Article Title:

Fungal community structure and seasonal trajectories respond similarly to fire across pyrophilic ecosystems

Running Title: Fire alters fungal communities similarly across systems

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Abstract

Fire alters microbial community composition, and is expected to increase in frequency due to climate change. Testing whether microbes in different ecosystems will respond similarly to increased fire disturbance is difficult though, because fires are often unpredictable and hard to manage. Fire recurrent or pyrophilic ecosystems, however, may be useful models for testing the effects of frequent disturbance on microbes. We hypothesized that across pyrophilic ecosystems, fire would drive similar alterations to fungal communities including altering seasonal community dynamics. We tested fire's effects on fungal communities in two pyrophilic ecosystems, a Longleaf pine savanna and tallgrass prairie. Fire caused similar fungal community shifts including a) driving immediate changes that favored taxa able to survive fire and take advantage of post-fire environments, and b) altering seasonal trajectories due to fire-associated changes to soil nutrient availability. This suggests that fire has predictable effects on fungal community structure and intra-annual community dynamics in pyrophilic ecosystems, and that these changes could significantly alter fungal function. Parallel fire responses in these key microbes may also suggest that recurrent fires drive convergent changes across ecosystems, including less fire frequented systems that may start burning more often.

Introduction

Fire is a consistent disturbance in terrestrial ecosystems that shifts the composition of microbial communities. The strength of these changes is largely determined by the historical fire regime, which can regulate how microbes and their associated functions change following individual fires (McMullan-Fisher et al. 2011; König et al. 2019; Pressler, Moore and Cotrufo 2019; Semenova-Nelsen et al. 2019). As predicted, anthropogenic influence (Balch et al. 2017) and

climate change (Liu and Wimberly 2016; Schoennagel *et al.* 2017) are increasing the frequency of wildfires. Understanding how increasing fire disturbance impacts microbial communities, which are foundational to terrestrial ecosystems, is critical to predict and mitigate ecosystem consequences. Using experimental fires in many ecosystems to test these responses, however, are often dangerous or impossible. In lieu of widespread, controlled fire experiments, pyrophilic (i.e. fire recurrent) ecosystems may provide models to test the potential effects of altered fire regimes since they are historically maintained by frequent fires (both natural and prescribed). Recurrent fires in these systems has driven long-term adaptation of their biological communities to fire (Bowman *et al.* 2009; Pausas 2015; Archibald *et al.* 2018). If repeated fires in distinct, pyrophilic ecosystems drive similar fire responses in microbial communities, it may support a more general framework to predict microbial responses to increased fire frequency.

Fungi are foundational microbes in most pyrophilic ecosystems, and fire directly alters their communities and function in ways that impact future fire disturbances. Since fungi directly modify available plant fuels through saprotrophic, pathogenic, and mutualist interactions, fire induced changes to fungal communities may drive feedbacks on the frequency and intensity of future fires. The ability of fungi to modify fire regimes through their effects on plant fuels makes understanding fire effects on fungi important to our comprehension of the ecological processes that underpin pyrophilic ecosystems. Across pyrophilic systems, fire restructures fungal communities by inducing mortality (Hamman, Burke and Stromberger 2007; Dooley and Treseder 2012), suppressing dominant taxa (Brown *et al.* 2013; Pressler, Moore and Cotrufo 2019; Semenova-Nelsen *et al.* 2019), and selecting for species that can withstand extreme fire temperatures and survive in harsh, post-fire environments (Hamman, Burke and Stromberger 2007; Peay, Garbelotto and Bruns 2009; Owen *et al.* 2019). Certain fungal taxa or groups may

be better at surviving fire than others, whether through thermotolerance and avoiding high temperatures via belowground sporocarps, or by taking advantage of post-fire conditions through stress tolerance, rapid colonization, and dispersal. This differential survival could alter community structure, and potentially shift fine fuel dynamics and feedbacks on future fires. Many of these community shifts may take place immediately with fire, but others may alter the normal intra-annual dynamics of fungal communities.

Many fungal taxa display seasonal trends, so apart from immediate community changes, fire could also change the seasonal trajectory of fungal communities. Fungal mutualists like mycorrhizal fungi are key examples. Both arbuscular (AM) and ectomycorrhizal (EM) fungi provide plant hosts with nutrients (mainly P and N respectively) in return for carbon. Both groups also display seasonal peaks in abundance (Harvey, Jurgensen and Larsen 1978; Santos-Gonzalez, Finlay and Tehler 2007), and are strongly impacted by fire (Klopatek, Debano and Klopatek 1988; Dhillion and Anderson 1993; Taudière, Richard and Carcaillet 2017). Fire is well known to decrease the richness and *in situ* colonization of AM and EM fungal communities (Dove and Hart 2017). In doing so, fire may suppress mycorrhizal fungi in ways that alter their function to plants at key life history stages (e.g. germination, growth, reproduction), thereby limiting future plant fuel production. Alternatively, fires early in the growing season are commonly used in ecosystem management and may benefit fuel production by reducing fungal plant pathogens in standing biomass (Hardison 1976; Katan 2000). Despite the potential interaction between fire and fungal seasonal trends, few studies have explored how fire alters fungal community seasonal trajectories. Natural seasonal turnover in fungal communities is expected regardless of fire, but fire may augment these seasonal dynamics. Since fungal communities are highly associated with environment conditions, it is likely that both the direct

(i.e. mortality) and indirect effects (i.e. changes to environmental conditions) of fire play important roles in shaping fungal communities across time.

