done because variation partitioning does not handle unknown values. The meter quadrat was visually divided into four quadrants and fire intensity was visually estimated within each quadrant on a scale from 1 to 5 (1 being most severe). Fire intensity measures were averaged for each quadrat.

Soil samples were stored in Whirlpak bags and that were moved to 4 °C within 24 hours. Within four days of sampling, samples were divided into three subsets for soil chemistry tests, soil fungal DNA extraction, and *F. roemeri* roots and shoots. Subset samples were stored at -80 °C for future analyses.

Soil Chemistry

Each soil chemistry subsample was analyzed for soil pH within four days of collection. To measure soil pH, we utilized a 2:1 deionized water to fresh soil slurry by mass. The remaining soil chemistry subsamples were kept frozen at -80 °C until they were sent to the Central Analytical Laboratory (CAL) at Oregon State University and analyzed for ammonium and nitrate concentrations, and total carbon and nitrogen to

generate C:N ratios. CAL used a potassium chloride (2M KCl) extraction protocol to extract nitrate (NO_3) and ammonium (NH_4) from approximately 10 g of dried and ground soil from the soil chemistry subsample we sent them. The filtered extract was analyzed using a Lachat 8500 Serries 2 Flow Infection Analysis system to assess ammonium and nitrate concentrations. Samples were analyzed for C and N by dry combustion using an Elementary vario MACRO cube (Elementary Analysensysteme GmbH, Langenselbold, Germany.

Soil Fungal DNA Extraction

Soil subsamples were sieved using a 2 mm soil sieve and 0.25 g soil was added to a bead tube provided by the PowerSoil DNA Isolation Kit (MoBio, Carlsbad, CA USA). Samples were homogenized by briefly vortexing and then DNA was extracted following the protocol given in the PowerSoil DNA Isolation Kit.

DNA extractions were analyzed for purity and concentration using a NanoDrop 2000 spectrophotometer prior to PCR. Extracts that fell above 20 ng/µl were diluted 1:10. Amplification of the fungal ITS region was carried out using ITS1F forward (5` CTT GGT CAT TTA GAG GAA GTA A 3`) and ITS2 reverse (5` GCT GCG TTC TTC ATC GAT GC 3`) primers (White et al. 1990; Kabir and Peay 2014) with Illumina adapter sequences and indexed barcodes appended to the reverse primer (Integrated DNA Technologies, Coralville, IA). A total of 84 soil samples and two controls, positive and negative, were amplified in duplicate using 84 unique reverse 12 bp barcodes for a total of two 84-well plates. A negative control was included with the ecological samples as well as a positive synthetic mock ITS community control (Palmer et al. 2017). Duplicate PCRs were performed in 20 µl volumes with the following proportion of reagents: 0.6 µl

of each forward and reverse primer (10 μM), 0.8 additional $MgCl_2$, 5.5 μl ultra-pure water, 2.5 μl of template DNA, and 10 μl of 2X PCR Master Mix, which contained Taq polymerase, dNTPs, and $MgCl_2$ (Bimake ©, Houston, TX). PCR amplification was carried out using the following parameters: initial denaturation of 94 °C for 5 min and then 30 cycles of 94 °C for 30 sec, 55 °C for 1 min, 72 °C for 30 sec., and a final elongation of 72 °C for 7 min (Thomas et. al. 2017). All PCR preparation was carried out in a Purifier Logic+ Class II A2 biological safety cabinet (Labconco ©, Kansas City, MO). Amplification was verified for all samples using gel electrophoresis.

Duplicate PCR product subsamples of 10 μl were pooled and cleaned using Mag-Bind Rxn PurePlus (OMEGA bio-tec ©, Norcross, GA) beads. Size selection and exclusion of primer-dimers was achieved mixing 0.8 volume beads to 1.0 volume PCR product. A second cleanup was performed to remove any residual primer-dimers and a final elution of 20 μl. Concentrations of each cleaned PCR product were carried out using the Quant-iT^{TM} dsDNA HS Assay Kit (Invitrogen, Thermofisher Scientific, Waltham, MA) and a Molecular Devices SpectraMax M5E Microplate Reader. Samples were normalized and pooled for a single Illumina MiSeq run. The final pool was submitted to the Genomics and Cell Characterization Core Facility for Illumina MiSeq platform sequencing, using a 600 cycle (2 x 300 bp) v3 MiSeq reagent kit.

Analysis of Sequencing Data Using the DADA2 Workflow

We used the DADA2 packages and workflow for the majority of the bioinformatics process to define sequence variants (SVs) (Callahan et al. 2016). Utilizing this pipeline allowed us to detect more fine-scale variation than would be expected using more traditional pipelines that group sequences into OTUs.

Quality profiles were examined for the forward and reverse reads to determine the quality cutoff in read length. To remove low quality regions, forward reads were trimmed at 225 bp and the reverse reads were trimmed at 200 bp. This allowed for sufficient sequence overlap for merging reads using PEAR.

DADA2 removes any sequences containing unnamed nucleotides (Ns), which results in a significant loss of reads during merging. To improve the number of reads recovered from merging the forward and reverse reads, we used QIIME to filter out the reverse reads to match the forward reads. Then, the forward and reverse reads were fed through PEAR for alignment, which retains any sequences that have significant overlap despite the presence of Ns and replaces these Ns with the base pair matching on the opposite read being merged. Any sequences that still had Ns after pairing through PEAR were dropped after a second filtering and trimming using DADA2.

Merged reads were dereplicated to combine all identical reads into unique sequences with a given abundance. Then, sequence variants from the dereplicated data were inferred using the DADA2 algorithm with error estimation. Chimeras were removed using the *removeBimeraDenovo* function using the "consensus" method. Taxonomy was assigned using the *assignTaxonomy* function and the fungal ITS designations from the UNITE database.

Statistical Analyses

Soil Chemistry

Two samples did not have sufficient quantity to acquire ammonium and nitrate concentrations. Unburned sample point 1 in North Eaton lacked a nitrate concentration value, and Wolf Haven unburned sample point 3 lacked an ammonium concentration

value. Because variation partitioning is sensitive to NA values, we used average nitrate and ammonium values from each of those treatment plots to replace this unknown value.

We conducted a One-Way ANOVA to compare differences in soil nutrients among burning treatments for soil pH, carbon and nitrogen ratios, and ammonium and nitrate concentrations. For any comparisons with heteroscedastic variances in soil nutrient data, found using Levene's test, we used White-Adjusted One-Way ANOVAs.

Trimming and Optimization of SV Tables

All processing and quality filtering of SV tables were carried out in the R package *phyloseq* (Mcmurdie and Holmes 2013). The inclusion of a positive synthetic ITS mock community and a negative control allowed us to gauge the rate of possible tag-switching and the ability to remove any potential contaminants (Thomas et al. 2017). To account for sequencing bias, we calculated counts of each synthetic mock community member that showed up in other samples. We calculated the geometric mean from these counts and used this as the estimated rate of tag-switching and subtracted this amount, approximately nine reads, from each sample. We also subtracted the maximum number of sequences that occurred in the negative control to remove any possible contaminants. Approximately 88 reads from negative control contaminants and nine reads from tag-switching were excluded from each sample to remove any bias from laboratory and sequencing error.

After filtering out the synthetic mock community members and negative control contaminants, all SVs with undetermined phyla designations were excluded from each sample. Rarefying sequences was not necessary, because we used DADA2 (Kraemer et al., 2018); however, given the widely different read abundances between samples, we

utilized variance stabilization methodology taken from the R package *DESeq* (Love et al. 2014; Thomas et al. 2017).

Fungal Community Structure

To investigate the differences in soil fungal communities between regions, sites, and time since burning, we visualized differences in community composition using non-metric multidimensional scaling (NMDS). We used the *ordinate()* function in the R package *phyloseq* to explore composition and generalized relationships between soil fungal communities. We used permutational multivariate analysis of variance (PERMANOVA) to compare soil fungal communities between regions, sites, and time since burning.

Predictors of Soil Fungal Communities

We used variation partitioning to estimate the contribution of abiotic, vegetation, spatial, and burning on soil fungal community composition. Variation partitioning estimates the relationships among community data and environmental predictors.

Redundancy analysis (RDA) was used to construct explanatory matrices for each environmental predictor. However, variation partitioning is very sensitive to multicollinearity between environmental predictors, so we calculated variance inflation factors (VIFs) for all combinations of environmental predictors. Any environmental variable with a VIF above 10 was excluded from further analysis, because a high VIF indicates this variable is highly correlated with other explanatory variables and may be redundant if included in downstream analysis (Gross 2003).

Community data was converted to presence/absence and Hellinger transformed to make the counts dimensionally homogeneous and create our community matrix for RDA and variation partitioning (Legendre and Gallagher 2001).

We ran RDA using the function *rda()* from the R package *vegan*. This function chooses the combination of environmental variables that explain the most variation in fungal community composition. These variables were then grouped into four environmental clusters: abiotic, vegetation, spatial, and burning regimen. Results of RDA were plotted to show the relative contributions of each environmental variable (Figure S1). Transformed community data and the four environmental clusters were then fed into the function *varpart()* from the R package *vegan*. This function generates adjusted R^2 values for all combinations of explanatory matrices to determine how much variation each combination explains. To test the significance of each explanatory fraction, we performed a permutation test similar to ANOVA using the function *anova.cca()*. A Venn diagram was constructed to visualize the relative contribution of each environmental cluster on soil fungal community composition.

Functional Group Assignment

FUNGuild is an online database that assigns functional information to sequence data (Nguyen et al. 2016). After filtering out possible contaminants and mock community members, we constructed an SV table that was run through the FUNGuild Assignment App based on the taxonomic assignments generated from the UNITE database. FUNGuild appends functional group information to the original sequence data. Functional group assignment includes a confidence ranking for that designation. Prior to exploring functional group data, we filtered out any taxa with confidence rankings below

'Possible'. Guild designations were reclassified to reduce the redundancy in classifications. For example, designations given a "-" or "NULL" were grouped and reclassified as "Unclassified." We explored functional group differences between regions, sites, and time since burning. We explored differences among four functional groups: arbuscular mycorrhizae (AMF), ectomycorrhizae (EcM), plant pathogens, and saprotrophs.

Fungal Diversity

Alpha diversity metrics were conducted on untransformed but variance stabilized data and measured using the Shannon index, which is a compound measurement of species richness and evenness and accounts for rare species (Spellerberg and Fedor 2003; Stratford et al. 2014). For total community diversity, we employed one-way ANOVAs using species richness values to compare differences between regions, sites, and time since burning and ran Tukey's HSD post-hoc tests to compare groups. For any comparisons with heteroscedastic variances in species diversity estimates, found using Levene's test, we used White-Adjusted one-way ANOVAs.

Shannon diversity of mycorrhizal groups, plant pathogens, and saprotrophs were also visualized for differences between region, site, and time since burning. We used the same method employed for complete community data to investigate differences in species diversity between regions, sites, and time since burning for each functional group. Differences between functional group percent abundances were compared using the *prop.test()* function from the base package in R, which is a test of equal proportions between groups.

All statistical analyses were performed in R 3.4.3 (R Core Team 2017) using the *vegan* package (Oksanen et al. 2017).

Results

Soil Chemistry

The treatments had minor and statistically insignificant effects on soil pH, C:N rations, and nitrate concentrations. The differences in soil ammonium concentrations among treatments was significant ($F_{2,78} = 7.92$, $p < 0.001$). Soil ammonium concentrations were highest in the most recently burned plots; however, ammonium concentrations were lower in the plots burned within 2 years than in the plots not burned in 150 years. Summary statistics for soil nutrient data are available in supplemental table 11.