The nature of fire's effects on fungal communities likely changes in the seasons following fire. While fire has well known short-term effects on fungal communities (Muñoz-Rojas *et al.* 2016; Semenova-Nelsen *et al.* 2019), how fire continues to alter fungal community dynamics after fires is less clear. One way fire may alter fungal recovery time is by local changes to limiting nutrients. Prescribed fires often drives a flush of nutrients immediately following the fire event (i.e. C, N, and P; Butler et al., 2018; Johnson and Curtis, 2001; Neary et al., 1999), but can drive a longer-term loss of nutrients due to erosion (Knelman *et al.* 2015), wind, and leaching, especially under high frequency fire regimes (Bell and Binkley 1989; Knelman *et al.* 2019). The lack of available nutrients could slow fungal activities like decomposition if C:N and C:P ratios increase due to nutrient loss (Raison 1979; Butler *et al.* 2019). Fire may also change the physical environment in ways that create inhospitable conditions for many fungal species (i.e. dry, hydrophobic soil; Iverson and Hutchinson, 2002; MacDonald and Huffman, 2004), thereby favoring stress tolerant taxa that can survive fire and/or take advantage of post-burn conditions through colonization and dispersal from nearby unburned areas. Despite strong, early effects on fungal communities, fire effects may attenuate with time as plants regrow, litter (e.g. fuels) replenishes, and fungi recolonize (Treseder, Mack and Cross 2004; Hart *et al.* 2005; Bastias *et al.* 2006; Holden, Gutierrez and Treseder 2013). These dynamic fire effects on fungal communities likely overlay, and interact with, seasonal changes in fungal communities (Averill *et al.* 2019; Štursová *et al.* 2020). Understanding these interactions is central to understand and predict fire's role in ecosystems and any feedbacks to future fires.

To address these questions, we sampled soil fungal communities and soil edaphic traits across the growing season following prescribed fires in two pyrophilic ecosystems: A Longleaf pine savanna and tallgrass prairie. These systems represent intact, late-succession ecosystems that have been historically managed with frequent prescribed burning (detailed below in methods). Soil samples were collected at one to two month intervals following prescribed fires, and fungal communities were characterized with amplicon sequencing that targeted the ITS2 rDNA region. We hypothesized that fire would drive similar shifts in fungal community structure in both pyrophilic ecosystems, and that these changes would persist throughout the year following fire, but attenuate with time. We expected that fire related differences in fungal community structure would reflect selection for fungal taxa that could survive the passage of fire and/or take advantage of post-fire environments. We further hypothesized that fire would alter fungal seasonal trajectories (i.e. compositional changes between successive sampling times) such that fungal communities that experienced fire would display larger changes across seasons compared to those that did not burn. We predicted these seasonal dynamics in burned communities would be linked to fire-related changes in nutrient availability and environmental conditions. Our findings suggest that fire causes parallel shifts in fungal community structure and seasonal trajectories across these pyrophilic ecosystems.

Methods

Study Sites: We conducted our study in two pyrophilic ecosystems: A Longleaf pine savanna and tallgrass prairie. Both represent key examples of pyrophilic ecosystems, and are maintained with frequent prescribed burns in order to mimic pre-colonial fire return intervals of 2-3 natural fires per decade (Platt 1999; Ford 2009). Despite the overstory Longleaf pines, both sites host similar

understory plant communities, including shared representative grass (*Schizachyrium*, *Andropogon*, and *Sorghastrum*) legume (*Chamaescrista*), and forb (*Liatris*) genera.

The Longleaf pine savanna is an old-growth pine savanna on the Wade Tract (30° 45' N; 84° 00' W; Thomas County, Georgia, USA; Fig.S1). Surface soils are acidic, fine-textured sands with 50-100 cm deep A horizons over clay hardpans (Carr *et al.* 2009; Levi *et al.* 2010). The open savanna/woodland physiognomy is characterized by overstory, EM fungal associated Longleaf Pines (*Pinus palustris*) and diverse herbaceous and AM fungal associated ground layer vegetation. Average annual precipitation for this site is 1350mm, with two peaks in seasonal rainfall (January - March, and June - August). Prescribed fires have been instrumental in maintaining old-growth aspects of the Wade Tract over the past century. Traditional "open woods burning" involved annual-biennial, low-intensity late dormant and early growing season fires, typically in February-March, from the early 1800s to 1978 (Crawford and Brueckheimer 2012). Records indicate 25 fires at the site during the 3.5 decades between 1982 and 2016. Fire return intervals averaged 1.5 years, with 90% occurring between mid-March and late June.

The two, remnant tallgrass prairies, Rockefeller (39° 2' N; 95° 12' W; Fig.S2) and Dogleg (39° 3' N; 95° 11' W; Fig.S2), are located at the University of Kansas Field Station (Leavenworth County, Kansas). Surface soils at these adjacent sites are Pawnee series and Grundy silty clay loam (Dickey *et al.* 1977). Both are characterized by diverse, AM fungal associated graminoid and forb vegetation, with Rockefeller approximately 2.5 times the size of Dogleg prairie. Average annual precipitation for these sites is 1013mm, with the majority occurring between April and September. Both prairies are managed with low-intensity, prescribed fires in March-April, with occasional mowing to sustain the grassland structure, and fire return intervals of 1.5-2 years.

2017 Prescribed Fires: Wade Tract prescribed fires took place on March 23rd (Keetch-Byram Drought Index = 150) and April 12th (Keetch-Byram Drought Index = 105) in the east and west management units respectively. Drip torches ignited both fires along a central access road. Back and flanking fires were ignited in the morning with winds of 11 – 30 km/hr and relative humidity's ranging from 37% - 83%. Flaming fronts were estimated at 0.5-1.5 m high, and fine fuel consumption was ~60-66% for both fires.

Prescribed fires at the Rockefeller prairie took place on April 7th. Drip torches started the fire along a paved walking trail. Flanking fires were used to ensure that the fire remained under control and limited to the Rockefeller prairie site. Flaming fronts were estimated at 1-2 m high. The prescribed fire was intense enough to remove most vegetation, with only charred Blackberry stems (*Rubus* sp.) remaining. Residual ash was primarily black in color with scattered white patches, and fine fuel consumption was approximately 50-60%.

Sampling of Fungal Communities: Experimental plots (4m^2) were established at the Wade Tract, Rockefeller prairie, and Dogleg prairies prior to prescribed fires in March (Rockefeller prairie and Wade Tract - East management unit) and April (Wade Tract - West management unit). Note that the Dogleg prairie served as a "no burn" comparison for the Rockefeller prairie, due to its close proximity to Rockefeller unburned status in 2017. This created 24 plots at the Wade Tract (15 burned, 9 no burn), and 15 plots across the Rockefeller and Dogleg prairies (10 burned, 5 no burn). "No burn" sites at the Wade Tract were determined based on management records, GPS fire maps, and on-site inspection. Given the importance of overstory Longleaf pines in the Wade Tract pine savanna (Platt 1999), we further classified the Wade Tract sites into "near" (<10m from nearest overstory pine) or "away" (>10m from nearest overstory pine) from pines. This classification was based on known differences in fire characteristics (e.g. temperature and

duration) and microbial communities that are caused by larger amounts of pine needle fuels near overstory pines (Platt *et al.* 2016; Semenova-Nelsen *et al.* 2019; Hopkins *et al.* 2020). This gave a final count of 13 "near" and 11 "away" plots.

A 2.5cm diameter soil corer was used to collect soil samples (~2.5cm deep) from the center of each plot. Soils were sampled to this depth, because temperature related effects of fire are known to decrease rapidly with depth at this site (Hopkins *et al.* 2020). Three total cores were collected (~50g total), and homogenized in sample bags to produce 1 sample per plot at each sampling time. At the Wade Tract sites, soils were sampled 2 weeks prior to fire, then, 1, 2, 3, 4, 5, 6, and 7 months post-fire. At the two prairie sites, soils were sampled 2 weeks prior to and following fires, then at 1, 2, 4, 7, and 8 month intervals. All samples were taken at least 30 cm from previous sample sites to avoid damage to soils. To avoid inter-sample contamination, soil corers were sterilized with 1:9, bleach:isopropyl alcohol solution between plots. Soil samples were deposited in sterile bags, kept on ice, and frozen at 20°C within six hours of sampling. When necessary, samples were shipped overnight to the University of Kansas where they were stored at -80°C until processing. Before downstream analyses, samples were thawed, homogenized, and subsampled. A two gram subsample was taken for molecular analyses, and the remaining soil was sent to Kansas State Soil Testing Lab for chemical analyses

Soil Chemical Analyses: Soil phosphorus content was measured using the Mehlich-3 method on a Lachat Quickchem 8000 (Lachat Instruments, Loveland, Colorado; (Mehlich, 1984). Total soil nitrogen and carbon samples were measured on a LECO TruSpec CN Carbon/Nitrogen combustion analyzer (LECO Corporation, St. Joseph, Michigan).

DNA extraction and PCR: DNA was extracted from 0.25g of the molecular subsample using Machery-Nagel NucleoSpin® Soil kits (Machery-Nagel, Düren, Germany) and following the

manufacturer's protocol. Then, a single step PCR was used to amplify the ITS2 rDNA region using the fITS7 (forward; Ihrmark et al. 2012) and ITS4 (reverse; White et al. 1990) primer pair. The PCR mix was: 0.8 µL of DNA, 8 µL of 5x Q5® buffer (New England Biosystems, Ipswich, Massachusetts), 0.8 µL of dNTPs (10mM), 2 µL each of forward and reverse primers (10mM), 0.4 µL of Q5® High-Fidelity DNA polymerase (New England Biosystems), 8 µL of enhancer (New England Biosystems), and 17.8 µL of ddH$_2$O to adjust reaction volume to 40 µL. The PCR scheme followed Semenova-Nelsen et al. 2019: an initial denaturation step at 98˚C for 30 sec, followed by 25 cycles of 98 ˚C for 10 sec, 57 ˚C for 30 sec, and 72 ˚C for 30 sec, and a final extension step at 72 ˚C for 2 min, then held at 4 ˚C. Products for all PCRs were checked using agarose gels to ensure successful amplification, and cleaned using Agencourt AMPure XP magnetic beads (Beckman Coulter, Indianapolis, Indiana).

Library Preparation and Sequencing: Illumina MiSeq Nextera protocol was used to sequence fungal community samples. Using a second PCR reaction, 12 bp sequence barcodes (Nextera indices, Illumina, San Diego, California) were added to samples. The second "barcoding" PCR was similar to the first, except 5 µL of the primary PCR amplicon was used instead of 8 µL of the original DNA template, and the number of PCR cycles was set to eight. Barcoded amplicons were purified with Agencourt beads (as above) and DNA concentrations were checked using a Qubit 2.0 (LifeTechnologies, Carlsbad, California). Samples were then pooled in equimolar concentrations into a single library and sequenced using an Illumina MiSeq (Illumina, San Diego, California) with 300bp paired-end reads and V3 chemistry at the Kansas State Integrated Genomics Center. Sequence data is deposited in the Genbank Sequence Read Archive (SRA) PRJNA626638.

Bioinformatics: Raw sequencing data were analyzed using Qiime v.1.9.1, following methods outlined in Caporaso et al. 2010. Quality and barcode filtering resulted in 5M (of 21M input sequences) reads for the prairie sites and 5.5M (of 21.5M input sequences) for the pine savanna sites, with median lengths between 270 and 280. Open-reference OTU picking was completed using Usearch 6.1 (Edgar 2010), and the UNITE fungal ITS reference database v7.2 "dynamic" (Abarenkov et al., 2010, accessed Sept. 2019) to cluster OTUs, and the Ribosomal Database Project Classifier 2.2 (Wang *et al.* 2007) to assign taxonomic identities to OTUs. OTUs with less than five reads were removed to reduce sequencing artefacts (Lindahl et al., 2013), and DESeq2 (Love et al., 2014) was used to normalize read counts across samples and OTUs due to differences in sequencing effort. Bioinformatics scripts are included in the appendix.