Bioinformatics

We recovered 8,707,376 total reads from Illumina sequencing. Following quality filtering without QIIME and PEAR and chimera removal, we were left with 4,000,430 reads. Merging forward and reverse reads using QIIME and PEAR resulted in 5,750,655 reads, a significant increase in usable reads. Following DADA2 and taxonomic assignment, 8,493 unique SVs were recovered. Following variance stabilization after the removal of possible contaminants, reads due to tag-switching, and unclassified phyla, we were left with 1602 SVs over 7 taxonomic ranks.

Fungal Community Structure

There were distinct differences in soil fungal communities between regions with the Washington communities clearly separated from the Oregon communities ($F_{1,80} = 16.64$, $p < 0.001$, $R^2 = 0.17$; Figure 1a). Soil fungal communities also differed significantly from one another by site ($F_{5,80} = 6.54$, $p < 0.001$, $R^2 = 0.30$; Figure 1b).

Despite the overlap of confidence intervals seen in the ordination by time since burning, the soil fungal communities differed significantly ($F_{2,80} = 2.51$, $p < 0.001$, $R^2 = 0.06$; Figure 1c).

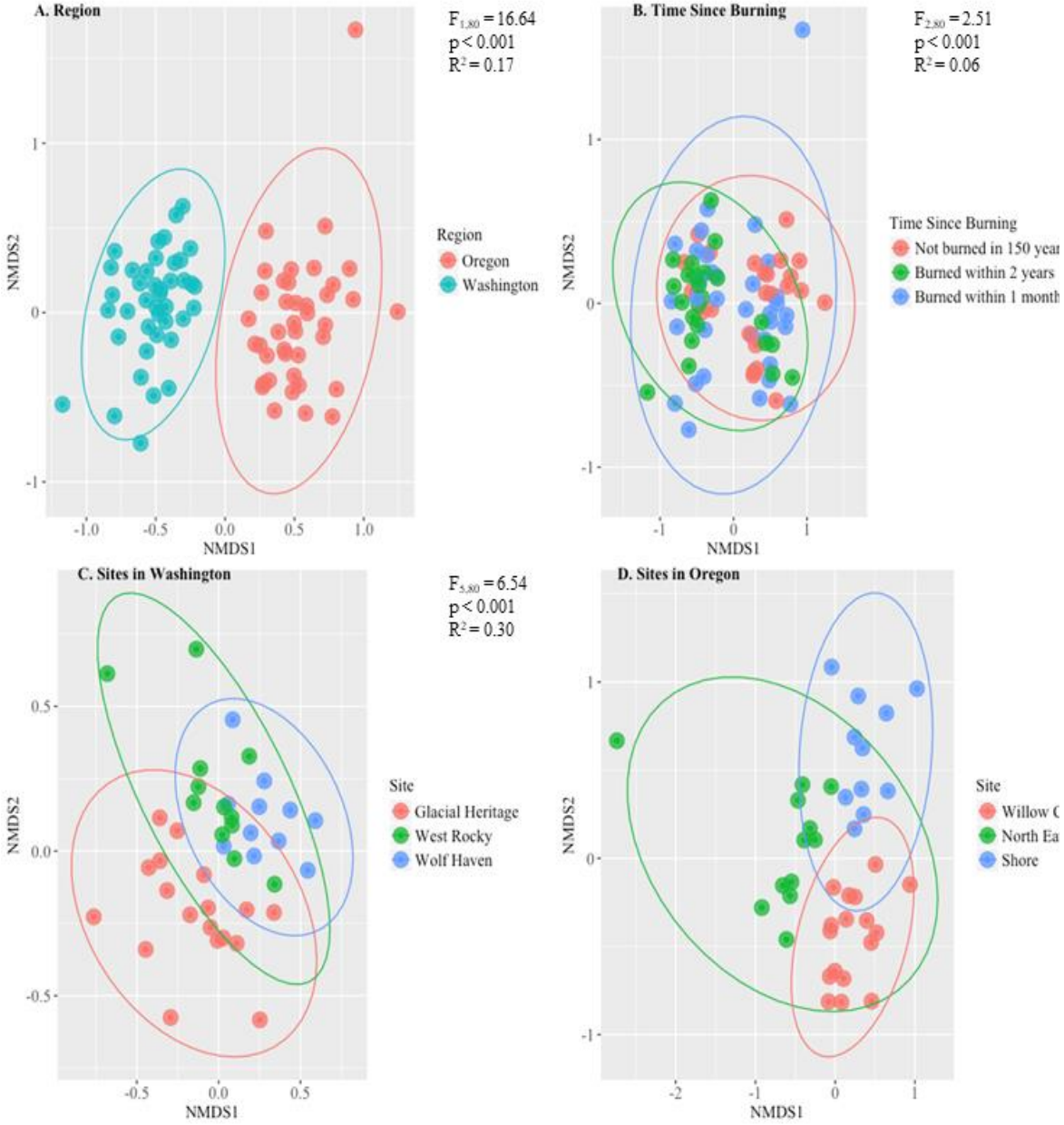

Figure 1. NMDS ordination of soil fungal communities. Ellipses illustrate 95% confidence intervals. Results of PERMANOVA given in box to the right of each plot. A. By region. B. By time since burning. C. By sites in Washington. The PERMANOVA results in the box to the right of Figure 1C represents results for all sites in Washington and Oregon. D. By sites in Oregon.

Soil Fungal Community Predictors

The environmental variables measured in this study explained 20% of the variation observed in the soil fungal communities (Figure 2). Spatial factors explained the most variation observed in total soil fungal communities ($F_{7,51} = 1.21$; $p = 0.001$; $R^2 = 0.07$). Spatial variables

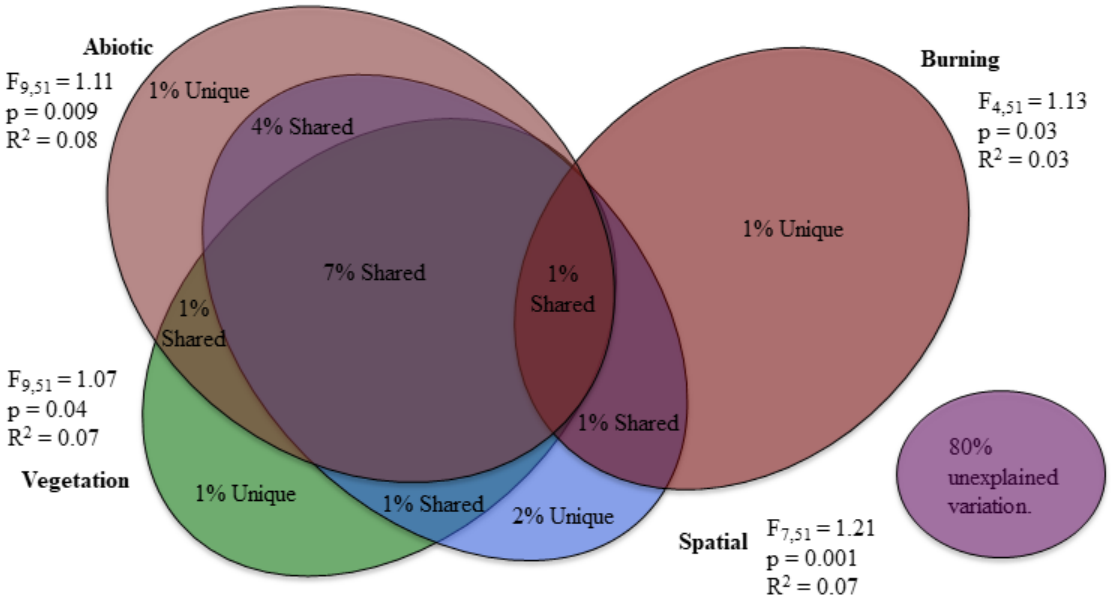

Figure 2. Venn diagram demonstrating the relative contribution of each environmental cluster to the variation observed in the soil fungal communities. Values are presented as a fraction of 1.0. Overlapping areas represent shared variation between those environmental clusters. Total variation explained by the variables measured was 20%.

included latitude, longitude, elevation, and site locations (Shore, North Eaton, Wolf Haven and West Rocky). Abiotic variables accounted for just 1% of the variation ($F_{9,51} = 1.11$; $p = 0.007$; $R^2 = 0.08$). Abiotic factors included climatic variables (MAT and MAP) as well as edaphic properties, which encompassed soil pH, nitrate and ammonium concentrations, and soil texture (loam, clay loam, and silt loam). Vegetation alone explained only 1% of the variation ($F_{9,51} = 1.07$; $p = 0.01$; $R^2 = 0.07$). Vegetation variables included total vegetative cover, litter cover, and the seven land use types, which were Grassland, Agriculture, Developed, Pasture, Tree Plantation, Shrubland, and

Wetland. Time since burning alone accounted for 1% of the variation ($F_{4,51} = 1.13$; $p = 0.02$; $R^2 = 0.03$) and included the variables bare soil cover, burning intensity, and whether the plot had been burned within one month or two years of sampling. The combination of spatial, vegetation, and abiotic factors explained the majority of the variation from our measured variables, 7% shared among all three. Time since burning explained the least variation observed in the soil fungal communities. The combination of burning variables with the other three variables was minimal, zero in most cases except when all four environmental clusters were taken into consideration. Shared variation for all four environmental clusters was only 1%.

Fungal Diversity for the Total Soil Fungal Community

There was a lot of variance in diversity, and thus there were no significant differences between regions ($F_{1,79} = 0.024$, $p = 0.88$) (Figure 3a), among treatments ($F_{2,78} = 0.70$; $p = 0.50$) (Figure 3b) or among sites ($F_{5,75} = 1.56$, $p = 0.18$) (Supplemental Figure S2).

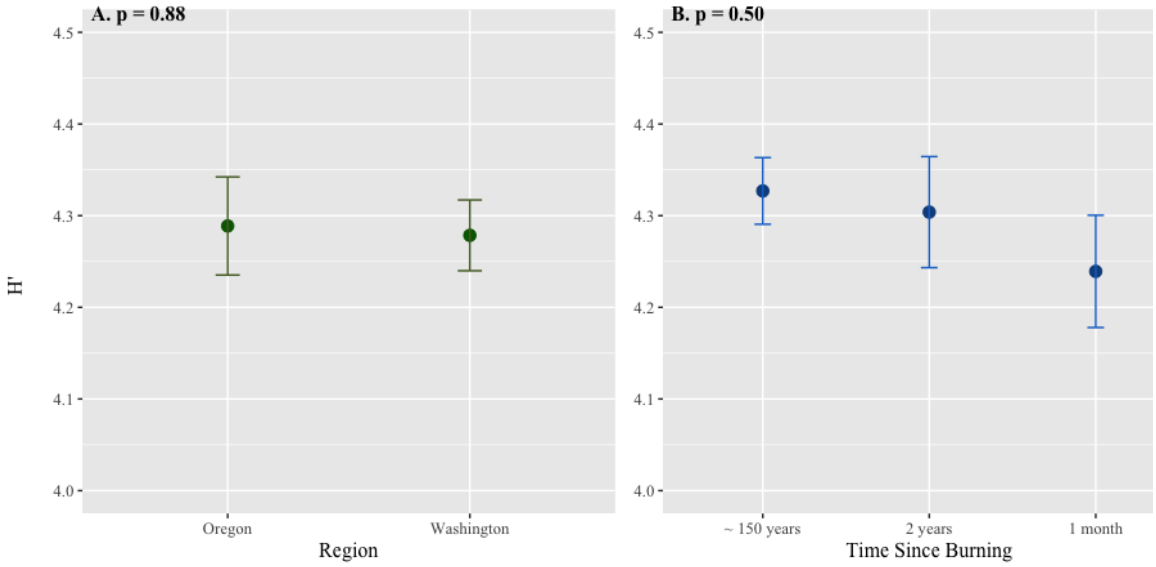

Figure 3. Mean species diversity (H' = Shannon index) comparisons for the total soil fungal community A. between Oregon and Washington and B. by time since burning. Error bars represent standard error (SE).