Statistical Analyses: To contrast fire driven and seasonal changes in fungal communities, we used PRIMER 6 & PERMANOVA+ (Clarke and Gorley 2006). Dissimilarity matrices from OTU tables were created using the Bray-Curtis index. PERMANOVAs and apriori contrasts testing specific differences in fungal community structure between successive (i.e. intra-seasonal) sampling times were used to assess the effects of fire and time since fire on fungal communities in the tallgrass prairie sites, and the effect of fire, pine proximity, and time since fire on fungal communities in the Longleaf pine savanna site. With tallgrass prairie samples, fire treatment and time since fire were treated as fixed effects, and plot as a random effect. Plot was included to account for natural differences between plots across time, and to separate location based effects between the two prairies from fire treatment effects. With Longleaf pine savanna communities, fire treatment, time since fire, and pine proximity were treated as fixed effects and fire management unit was accounted for as a fixed effect. Fire management unit was treated as a fixed versus random effect, as there were only two levels (e.g. East & West), instead of the 5

plus suggested for use as a random effect (Crawley 2002; Gelman 2005). To assess fungal community shifts following fire, Longleaf pine savanna samples were grouped together across fire management units by time since fire. Grouping Longleaf pine savanna samples in this way made them comparable to analyses in the tallgrass prairie sites. Following PERMANOVAs, custom, apriori contrasts were used to test short-term or intra-seasonal (between successive sampling times) differences in fungal communities. Results for both ecosystems were independently visualized using non-metric multidimensional scaling (NMDS). Additionally, fire's effect on fungal community dispersion was assessed using the PERMdisp function, which calculates the OTU dispersion heterogeneity (average intra-group variance) with respect to burn treatment.

Using R v. 3.5.1 (R Core Team, 2013), differences in fungal communities, environmental effects, and species diversity were assessed and visualized based on fire treatment and time since fire using the envfit, CCA, and specnumber functions in the Vegan package (Oksanen *et al.* 2013). Fungal community diversity was calculated by quantifying raw OTU richness and the Inverse Simpson Index for each sample using the specnumber function, then treatment based differences in community diversity for post-fire samples were assessed using type III analysis of variance (ANOVAs) in the Emmeans package (Lenth 2018). Environmental variables were projected onto ordinations using Pearson correlation coefficients, and experimental treatment effects on nutrient availability were assessed similarly to species diversity. The resulting means were used to plot changes in nutrient availability over time. We also used the indicspecies package (De Caceres and Jansen 2016), to identify fungal OTUs that were associated with burned and unburned sites. In longleaf pine savanna site, burned and unburned plots were interspersed, and we were able to directly compare indicator species between burned and

unburned plots. In the prairie, where burned plots were adjacent to unburned plots, we were concerned that spatial effects might lead to indicator species differences beyond those caused by fire alone. To account for this, we choose instead to contrast pre-fire and 2wk post fire samples in the burned plots alone. Using the mulitpatt function in the indicspecies package, we inserted both OTU tables and allowed for 999 permutations and a p-value cutoff of 0.05. The indicspecies function tests the indicator value index of each OTU for a given treatment group by assessing 1) the OTU's "specificity," or the probability that a site belongs to the treatment group, given that the OTU is present (A statistic), and 2) the OTU's "fidelity," or the probability of finding a species in a site belonging to the specific treatment group (B statistic). Note that the indicspecies function handles indicator species analysis for all taxa independently. To help further avoid spurious results, species were only considered indicator species if their A statistic was at least 50%, their B statistic was at least 40%, and their indicator value index significance was below 0.05. For species meeting these criteria, available taxonomic and ecological data are mentioned in the results and provided in the appendix (Tables S9-S12).

Results

Fungal Data: Community sequence data revealed a highly diverse fungal community in the Longleaf pine savanna site. A total of 8749 OTUs were identified, with 80% of classifiable OTUs representing 5 phyla, 25 classes, 87 orders, 195 families, and 2647 genera. 1686 OTUs were identified only to the kingdom level (Fungi), and 88 OTUs were either not fungi or unclassifiable and removed from downstream analyses. Pine savanna communities were dominated by the Basidiomycota class Agaricomycetes (~26%), followed by the Ascomycota classes Sordariomycetes (~17%), and Dothideomycetes (~10%), and four orders: Basidiomycota

orders Agaricales (~8%) and Russulales (~6%), and Ascomycota orders Hypocreales (~6%) and

Pleosporales (~5%). The five most abundant OTUs were an unidentified Trichocomaceae

species, the endophyte *Umbelopsis diamorpha*, an unidentified *Geminibasidium* species, the

capsule forming *Cryptococcus podzolicus*, and an unidentified, basal lineages fungal species.

Note that many members of the family Aspergillaceae (i.e. Trichocomaceae) and genus

Geminibasidium are able to survive in extreme conditions and are thermotolerant (McGee *et al.*

2006; Nguyen, Nickerson and Seifert 2013).

In the tallgrass prairies, a total of 8425 OTUs were identified, with ~76% classifiable

OTUs representing 5 phyla, 24 classes, 90 orders, and 483 genera. 2036 OTUs were identified

only to the kingdom level (*Fungi*), and 96 OTUs were either not fungi or unclassifiable and

removed from downstream analysis. Prairie fungal communities were dominated by three

classes: Ascomycota classes Sordariomycetes (~27%) and Dothideomycetes (~18%), and the

Basidiomycota class Agaricomycetes (~22%), and three orders: Ascomycota orders Hypocreales

(~14%) and Pleosporales (~13%), and the Basidiomycota order Agaricales (~12%). The five

most abundant OTUs corresponded to an unidentified Pleosporales sp., an unidentified *Periconia*

species, an unidentified Ascomycete, an unidentified Nectriaceae species, and an unidentified

Sordariaceae species.

Direct Effects of Prescribed Fire on Fungal Communities: In the longleaf pine savanna site,

fungal communities varied based on the presence/absence of fire and location relative to

Longleaf pines. As expected, burned fungal community composition was different than

unburned community composition (PERMANOVA: $F_{1,102} = 2.13$, $P = 0.001$, $R^2 = 1.9\%$; Fig.1a,

Table 1). However, despite apparent fire driven shifts to fungal community structure, fire did not

homogenize communities found in burned vs. no burn plots (Beta-dispersion: $F_{1,22} = 0.013$, $P =$

0.931; Table 2), or alter alpha diversity (OTU Richness: $F_{1,19}$ = 1.33, P = 0.264, Fig.2a; Inverse

Simpson: $F_{1,19}$ = 1.85, P = 0.189, Fig.2c; Table 3). Proximity to overstory Longleaf pines also

suggested that "near" and "away" fungal communities differed marginally ($F_{1,102}$ = 1.35, P =

0.078, R^2 = 1.2%; Table 1), and that this effect was modified by the presence/absence of fire

($F_{1,102}$ = 1.64, P = 0.013, R^2 = 1.4%; Table S1). Specifically, differences between burned and

unburned fungal communities were larger in near pines plots than in away from pines plots.

Within the tall grass prairie sites, fungal communities were also altered by prescribed

fire. Fire treatment described the largest differences between fungal communities ($F_{1,102}$ =

8.6781, P = 0.001, R^2 = 15%; Fig.1b, Table 4), but some of this was likely due to inherent site-

based variation in fungal communities between the two prairies. As in the Longleaf pine

savanna, fire did not homogenize communities as both types of plots (i.e. burned and unburned)

showed similar average dispersion of fungal communities ($F_{1,20}$ = 1.939, P = 0.21; Table 5).

Fungal diversity, however, was marginally higher in burned sites when the Inverse Simpson

Index was taken into account (OTU Richness: $F_{1,13}$ = 2.41, P = 0.14, Fig.2b; Inverse Simpson,

Fig.2d: $F_{1,13}$ = 3.66, P = 0.078; Table 6). In summary, fungal communities were altered by

prescribed fire in both the Longleaf pine savanna and tallgrass prairie sites, with fire-driven

changes primarily related to changes in community composition, and not the dispersion or

richness of fungal communities.

Fungal Community Seasonal Trajectory: In the Longleaf pine savanna, fungal communities

exhibited seasonal changes that were modified by the presence/absence of fire. Seasonal

changes in fungal community composition were apparent across fungal communities ($F_{7,102}$ =

2.08, P = 0.001, R^2 = 12.7%; Fig.3a-b, Table 1), with significant overall differences between

successive sampling times (P < 0.05, Table S2-S3). Specifically, fungal communities displayed

substantial shifts from previous sampling times until the 6-7 months (October-November) after the start of the experiment. Also, despite no significant overall interaction between time since fire and fire treatment ($F_{6,102} = 0.973$, P = 0.61; Table 1), apriori contrasts revealed significant fungal community shifts in burned plots, which exhibited distinct compositional changes between successive sampling times early after fire (e.g. pre-fire vs. 1month, 2 vs. 3 months, 3 vs. 5 months), however these differences were no longer apparent 4-6 months after fire (P < 0.05; Table S4-S5). Fungal communities in non-burned plots also displayed some changes between successive sampling times, however, unburned communities varied less across time and displayed smaller inter-sampling differences than burned fungal communities(Table S4-S5).

There were also strong seasonal changes in tallgrass prairie fungal communities that were altered in the presence of fire. Fungal communities shifted seasonally ($F_{6,102} = 2.1448$, $P = 0.001$, $R^2 = 7.2\%$; Fig.3c-d, Table 4), but now there was a significant overall interaction between fire treatment and sampling time ($F_{6,102} = 1.47$, $P = 0.001$, $R^2 = 5\%$; Table 4, Table S6). Community shifts between pairs of successive sampling times were significant in burned, but not unburned prairie plots (P < 0.05, Table S7-S8). Similar to fungal community shifts in the Longleaf pine savanna, fungal community turnover slowed around 7 months following fire (November). In conclusion, fire altered the seasonal trajectories of fungal communities across the two pyrophilic ecosystems by making differences between successive sampling times larger in burned vs. unburned plots, and driving longer term (i.e. 1 yr.) differences between burned and unburned fungal communities.

Indicator Species Analyses: Longleaf pine savanna indicator species reflected fire driven community shifts that favored fungi able to survive the short and longer term effects of fire. Taxa identified as indicators in the burned plots (Table S9) were either truffle forming

mycorrhizae like Hydnangiaceae and *Rhizopogon*, or able to survive in the extreme conditions following the passage of fire like *Geminibasidium* (Nguyen, Nickerson and Seifert 2013), Chaetothyriales (Sterflinger, De Hoog and Haase 1999; Villaseñor 2004), and Aspergillaceae (McGee *et al.* 2006). Taxa identified as indicators in unburned plots (Table S10), however, were largely plant pathogens like *Trimmatostroma* (Dick and Gadgil 2009), *Myrothecium* (Chen 2016), and Mycosphaerellaceae (Taylor, Groenewald and Crous 2003), or saprotrophs like *Preussia* (Kirk *et al.* 2008) and many Dothideomycetes.