Functional Group Differences

Percent abundances of functional groups did not differ significantly between regions or among treatments (see supplemental tables 1 and 2 and Figure S3A and B); however, this overall test does not examine species differences, which evalulated within each functional group.

Arbuscular Mycorrhizae

Glomus was the dominant genus in Oregon and *Acaulospora* was the dominant genus in Washington. Both were present in each region at varying abundances. Several AMF genera were absent in Washington, including *Dominikia, Funneliformis, Claroideoglomus,* and *Scutellospora* (Figure 4a).

Only three genera were present in the prairies not burned for approximately 150 years, *Glomus, Funneliformis,* and *Archaeospora*. *Acaulospora* and *Scutellospora* were only present in the most recently burned prairies (Figure 4b). Results of proportions tests showed significant differences in abundances by region for all AMF genera except *Scutellospora* and *Funneliformis* (Supplemental Table 4). Proportions tests of abundances by time since burning showed significant differences between genera for all AMF genera except *Scutellospora* (Figure 4b).

There were significant differences in AMF diversity between Washington and Oregon ($F_{1,79} = 5.49$, $p = 0.022$; Figure 5A) and among sites ($F_{5,75} = 5.46$, $p < 0.001$; Supplemental Figure S8A). Though AMF communities showed no significant differences in species diversity with time since burning ($F_{2,78} = 0.86$, $p = 0.43$; Figure 5B), the most recently burned plots had the lowest species diversity.

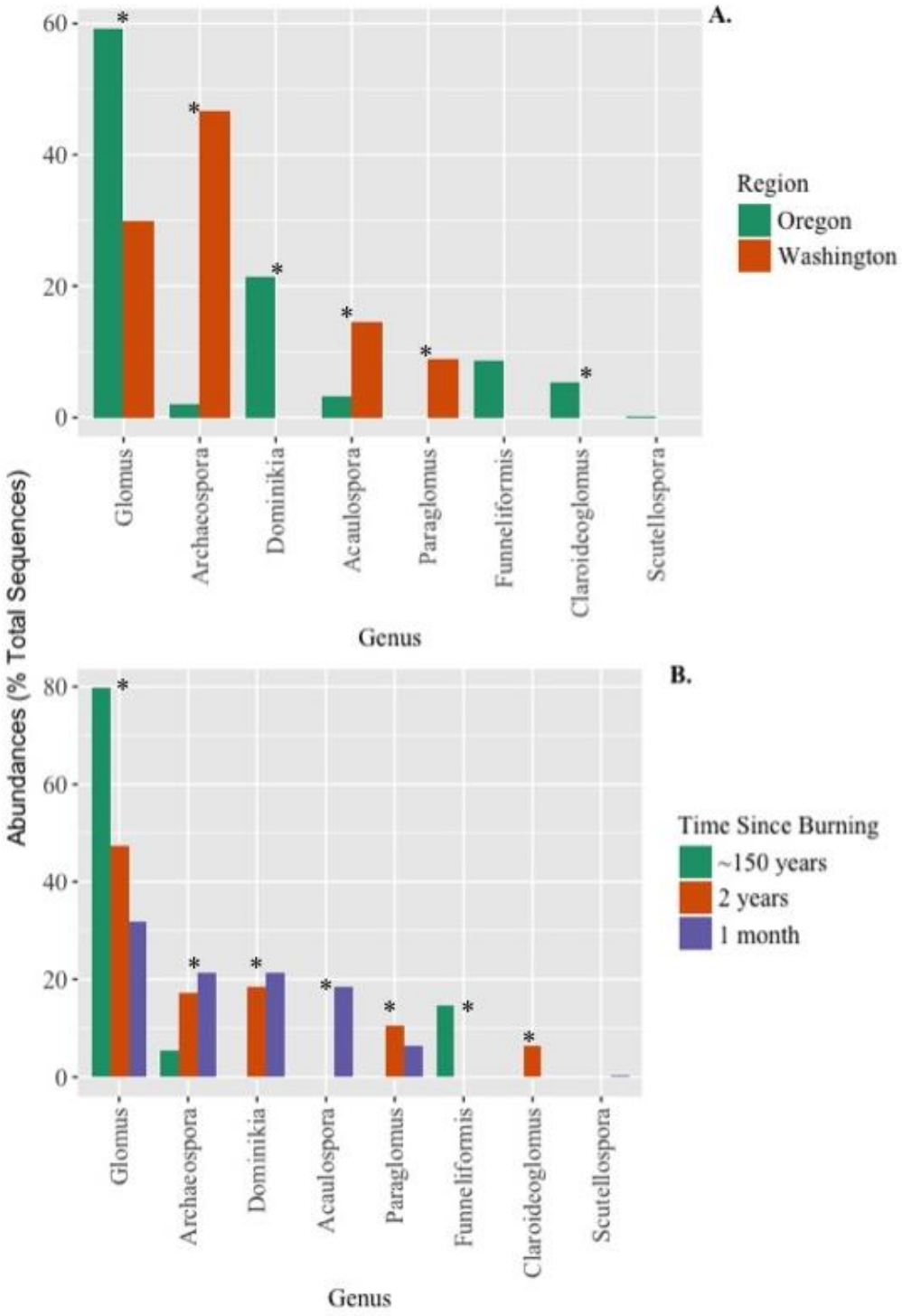

Figure 4. Relative percent abundances of arbuscular mycorrhizae by genus A. by region and B. by time since burning. Asterisks indicate significant differences between regions or by time since burning for abundance of that genus ($p < 0.05$).

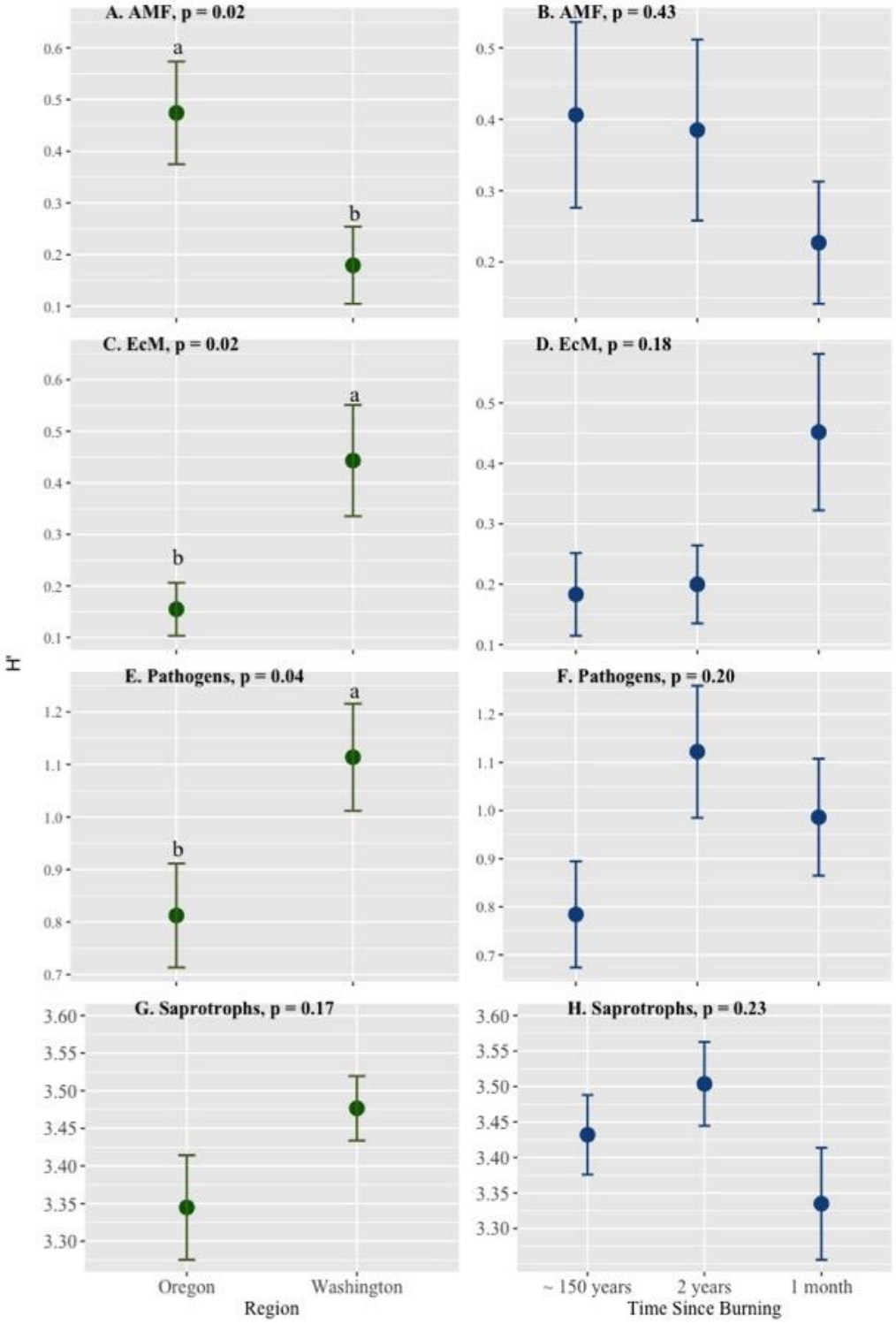

Figure 5. Mean species diversity (H') comparisons. Error bars represent standard error (SE). Results of post-hoc Tukey tests designated by letters above error bars. A. AMF by region. B. AMF by time since burning. C. EcM by region. D. EcM by time since burning. E. Plant pathogens by region. F. Plant pathogens by time since burning. G. Saprotrophs by region. H. Saprotrophs by time since burning.

Ectomycorrhizae

Hymenoscyphus was the most abundant ectomycorrhizal genus in Oregon, and *Pezoloma* was the most abundant genus in Washington (Figure 6A). *Russula, Sistotrema, Lactarius, Chloridium, Scleroderma,* and *Wilcoxina* were only present in Oregon, while *Amanita, Xerocomellus, Cenococcum, Laccaria,* and *Cortinarius* were only present in Washington.

Examination of EcM genera abundances by time since burning reveals *Amanita, Xerocomellus, Chloridium, Cenococcum, Laccaria,* and *Cortinarius* were only present in the most recently burned plots in Washington (Figure 6B), specifically in Wolf Haven (Supplemental Figure S5). *Lactarius, Scleroderma,* and *Wilcoxina* were only present in more recently burned plots (1month and 2 years post-burn) in Oregon. No single genus was present in every burning regimen.

Wolf Haven had the most genera, which were represented at varying abundances (Supplemental Figure S5). Diversity of EcM communities differed significantly between regions ($F_{1,79} = 5.68$, $p = 0.02$; Figure 5C), with Oregon having lower species diversity than Washington. Species diversity of EcM communities tended to differ among sites ($F_{5,75} = 2.21$, $p = 0.06$; Supplemental Figure S8B) but not by time since burning ($F_{2,78} = 1.74$, $p = 0.18$; Figure 5D).