Tallgrass prairie indicator species reflected similar trends as pine savanna taxa, as the burned sites contained species known to survive extreme temperatures and thrive in post-fire environments. Two weeks after fire, taxa known to rapidly colonize burned soils like *Cortinarius* (McMullan-Fisher *et al.* 2011), *Talaromyces* (Sharma 1981), and Pyronemataceae (Hansen *et al.* 2013), as well as wood associated saprotrophs like *Lophiostoma* (Holm 1988), *Coprinellus* (Peiris *et al.* 2007), Lasiosphaeriaceae (Cannon and Kirk 2007), and *Urnula* (Huffman 2008) were representative of burned communities (Table S11). Unburned, pre-fire indicator taxa (Table S12) were taxa known to contain plant-associated pathogens like Tubeufiaceae (Rossman 1987), *Trimmatostroma* (Dick and Gadgil 2009), *Phoma* (Kirk *et al.* 2008), *Zymoseptoria* (Quaedvlieg *et al.* 2011), and Mycosphaerellaceae (Taylor, Groenewald and Crous 2003), as well as saprotrophic taxa like *Cryptococcus* (May *et al.* 2016), Phaeosphaeriaceae (Cannon and Kirk 2007), *Periconia* (Markovskaja and Kačergius 2014), and *Bullera* (Nakase and Suzuki 1986). In summary, the indicator taxa for burned plots were taxa known to rapidly colonize and survive in post-fire environments, while the unburned indicators were predominantly plant pathogenic and saprotrophic taxa.

Indirect Effects of Prescribed Fire on Fungal Communities: Fire altered nutrient levels in Longleaf pine savanna sites, however these changes were only correlated with shifts in fungal communities 3 months after fire (Table S13). Soil carbon, nitrogen, and phosphorus levels did not vary significantly with time (C: $F_{6,63}=0.003$, $p = 1$; N: $F_{6,63}=0.082$, $p=0.99$; P: $F_{6,63}=0.074$; $p=0.99$; Fig.4a,c,e; Tables S14-16), but C and N were generally higher in burned sites (C: $F_{1,63}=6.68$, $p=0.01$; N: $F_{1,63}=5.34$, $p=0.02$; Fig.4a,c), and P levels were higher in burned plots near overstory Longleaf pines ($F_{1,63}=8.18$, $p<0.001$; Fig.4e). Despite fire induced differences in nutrient availability between treatments, nutrients were only associated with fungal community structure three months after fire ($P<0.05$; Table S13), when decreased P in burned sites was associated with fungal community structure.

Unlike in the savanna system, fire induced changes to nutrient availability were clearly associated with fungal community structure in the tallgrass prairie sites (Table S17). The largest differences in C, N, and P levels were due to differences between fire treatments (C: $F_{1,89}=55.3$, $p<0.001$; N: $F_{1,89}=61.2$, $p=0.001$; P: $F_{1,89}=13.4$; $p<0.001$; Fig.4b,d,f, Table S18-20) and sampling times (C: $F_{6,89}=8.3$, $p<0.001$; N: $F_{6,89}=7.1$, $p<0.001$; P: $F_{6,89}=3.2$, $p<0.001$). C, N, and P levels were generally higher in the burned, Rockefeller prairie, but followed similar seasonal patterns in both prairies (Fig.4b,d,f). Carbon, nitrogen, and phosphorus levels decreased early after fire (2 weeks – 2 months), but began to increase starting around 3 months. Despite similar seasonal changes in nutrient levels between the burned and non-burned prairies, fungal community shifts from 2 weeks – 2 months were tightly correlated with C, N, and P loss in the burned prairie plots, and this relationship attenuated with time (Table S17). Similar associations between fungal communities and C, N, and P were not observed in the unburned prairie plots. In summary, fires

altered nutrient availability similarly across both pyrophilic systems but changes to nutrient availability were more associated with fungal communities in the tallgrass prairie sites.

Discussion

Fire altered the structure and seasonal trajectories of fungal communities in a similar manner across Longleaf pine savanna and tallgrass prairie sites. The fungal community responses to fire observed here mirror changes in other pyrophilic systems: Mediterranean shrublands (Goberna *et al.* 2012), oak savannas (Ponder, Tadros and Loewenstein 2009), Loblolly pine forests (Brown *et al.* 2013), and Ponderosa pine forests (Stendell, Horton and Bruns 1999; Hamman, Burke and Stromberger 2007), suggesting there are generalizable fungal community responses to fire. The Longleaf pine savanna and tallgrass prairie ecosystems are distinct in several ways, including vegetation, (overstory pines vs grassland vegetation only) mycorrhizal status of plants (mixed EM and AM vs AM dominated), and climate (sub-tropical versus temperate). Despite these key differences, fire favored fungal taxa with similar traits (e.g. thermotolerance, drought tolerance, or effective post-fire colonization/dispersal ability) in both systems and caused similar shifts in fungal seasonal trajectories. These shifts were largely due to compositional turnover and suppression of dominant taxa, rather than mortality and loss of taxa alone. These effects are distinct from wildfire effects in less fire tolerant systems (Glassman *et al.* 2016; Dove and Hart 2017; Day *et al.* 2019), where rarer, high intensity fires often drive fungal mortality and declines in species richness (Treseder, Mack and Cross 2004; Glassman *et al.* 2016; Dove and Hart 2017; Day *et al.* 2019). However, both pyrophilic (Brown *et al.* 2013; Semenova-Nelsen *et al.* 2019) and less fire tolerant ecosystems (Bastias *et al.* 2006; Glassman *et al.* 2016; Smith *et al.* 2016;

Owen *et al.* 2019) contain fungal taxa able to survive fire and rapidly take advantage of post-fire environments in ways that may alter their seasonal trajectory as they did here.

Fire interacted with seasonal trends in fungal community structure to influence fungal seasonal trajectories following fire. As expected, fungal community composition in burned and unburned plots changed across time (i.e. natural seasonality; Dhillion and Anderson 1993; Averill *et al.* 2019; Štursová *et al.* 2020), however, changes in burned fungal communities were often larger, and varied more between successive sampling times than did unburned communities. The larger, fire-associated changes in burned communities altered their seasonal trajectories from fungal communities in nearby unburned plots, producing distinct burned and unburned communities that were maintained throughout the year following fire. These larger shifts in burned communities were likely due to the suppression of dominant fungi (Hansen *et al.* 2019; Semenova-Nelsen *et al.* 2019) that allowed for greater turnover with the growing season and new fungal dispersal and growth. Fire's effects on fungal communities did attenuate with time, however, likely reflecting the regrowth of host plants, replenishing litter fuel loads, and post-fire fungal recovery and dispersal effects (Treseder, Mack and Cross 2004; Hart *et al.* 2005; Bastias *et al.* 2006; Bárcenas-Moreno *et al.* 2011; Holden, Gutierrez and Treseder 2013). Since burned and unburned communities remained distinct throughout the study (~1 yr.), this may suggest that post-fire priority effects (Kennedy and Bruns 2005; Glassman *et al.* 2016) can promote alternative fungal assemblages. As fires continue to increase, these priority effects may drive larger and larger differences in seasonal trajectories between burned and unburned fungal communities, and contribute to inter-annual variations in fungal community structure (Bastias *et al.* 2006; Cairney and Bastias 2007; Egidi *et al.* 2016).

Fire related shifts in fungal community structure, were at least in part associated with fire-driven changes to nutrients, possibly reflecting an initial, post-fire nutrient flush (Certini 2005). This relationship was only clear in tallgrass prairie fungal communities which were linked to early shifts in C, N, and P levels, whereas these relationships were only present in in the Longleaf pine savanna communities at 3 months after fire. The differences in fungal responses to nutrient levels may be due to differences in soil type. As across the Southeast Coastal Plain, these pine savanna sites have nearly pure-sand top soils (~99% sand, 1% silt), which can accelerate post-fire nutrient leaching and loss, particularly following annual prescribed fires (Bell and Binkley 1989; Certini 2005). The importance of fire driven changes to nutrients declined with time, but fungi able to survive fire and thrive in these post-fire environments created persistent community differences.

Indicator taxa in burned plots possessed traits that can help resist high temperatures and post-fire conditions. The indicator taxa for fire differed between pine savanna and tallgrass prairie sites, likely due to differences in available species pools between the two systems (i.e. mix of AM and EM associated species in pine savannas, and predominantly AM associated species in prairies). Yet in both systems, indicator species represented known thermo- and drought tolerant fungal species including such as Geminibasidium in burned Longleaf pine savanna plots, as well as rapid post-fire colonizers like *Talaromyces* (Sharma 1981; McMullan-Fisher *et al.* 2011) and Pyronemetaceae (Hansen *et al.* 2013) in the post-fire tallgrass prairie plots. In both sites, fires remove most aboveground plant biomass and left sites exposed to the wind and sun, which likely favored drought-tolerant taxa. Drought-tolerant fungi proliferate after fires (Persiani and Maggi 2013), and our data suggest that adaptation to post-fire conditions is just as important as fire resistance alone, given that drought tolerant taxa were indicative of

burned sites at all sampling times in our study. Many indicator taxa of burned plots were also either wood decomposers or truffle forming fungi (mycorrhizal). Wood decomposers may be shielded from low-severity prescribed fires, and fires may modify substrates in ways that allow them to proliferate or readily disperse after fire (McMullan-Fisher *et al.* 2011; Hanula, Ulyshen and Wade 2012). Similarly, truffle forming mycorrhizal species may be insulated from fire both by the soil and within plant roots (Carson *et al.* 2019), which may explain why *Rhizopogon* and Hydnangiaceae species were indicative of burned pine savanna sites, a pattern well-supported in other systems (Klopatek, Debano and Klopatek 1988; Horton, Cázares and Bruns 1998; Baar *et al.* 1999; Glassman *et al.* 2016; Owen *et al.* 2019). If low-severity fire favors functional groups like wood rot and truffle forming mycorrhizal fungi, decomposition of fine plant fuels (i.e. non-woody litter) may slow and future plant fuel production may be favored respectively (Ficken and Wright 2017; Semenova-Nelsen *et al.* 2019; Hopkins *et al.* 2020). These changes could increase fine fuel loads over time, and increase the likelihood or spread of future fires in pyrophilic ecosystems.

Over time, as recurrent fires reduce fuel loads in long unburned systems (Kalies and Yocom Kent 2016), and fires decrease in intensity, less fire tolerant systems may start to approximate fire recurrent, pyrophilic systems. High intensity wildfires differ significantly from lower intensity prescribed fires, yet frequent fires in pyrophilic systems may be the safest method for testing the effect of altered fire regimes on fungal communities. Non-pyrophilic systems may start to experience increased fires both as a result of climate change (Liu and Wimberly 2016) *and* the increased usage of prescribed burns in land management to prevent wildfires (Kolden 2019). As these systems burn, soil microbial dynamics in less fire tolerant and long unburned systems may start to parallel those seen in pyrophilic systems, with concomitant changes to

ecological processes like nutrient cycling (Ficken and Wright, 2017; Hopkins et al., 2020). Recent wildfires have shown that fire tolerant taxa are already present in long unburned ecosystems and increase in abundance following fire (Reazin *et al.* 2016; Smith *et al.* 2016; Hughes *et al.* 2020). Improving our understanding between microbial community structure, seasonality, and function in fire recurrent ecosystems may therefore provide a more generalizable model for predicting future changes with more frequent fires.