Washington had significantly higher species diversity than Oregon, which may be related to the particularly high species richness observed at Wolf Haven. Though the differences were not significant, ectomycorrhizal species richness was highest in the most recently burned plots as compared to the plots burned within 2 years and within 150 years (Figure 5D).

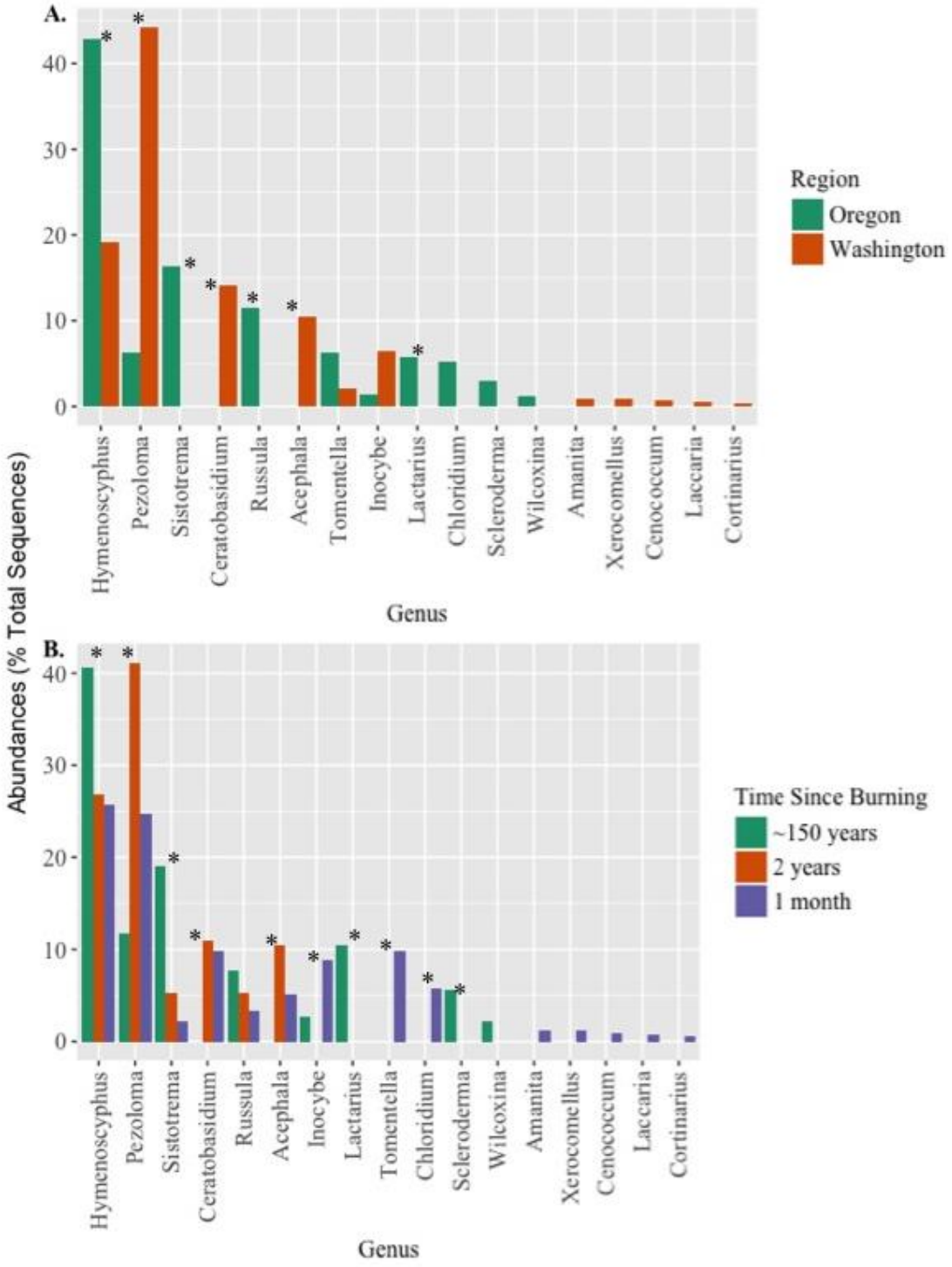

Figure 6. Ectomycorrhizal percent abundances by genus A. by region and B. by time since burning. Asterisks represent significant differences between percent abundances for that genus ($p < 0.05$).

Plant Pathogens

Dothideomycetes and *Sordariomycetes* were the most abundant ascomycetous plant pathogen classes in Oregon and Washington. *Agaricomycetes* were the most abundant plant pathogenic basidiomycetes in Oregon and Washington (Figure 7A). There were no significant differences between Oregon and Washington abundances for any class of plant pathogen (Supplemental Table 7).

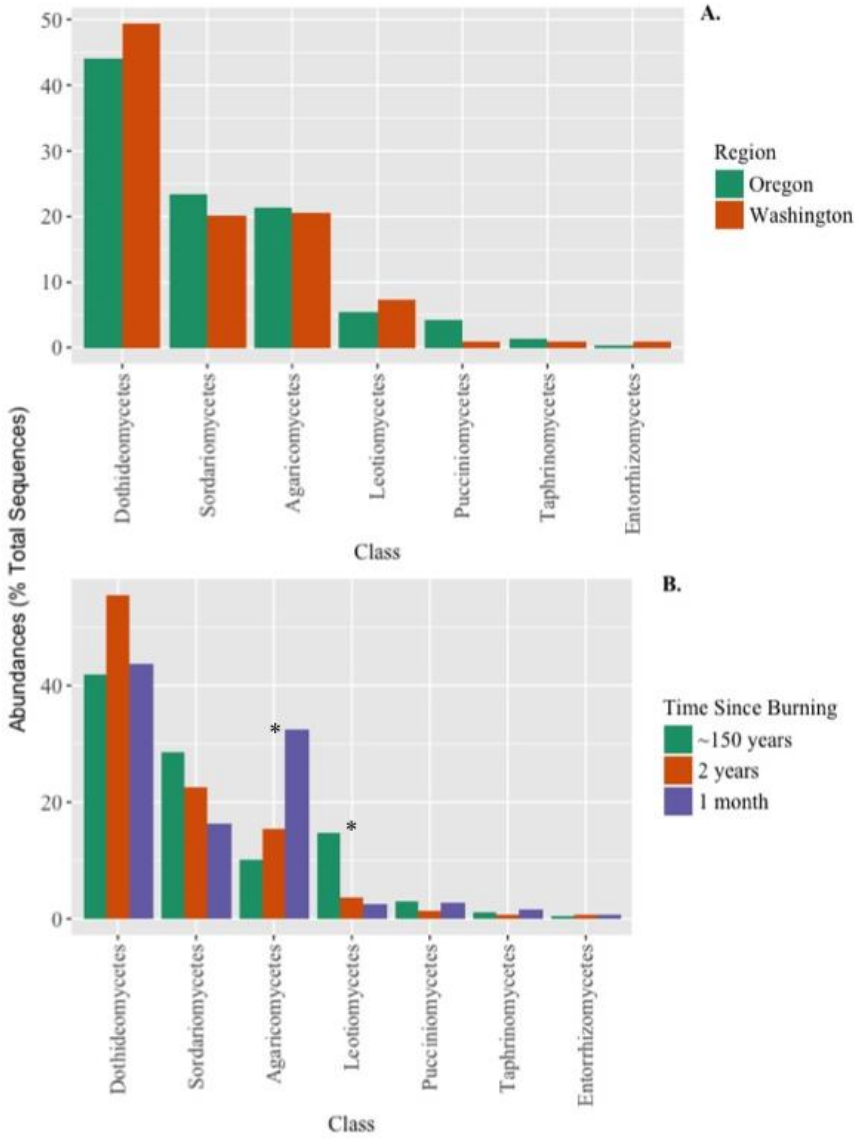

Figure 7. Abundances of plant pathogen by class A. by region and B. by time since burning. Asterisks represent significant differences between percent abundances for that class ($p < 0.05$).

There were significant differences in plant pathogen abundances of *Agaricomycetes* and *Leotiomycetes* by time since burning (Figure 7B). Pathogenic *Agaricomycetes* were most abundant in the most recently burned plots. Certain plant pathogen class abundances were lower in the most recently burned plots, particularly *Sordariomycetes* and *Leotiomycetes*, but the differences were not significant for the *Sordariomycetes* (Supplemental Table 8). Percent abundances of *Pucciniomycetes, Taphrinomycetes,* and *Entorrhizomycetes* remain relatively constant across burning regimens (Figure 7B).

There were significant differences in plant pathogen diversity between Washington and Oregon ($F_{1,79} = 4.49$, $p = 0.04$; Figure 5E), with Washington having significant higher plant pathogen diversity. There were no significant differences in species richness between sites ($F_{5,75} = 1.60$; $p = 0.17$; Supplemental Figure S8C) or time since burning ($F_{2,78} = 1.63$; $p = 0.20$; Figure 5F). Examination of differences in plant pathogen diversity between burning regimens shows that species richness was marginally but not significantly higher in the most recently burned prairies when compared to the prairies burned within two years. However, prairies not burned within 150 years had the lowest plant pathogen species diversity (Figure 5F).

Saprotrophs

Eurotiomycetes was the most abundant class of saprotroph for both regions. There were slight differences between saprotrophic soil fungi by region (Figure 8A) and by time since burning (Figure 8B), but these differences were not significant (Supplemental Table 9). Figure 8B shows the abundances of soil saprotrophs by time since burning. *Geminibasidiomycetes* and *Orbiliomycetes* were not present in the plots not burned

within 150 years, but differences in abundance by class were not significant (Supplemental Table 10).

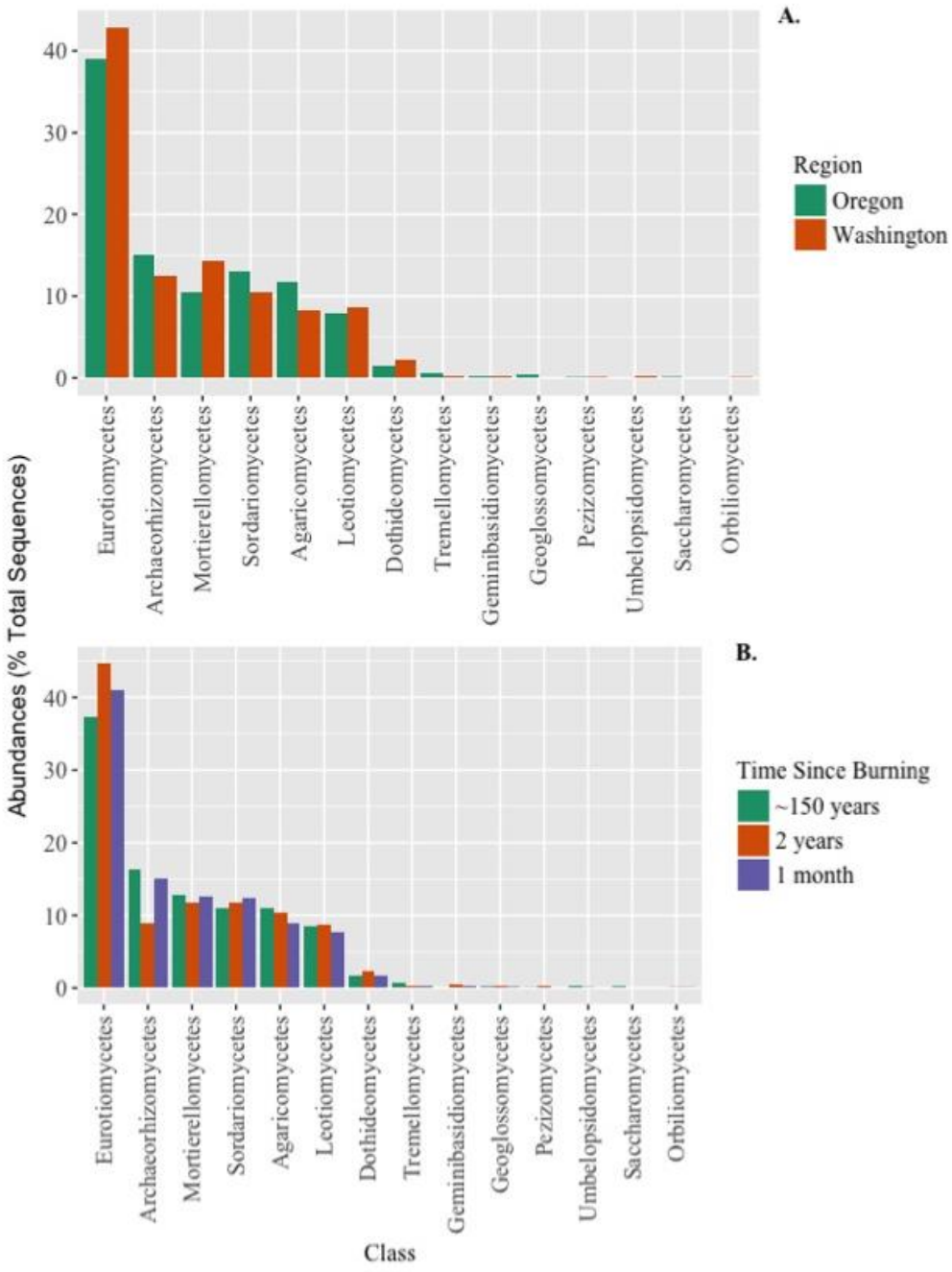

Figure 8. Abundances of saprotroph by class A. by region and B. by time since burning.

There were no significant differences in soil saprotroph diversity between sites ($F_{5,75}$ = 1.58, p = 0.175; Supplemental Figure S8D) or between regions ($F_{1,79}$ = 2.64; p =

0.11; Figure 5G). Examination of differences in saprotroph diversity by time since burning shows prairies burned within two years have the greatest species diversity while the most recently burned prairies have the lowest species diversity. However, these differences in diversity by burning were not significant ($F_{2,78} = 1.51$; $p = 0.23$; Figure 5H).

Discussion

Do soil fungal communities differ significantly between regions and with time since burning?

The total soil fungal communities in Washington and Oregon were distinct from one another and total soil fungal community composition was significant different among burning treatments. These findings are consistent with an aboveground study surveying mushrooms in similar upland prairies within these regions, which found the community composition of fruiting bodies in Washington did not resemble the community makeup found in Oregon (Roy et al. 2016), and that fire affected fruiting. The results of the current study shows these patterns are reflected belowground in the soil fungal community.

While there were differences in species composition as a result of region, site, and treatment, none of these factors explained variation in species diversity. In fact, there were no significant differences in diversity as indicated by Shannon's H or by species richness (not shown). Though the differences were not significant, the most recently burned plots (1 month) had the lowest species diversity, and as time since burning increased, we observed a general trend of species diversity recovering (Figure 3B). Our results are congruent with previous studies in grasslands that have detected similar

patterns of recovery for the general soil fungal community post-fire (Dangi et al. 2010; Docherty et al. 2011).

How much do abiotic, biotic, spatial, and burning factors contribute to the variation observed in soil fungal communities?

Soil fungal communities differed by time since burning; however, burning regimen only explained 1% of the unique variation observed in the communities (Figure 2). Numerous studies in different grassland types have found fire altering soil chemical and physical properties, but these effects are rarely consistent across studies and do not show the same trends (Wicklow 1973; Raison 1979; Picone et al. 2003; Hartnett et al. 2004; Sherman et al 2005; Docherty et al. 2012; Augustine et al. 2014; Pereira et al. 2014; Fultz et al. 2016). Interestingly, we did not observe any shared variation explained by the combination of burning and abiotic variables (Figure 2), suggesting variation of edaphic properties in prairies with or without burning may have a significant effect on soil fungal community variation.

The combination of abiotic, vegetation, and spatial factors explained the most variation observed in the soil fungal communities (Figure 2). These three environmental clusters interacted heavily with each other, while burning variables did not interact at all with abiotic or vegetation factors. The absence of interaction between burning and vegetation was interesting, because burning removes much of the aboveground vegetation and can shift plant community structure. However, vegetation variables were mostly comprised of land use prior to restoration. Land use types were vastly different between regions with the Washington prairies containing more remnant grasslands, and the Oregon prairies largely restored from agriculture, pastureland, and urban

development. This could explain why there was little interaction between burning and vegetation. If we had included more information about plant community structure within each burning treatment, the interaction between these two environmental clusters may have been stronger.

Given that land use types differed between regions and the Washington soils were also quite different from the Oregon soils, regional differences are likely driving the dissimilarities in the abiotic and biotic variables we measured. The interactions between spatial, abiotic, and biotic factors were the dominant environmental drivers of soil fungal community variation.

How does fire affect fungal functional groups in these prairies?

We found no significant differences among fungal functional groups between Washington and Oregon, but there were significant differences at finer taxonomic scales within each functional group. This suggests there is some local adaptation to the specific conditions in each locality and redundancy at upper levels of classification. This pattern of redundancy at the functional level, but local endemism at the species level, is one of the strong patterns found in soil fungi at continental scales (Talbot et al. 2014). Among burning intervals, relative abundances were somewhat consistent across functional groups, suggesting these communities recover following fire. We focused on four fungal functional groups that serve important roles within grassland ecosystems: two mycorrhizal groups, plant pathogens, and saprotrophs.

Arbuscular Mycorrhizae

We observed larger effects of fire on the arbuscular mycorrhizal communities within the sampled prairies compared to other functional groups. Arbuscular mycorrhizae

provide host plants access to limiting mineral resources (Smith, 2003), confer pathogen resistance (Liu et. al. 2007), and increase tolerance of abiotic stresses (Finlay, 2008). AMF influence the outcome of intraspecific and interspecific competition by differentially affecting the growth of neighboring competing plant species, thereby affecting plant community structure and coexistence (Smith et. al 1999).

AMF abundances did not change significantly with time since burning, but it is clear from the results of this study that burning affects AMF community composition. Shifts in mycorrhizal community composition and colonization can be reflected in plant community composition differences (Smith et al. 1999; Azul et al. 2010); however, there may be functional redundancies between the mycorrhizal communities of more recently burned prairies and prairies not burned in 150 years regardless of community composition differences. We also hypothesize that burning may select for AMF species that are more adapted to disturbance, which may explain the AMF community compositional differences by time since burning.

While not statistically significant, plots that were burned within one month of sampling had the lowest AMF species diversity, which is consistent with previous studies in predominantly C4 grasslands (Eom et al. 1999), suggesting our C3 grasslands are responding similarly. Increasing AMF diversity can increase plant biodiversity (van der Heijden et al. 1998), so decreasing AMF species diversity with burning may shift host-plant community structure in the long term.

Nitrogen and phosphorus are considered limiting nutrients in soil and key nutrients exchanged between host plants and mycorrhizal symbionts (Mader et al. 2000; Smith, 2003; Karandashov and Bucher 2005; Hodge and Fitter 2010). Burning can

increase phosphorus levels in soils (Wicklow 1973; Picone et al. 2003). Previous studies have shown increases in soil ammonium following fire (Docherty et al 2011; Augustine et al. 2014; Fultz et al. 2016) while soil nitrate levels have been differentially affected by fire (Hartnett et al. 2004; Augustine et al. 2014; Fultz et al. 2016). Our results showed a significant increase in soil ammonium with burning ($F_{2,78} = 7.92$, $p < 0.001$) but no discernible effect of burning on soil nitrates ($F_{2,78} = 1.97$, $p = 0.27$), which is consistent with another study in a semi-arid grassland within the southwestern United States (Fultz et al. 2016). Fluctuations in nitrogen and phosphorus levels can affect the relationship between mycorrhizae and their host (Egerton-Warburton et al. 2007) and these fluctuations following fire can reduce mycorrhizal colonization rates in grasses (Hartnett et al. 2004). We hypothesize that fluctuations in nutrient abundances may affect host-mycorrhizal dynamics, where increasing nitrogen and phosphorus content in soils following fire may make it energetically unfavorable to maintain the symbiosis. This may result in a decrease in AMF species diversity as well as distinct differences in AMF species present between burned and unburned prairies.

Fire can fully remove the aboveground community in grasslands, which may include host plants with mycorrhizal associations. Disrupting AMF linkages between plants can alter plant community competition dynamics (Smith et. al. 1999) and AMF recovery may be tied to the recovery of the grass community (O'Dea 2007b); therefore, successful plant community recovery may depend on the recovery of AMF communities. Many studies have found significant reductions in mycorrhizal colonization rates following fire (Habte, 1989; Gibson and Hetrick 1988; Dhillion and Anderson, 1993; Smith et al. 1999; O'Dea 2007a). However, several studies have observed the AMF

community recovering over time following low severity grassland fires (Dangi et al. 2010; Docherty et al. 2011). There was an observable trend towards recovery in AMF species diversity from the most recently burned plots (1 month) to the plots burned within two years (Figure 5B). Given there were no significant differences in the relative abundances of AMF in our chronosequence and an increase in AMF diversity over time post-fire, our results suggest the AMF community does recover over time and species abundances are not significantly affected by burning.

Ectomycorrhizae

Ectomycorrhizal (EcM) fungi are phylogenetically distinct from AMF and have different methods of reproduction and growth forms, commonly associating with woody plant species, but both mycorrhizal groups can confer similar benefits to their host plants (Smith et al. 2008). The composition of EcM communities differed significantly regionally and with time since burning. This may have partially been an artifact of the high species diversity at Wolf Haven's burned plot, where there were many trees and some of the EcM genera were only present at this site (Supplemental Figure S7). However, there were several genera that were present in multiple sites but only found in recently burned plots, such as *Tomentella* and *Chloridium. Tomentella* are very common ectomycorrhizae in temperate and boreal forests that associate with a range of host trees, including *Populus, Picea,* and *Quercus* (Jakucs et al. 2005). This genus has been shown to persist following forest fire (Baar et al. 1999; Stendell et al. 1999). *Chloridium* species have been identified as ectomycorrhizal with some endophytic growth characteristics, termed ectendomycorrhizal (Wilcox 1983; Alberton et al. 2010). The recovery or persistence of this genus following fire has not been addressed in previous studies.

Plant Pathogens

Plant pathogens had higher species diversity in Washington compared to Oregon, which could result from differences in time since restoration and because the Washington prairies contain more remnants with more diverse plant communities (Rottstock et al. 2014). Though the differences were not significant, there was higher plant pathogen diversity in the most recently burned prairies compared to the prairies not burned in approximately 150 years. Fire has been shown to increase plant community diversity by removing competitive and invasive plant species (DiTomaso et al. 1999), but increased plant community diversity has been linked to higher plant pathogen diversity (Rottstock et al. 2014). This could explain the increase in plant pathogen diversity with more recent burns.

There was also a notable increase in plant pathogen relative abundances for certain classes with time since burning, which was significant for *Leotiomycetes*, host to numerous serious plant pathogens. This suggests burning can control abundances of pathogenic *Leotiomycetes*. Alernatively, there was a significant increase in the relative abundance of *Agaricomycetous* pathogens in the most recently burned plots. Fire is used to control fungal pathogens in grasslands, because it temporarily removes the host plant, decreases litter where pathogen reproductive structures typically reside, and disrupts the disease cycle (Shearer and Tiffany, 1989; Roy et al. 2014). However, the results of the current study show differential effects of fire on certain plant pathogen groups, suggesting prescribed burning to control plant disease may not be a panacea.

Saprotrophs

We did observe distinct regional differences in soil saprotroph diversity with Washington having significantly higher species diversity than Oregon. This is consistent with a previous aboveground study surveying fungal fruiting (mushrooms) following fire in these same prairies of Washington and Oregon (Roy et al. 2016). There were no discernible effects of time since burning on soil saprotroph relative abundances nor were there notable regional differences in relative abundances. The same aboveground fruiting body study by Roy et al. 2016 found significant increases in fungal fruiting following fire; however, our results indicate this is not reflected belowground with the soil fungal community. This suggests abundances of soil saprotroph groups belowground remain relatively constant among burning intervals but some may remain dormant until fire, or some other disturbance that reduces aboveground plant biomass. Further, fungal fruiting does not indicate dominance within the general soil fungal community.

Some studies have witnessed trends towards recovery of the general soil fungal community post-fire (Dangi et. al. 2010; Docherty et. al. 2011), suggesting saprotrophic fungi may persist in the soil despite burning. Prescribed fires in grasslands tend to be low severity (Martin and Hamman, 2016) and so may have less effect on belowground communities, including the general saprotrophic community. Grassland soils have lower surface litter inputs compared to other ecosystems such as woodland soils (Griffith and Roderick, 2008). Soil organic matter has been shown to increase following fire in grasslands (Wicklow 1973; Docherty et al. 2011; Pereira et al. 2014). Low intensity burning may insulate the saprotrophic community from the detrimental effects of fire but increases in soil organic matter input may indicate reduced decomposition rates and

suppression of the microbial community. However, our results indicate relative saprotrophic abundances and species diversity did not differ among burning intervals, which suggests the saprotrophic community may not be severely affected by burning or recovers quickly following fire. Perhaps this increase in soil organic matter input in an ecosystem that receives infrequent litter inputs may buffer the effects of fire on the saprotrophic community.

Implications

This study is a first look at how burning affects soil fungal functional groups and the relative contributions of environmental drivers in soil fungal community variation in prairies of the PNW where fire is a common management tool in restoration. Our results suggest burning has short-lived consequences for soil fungal communities, and other environmental variables are driving the variation observed in these communities, mainly regional differences. However, the direction of how the soil fungal community is altered by burning is not consistent across functional groups, and we did find differences in species diversity and community composition among burning intervals for several functional groups that are important in restorations, mycorrhizae and some plant pathogen groups.

Management of these prairies involves rotational partial burns of low severity. Low intensity burns may not penetrate the soil deep enough to drastically alter the soil fungal communities, but it appears burning does affect the structure of these communities to some extent. We observed this in some key shifts in arbuscular mycorrhizal community composition. However, consistent relative abundances of AMF across burning intervals suggests there is functional redundancy at regional scales with different

actors at local scales. For each site, only part of the prairie is burned each year. This may allow for the persistence and recovery of less resilient soil fungal species in the part left unburned. Employing a fire mosaic management strategy may also aid in the recovery of key functional groups, especially saprotrophs and arbuscular mycorrhizae. The burning frequency and intensity utilized in this study appears to allow total soil fungal communities time to recover following fire. However, current prescribed burning practices may not control for plant pathogens in a consistent manner, suggesting further studies are necessary to determine the right burning frequency and severity to control particular pathogens.

Future research could explore the internal fungal communities of *Festuca roemeri,* the dominant native grass in these prairies. A closer look at the root and shoot fungal communities may help us understand which mycorrhizal species and plant pathogens are associated with *F. roemeri,* and how these communities shift within the plant at different burning intervals. This will inform site managers about mycorrhizal inoculation practices that have already begun (Sarah Hamman, pers. comm.) and the persistence of plant pathogens following fire.

APPENDIX A

SUPPLEMENTARY TABLES

Supplemental Table 1. Results of proportions tests comparing percent abundances of functional groups by region.

Functional Group	**p-value**	**X^2**
Animal/Insect Pathogen	0.8	0.06
Arbuscular Mycorrhizal	0.78	0.08
Bryophyte Parasite	1	<0.001
Ectomycorrhizal	0.67	0.18
Endophyte	0.97	0.001
Epiphyte	1	<0.001
Ericoid Mycorrhizal	0.99	<0.001
Fungal Parasite	0.25	1.31
Lichen	0.97	0.001
Plant Pathogen	0.69	0.15
Saprotroph	1	<0.001

Supplemental Table 2. Results of proportions tests comparing percent abundances of functional groups by time since burning.

Functional Group	**p-value**	**X^2**
Animal/Insect Pathogen	0.82	0.39
Arbuscular Mycorrhizal	0.88	0.25
Bryophyte Parasite	0.92	0.16
Ectomycorrhizal	0.67	0.8
Endophyte	0.79	0.48
Epiphyte	0.94	0.13
Ericoid Mycorrhizal	0.89	0.22
Fungal Parasite	0.7	0.72
Lichen	0.6	1
Plant Pathogen	0.75	0.57
Saprotroph	0.82	0.39

Supplemental Table 3. Results of proportions tests comparing percent abundances of arbuscular mycorrhizae by region. Bolded values indicate significance ($p < 0.05$).

Genus	**p-value**	**X^2**
Acaulospora	**0.007**	7.17
Archaeospora	**<0.001**	51.7
Claroideoglomus	**0.007**	7.31
Dominikia	**<0.001**	21.78
Funneliformis	0.057	3.63
Glomus	**<0.001**	16.1
Paraglomus	**0.01**	6.66
Scutellospora	1	<0.001

Supplemental Table 4. Results of proportions tests comparing percent abundances of arbuscular mycorrhizae by time since burning. Bolded values indicate significance ($p < 0.05$).

Genus	**p-value**	**X^2**
Acaulospora	**<0.001**	36.07
Archaeospora	**0.004**	10.78
Claroideoglomus	**0.001**	13.26
Dominikia	**<0.001**	23.47
Funneliformis	**<0.001**	31.04
Glomus	**<0.001**	47.77
Paraglomus	**0.005**	10.58
Scutellospora	0.75	0.57

Supplemental Table 5. Results of proportion tests comparing percent abundances of ectomycorrhizae by region. Bolded values indicate significance ($p < 0.05$).

Genus	**p-value**	**X^2**
Acephala	**0.003**	8.98
Amanita	1	<0.001
Cenococcum	1	<0.001
Ceratobasidium	**<0.001**	13.17
Chloridium	0.06	3.53
Cortinarius	1	<0.001
Hymenoscyphus	**<0.001**	11.98
Inocybe	0.14	2.21
Laccaria	1	<0.001
Lactarius	**0.04**	4
Pezoloma	**<0.001**	35.95
Russula	**0.001**	10.08
Scleroderma	0.24	1.39
Sistotrema	**<0.001**	15.81
Tomentella	0.25	1.33
Wilcoxina	0.85	0.04
Xerocomellus	1	<0.001

Supplemental Table 6. Results of proportions tests comparing percent abundances of ectomycorrhizae by time since burning. Bolded values indicate significance ($p < 0.05$).

Genus	**p-value**	**X^2**
Acephala	**0.004**	11.21
Amanita	0.28	2.55
Cenococcum	0.39	1.87
Ceratobasidium	**0.003**	11.29
Chloridium	**0.003**	11.68
Cortinarius	0.6	1.03
Hymenoscyphus	**0.04**	6.38
Inocybe	**0.003**	11.34
Laccaria	0.49	1.41
Lactarius	**<0.001**	21.84
Pezoloma	**<0.001**	22.68
Russula	0.4	1.83
Scleroderma	**0.003**	11.45
Sistotrema	**<0.001**	20.07
Tomentella	**<0.001**	20.2
Wilcoxina	0.1	4.5
Xerocomellus	0.29	2.47

Supplemental Table 7. Results of proportions tests comparing percent abundances of plant pathogens by region.

Class	**p-value**	**X^2**
Agaricomycetes	1	<0.001
Dothideomycetes	0.55	0.36
Entorrhizomycetes	1	<0.001
Leotiomycetes	0.8	0.06
Pucciniomycetes	0.3	1.09
Sordariomycetes	0.69	0.15
Taphrinomycetes	1	<0.001

Supplemental Table 8. Results of proportions tests comparing percent abundances of plant pathogens by time since burning. Bolded values indicate significance ($p < 0.05$).

Class	**p-value**	**X^2**
Agaricomycetes	**<0.001**	17.31
Dothideomycetes	0.11	4.35
Entorrhizomycetes	0.95	0.11
Leotiomycetes	**<0.001**	14.38
Pucciniomycetes	0.74	0.59
Sordariomycetes	0.13	4.14
Taphrinomycetes	0.82	0.39

Supplemental Table 9. Results of proportions tests comparing percent abundances of saprotrophs by region.

Saprotrophs by Region		
Class	**p-value**	**X^2**
Agaricomycetes	0.58	0.3
Archaeorhizomycetes	0.76	0.09
Dothideomycetes	1	<0.001
Eurotiomycetes	0.68	0.17
Geminibasidiomycetes	1	<0.001
Geoglossomycetes	1	<0.001
Leotiomycetes	1	<0.001
Mortierrellomycetes	0.56	0.34
Orbiliomycetes	1	<0.001
Pezizomycetes	1	<0.001
Saccharomycetes	1	<0.001
Sordariomycetes	0.72	0.12
Tremellomycetes	1	<0.001
Umbelopsidomycetes	1	<0.001

Supplemental Table 10. Results of proportions tests comparing percent abundances of saprotrophs by time since burning.

Saprotrophs by Time Since Burning		
Class	**p-value**	**X^2**
Agaricomycetes	0.88	0.25
Archaeorhizomycetes	0.24	2.82
Dothideomycetes	0.92	0.16
Eurotiomycetes	0.56	1.17
Geminibasidiomycetes	0.83	0.38
Geoglossomycetes	0.97	0.07
Leotiomycetes	0.96	0.08
Mortierrellomycetes	0.98	0.05
Orbiliomycetes	0.97	0.05
Pezizomycetes	0.86	0.29
Saccharomycetes	0.85	0.32
Sordariomycetes	0.95	0.09
Tremellomycetes	0.91	0.19
Umbelopsidomycetes	0.82	0.4

Supplemental Table 11. Means and standard deviations of soil nutrient data.

Mean Soil Nutrient Values				
Time Since Burning	**Soil pH**	**C:N Ratio**	**Nitrate (mmol/g)**	**Ammonium (mmol/g)**
150 years	5.18	11.4	4.15 x 10^-5	0.000836
2 years	5.31	12.4	4.33 x 10^-5	0.000513
1 month	5.3	12	6.94 x 10^-5	0.00116
Standard Deviation of Soil Nutrient Values				
Time Since Burning	**Soil pH**	**C:N Ratio**	**Nitrate (mmol/g)**	**Ammonium (mmol/g)**
150 years	0.209	1.49	6.48 x 10^-5	0.00063
2 years	0.334	1.693	3.75 x 10^-5	0.000297
1 month	0.454	1.684	1.39E-04	0.000941

APPENDIX B

SUPPLEMENTARY FIGURES

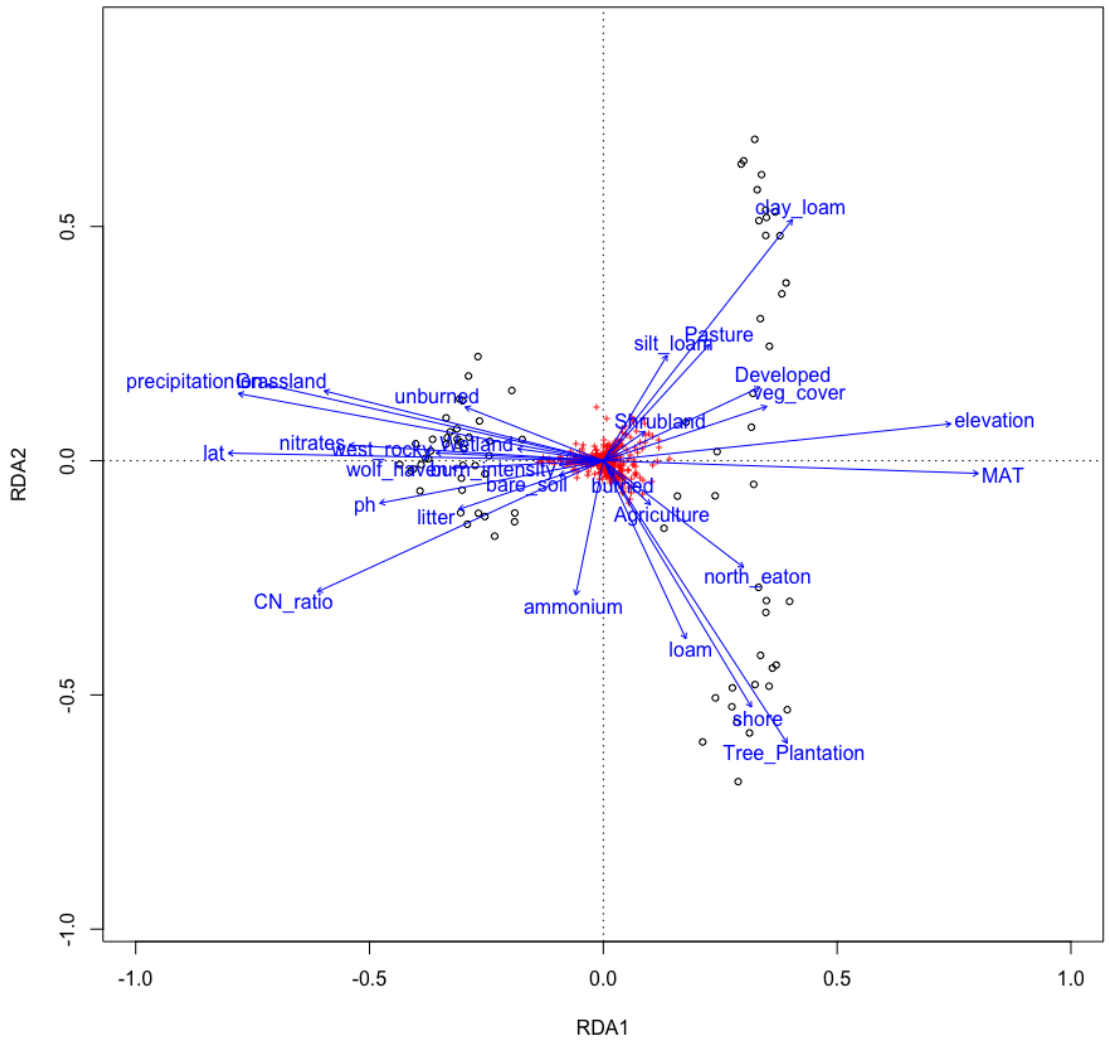

Figure S1. RDA plot of environmental variables.

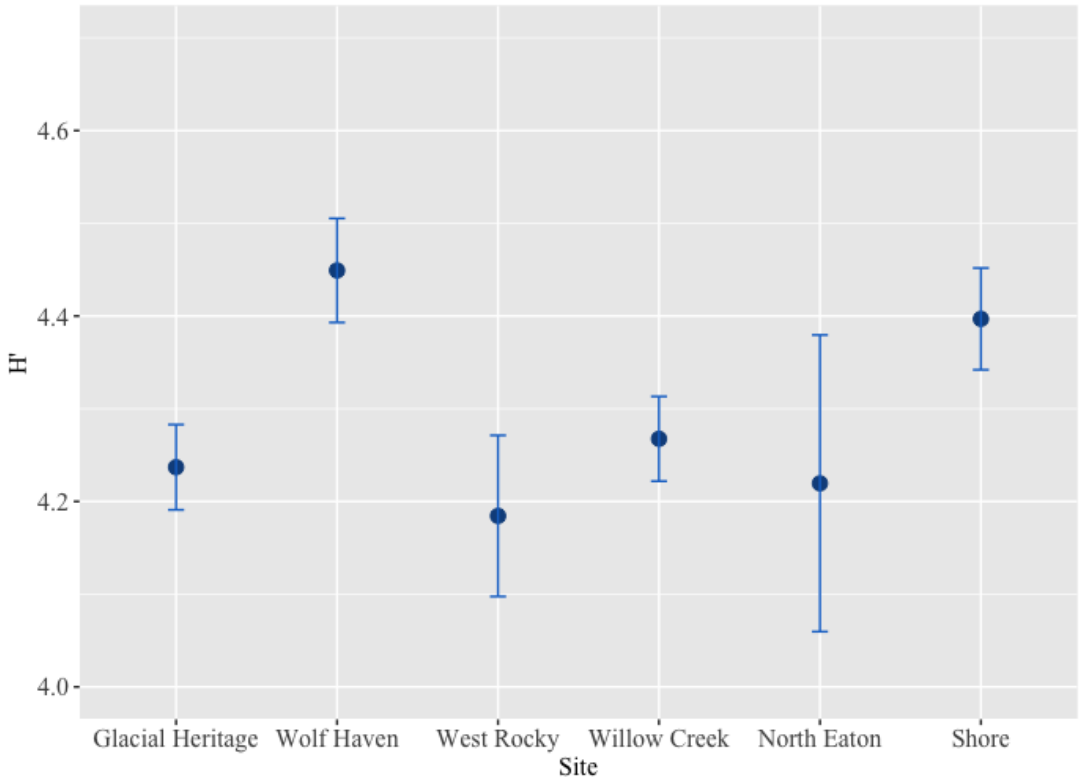

Figure S2. Mean Shannon diversity of complete soil fungal community by site. Error bars represent SE.

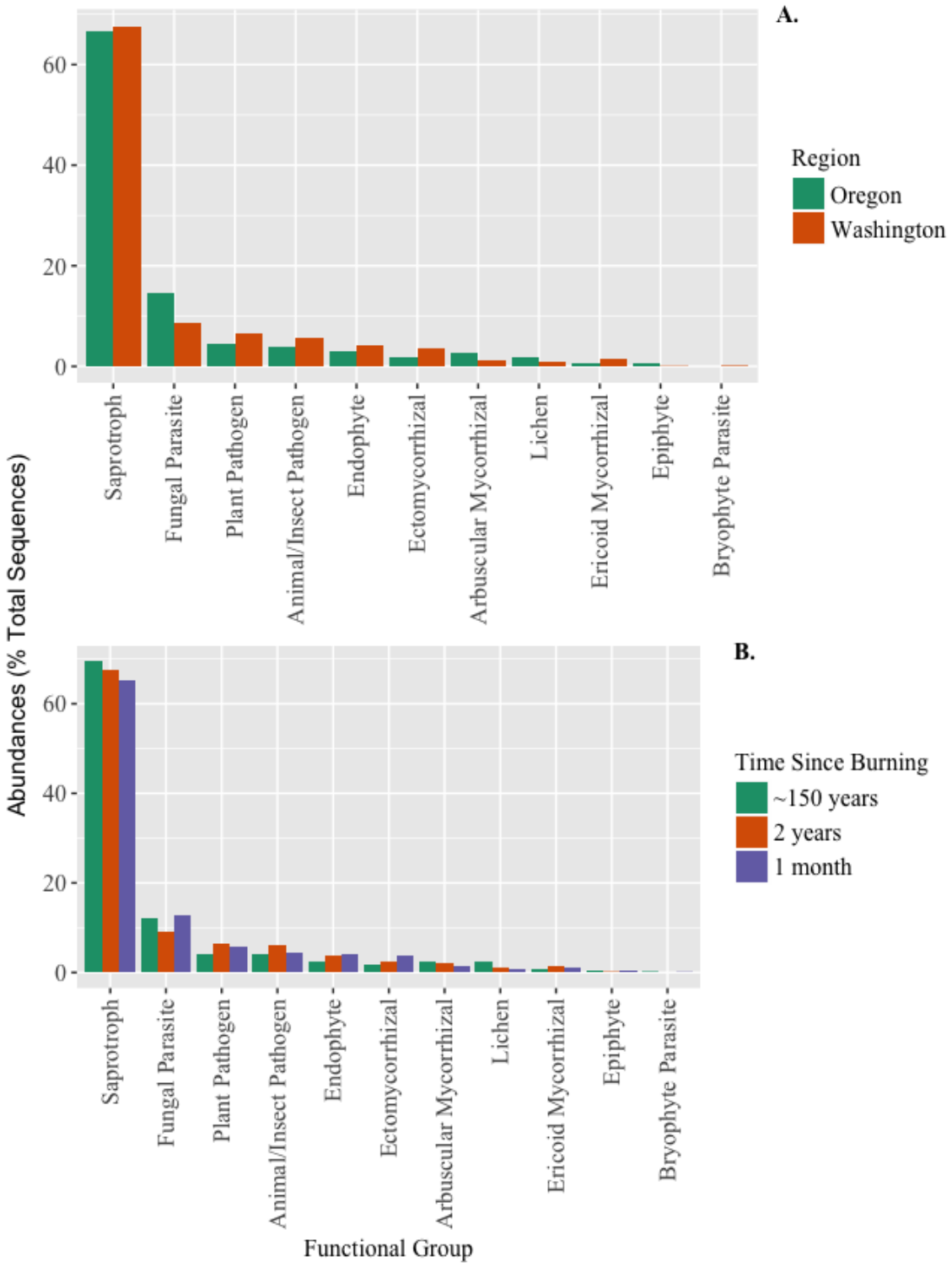

Figure S3. Functional group percent abundances. A. By region. B. By time since burning.

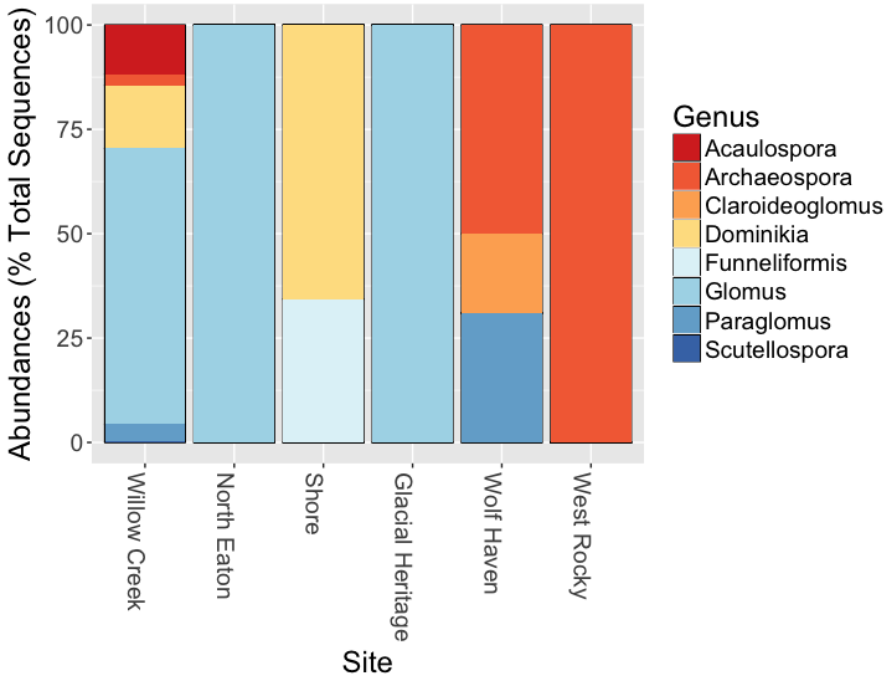

Figure S4. Arbuscular mycorrhizae proportions of genera by site.

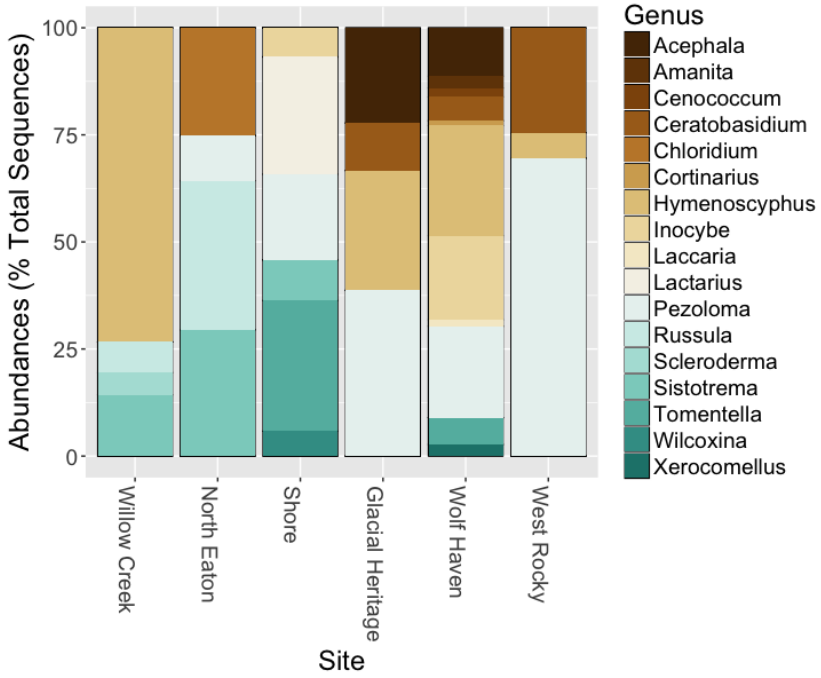

Figure S5. Percent abundances of EcM fungi by site.

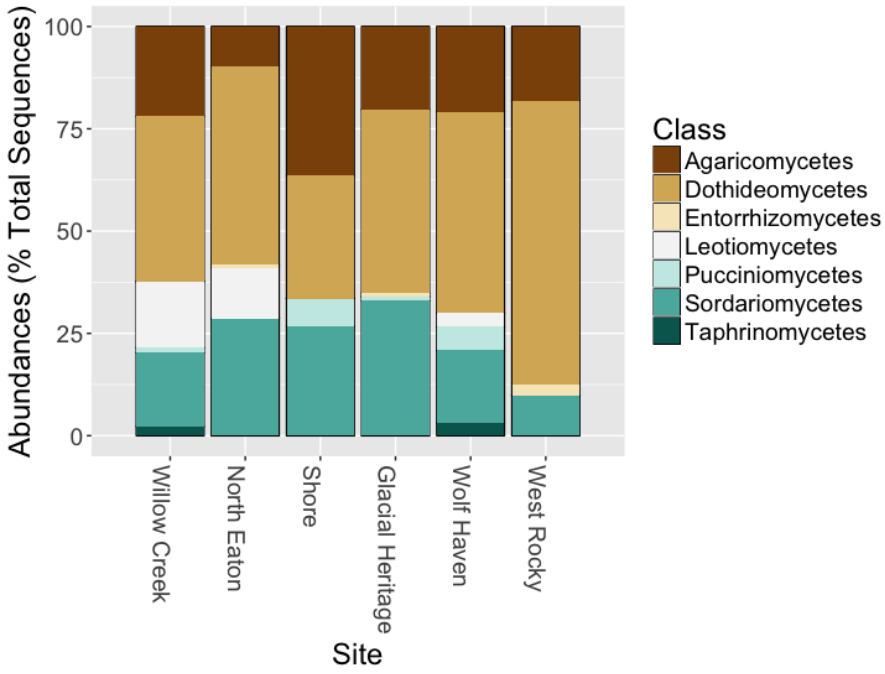

Figure S6. Percent abundances of plant pathogens by site.

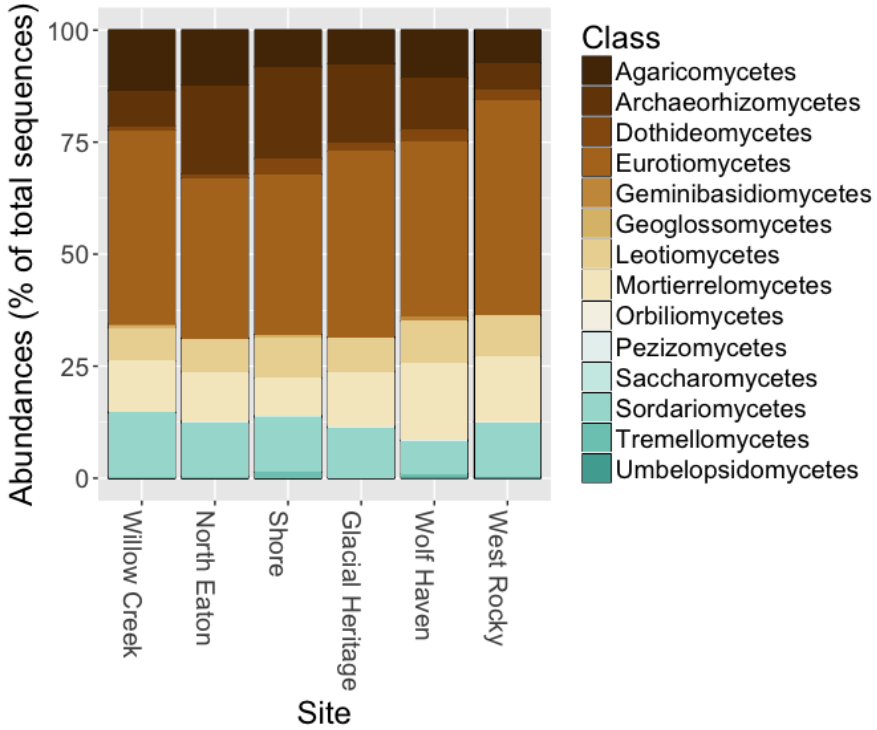

Figure S7. Percent abundances of saprotrophs by site.

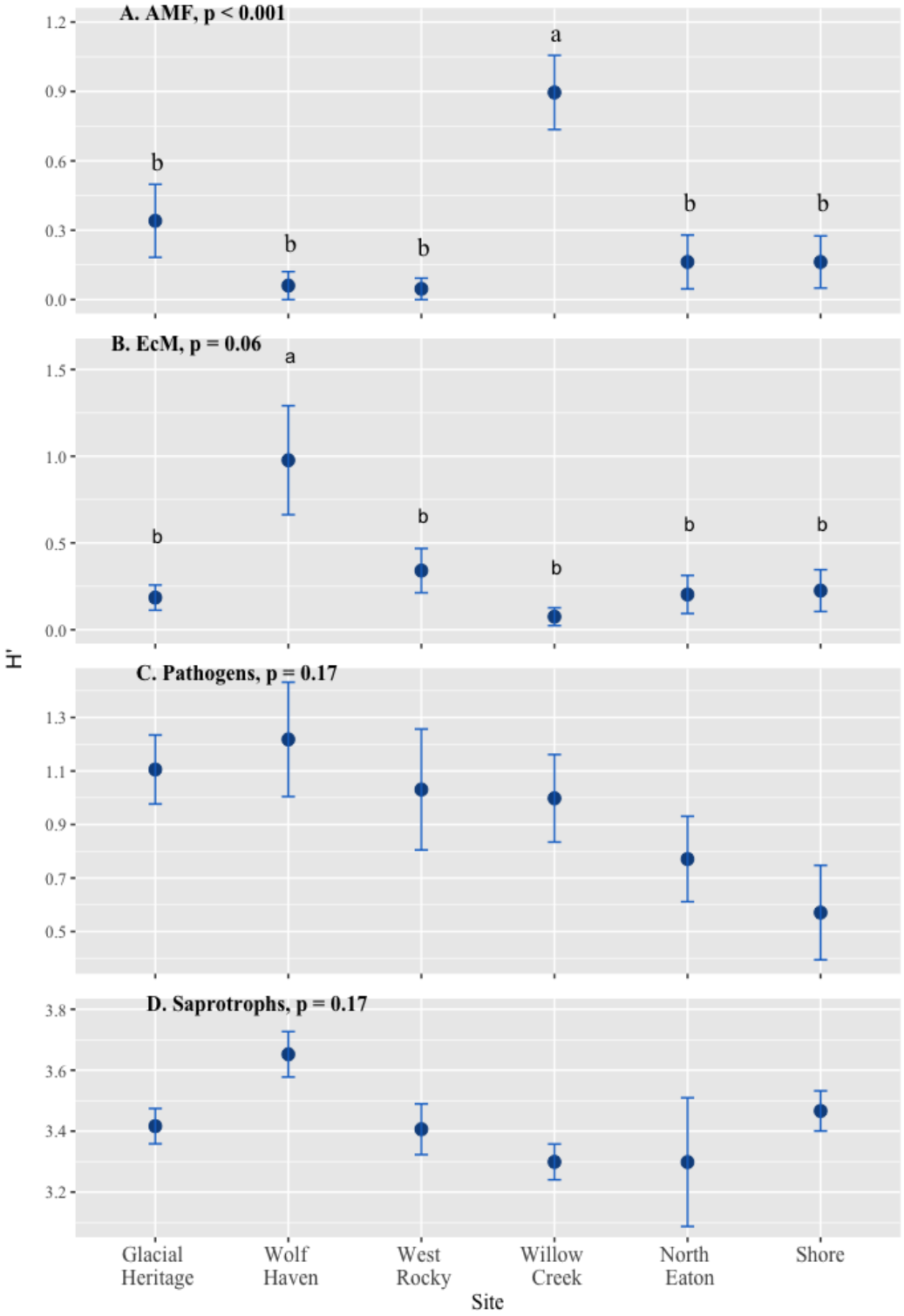

Figure S8. Mean Shannon species diversity comparisons by site. Error bars represent SE. A. AMF. Results of post-hoc Tukey tests designated by letters above error bars. B. EcM. C. Plant Pathogens. D. Saprotrophs.

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