In conclusion, our research demonstrates that fire drives comparable changes to fungal communities across pyrophilic ecosystems. In both prairie and pine savanna systems, fire driven changes reflected patterns consistent with selection for fire tolerant traits, community turnover, and changes to the local environment. Furthermore, fire driven changes altered the seasonal dynamics of fungal communities that naturally occur in the absence of fire. The similarity of fire driven shifts to fungal communities (Bárcenas-Moreno *et al.* 2011; Carson *et al.* 2019; Owen *et al.* 2019), suggests that pyrophilic systems may provide a useful model for assessing the influence of increased fires on microbial communities in less fire tolerant ecosystems, even if fires there are initially high intensity. Understanding fire's effects on microbes like fungi can improve our knowledge of the ecological processes that underpin terrestrial ecosystems and help ensure their resilience.

Conflicts of Interest

Authors have no conflicts of interest to declare.

References

Abarenkov K, Nilsson RH, Larsson KH *et al.* The UNITE database for molecular identification of fungi-recent updates and future perspectives. *New Phytologist* 2010;**186**:281–5.

Archibald S, Lehmann CER, Belcher CM *et al.* Biological and geophysical feedbacks with fire in the Earth system. *Environmental Research Letters* 2018;**13**:1–19.

Averill C, Cates LL, Dietze MC *et al.* Spatial vs. temporal controls over soil fungal community similarity at continental and global scales. *The ISME Journal* 2019;**13**:2082–93.

Baar J, Horton TR, Kretzer AM *et al.* Mycorrhizal colonization of Pinus muricata from resistant propagules after a stand-replacing wildfire. *New Phytologist* 1999;**143**:409–18.

Balch JK, Bradley BA, Abatzoglou JT *et al.* Human-started wildfires expand the fire niche across the United States. *Proc Natl Acad Sci USA* 2017;**114**:2946–51.

Bárcenas-Moreno G, García-Orenes F, Mataix-Solera J *et al.* Soil microbial recolonisation after a fire in a Mediterranean forest. *Biology and Fertility of Soils* 2011;**47**:261–72.

Bastias BA, Huang ZQ, Blumfield T *et al.* Influence of repeated prescribed burning on the soil fungal community in an eastern Australian wet sclerophyll forest. *Soil Biology and Biochemistry* 2006;**38**:3492–501.

Bell R, Binkley D. Soil nitrogen mineralization and immobilization in response to periodic prescribed fire in a loblolly pine plantation. *Canadian Journal of Forest Research* 1989;**19**:816–20.

Bowman DMJS, Balch JK, Artaxo P *et al.* Fire in the Earth System. *Science* 2009;**324**:481–4.

Brown SP, Callaham MA, Oliver AK *et al.* Deep Ion Torrent sequencing identifies soil fungal community shifts after frequent prescribed fires in a southeastern US forest ecosystem. *FEMS Microbiology Ecology* 2013;**86**:557–66.

Butler OM, Elser JJ, Lewis T *et al.* The phosphorus-rich signature of fire in the soil-plant system: a global meta-analysis. Niu S (ed.). *Ecology Letters* 2018;**21**:335–44.

Butler OM, Lewis T, Rashti MR *et al.* The stoichiometric legacy of fire regime regulates the roles of micro-organisms and invertebrates in decomposition. *Ecology* 2019, DOI: e02732.

Cairney JWG, Bastias BA. Influences of fire on forest soil fungal communities. *Can J For Res* 2007;**37**:207–15.

Cannon PF, Kirk PM. *Fungal Families of the World*. Wallingford, UK: CAB International, 2007.

Carr SC, Robertson KM, Platt WJ *et al.* A model of geographical, environmental and regional variation in vegetation composition of pyrogenic grasslands of Florida - Carr - 2009 - Journal of Biogeography - Wiley Online Library. *Journal of Biogeography* 2009;**36**:1600–12.

Carson CM, Jumpponen A, Blair JM *et al.* Soil fungal community changes in response to long-term fire cessation and N fertilization in tallgrass prairie. *Fungal Ecology* 2019;**41**:45–55.

Certini G. Effects of fire on properties of forest soils: a review. *Oecologia* 2005;**143**:1–10.

Chen Y. Myrothecium. *Mycosphere* 2016;**7**:64–80.

Clarke KR, Gorley RN. *PRIMER Version 6: User Manual/Tutorial*. Plymouth, UK: PRIMER-E, 2006.

Crawford RL, Brueckheimer WR. *The Legacy of a Red Hills Hunting Plantation: Tall Timbers Research Station & Land Conservancy*. University Press of Florida, 2012.

Crawley MJ. *Statistical Computing: An Introduction to Data Analysis Using S-Plus*. New York: Wiley, 2002.

Day NJ, Dunfield KE, Johnstone JF *et al.* Wildfire severity reduces richness and alters composition of soil fungal communities in boreal forests of western Canada. *Glob Change Biol* 2019:gcb.14641.

De Caceres M, Jansen F. Indicspecies: relationship between species and groups of sites. 2016;**R package version 1.7.6**.

Dhillion SS, Anderson RC. Seasonal dynamics of dominant species of arbuscular mycorrhizae in burned and unburned sand prairies. *Canadian Journal of Botany* 1993;**71**:1625–30.

Dick M, Gadgil PD. Eucalyptus Leaf Spots. *Forest Pathology in New Zealand*. 2nd ed. Rotorua, NZ: Forest Research Institute, 2009.

Dickey HP, Zimmerman JL, Plinsky RO *et al. Soil Survey of Douglas County, Kansas*., 1977.

Dooley SR, Treseder KK. The effect of fire on microbial biomass: a meta-analysis of field studies. *Biogeochemistry* 2012;**109**:49–61.

Dove N, Hart S. Fire Reduces Fungal Species Richness and In Situ Mycorrhizal Colonization: A Meta-Analysis. *Fire Ecology* 2017;**13**:37–65.

Edgar RC. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 2010;**26**.

Egidi E, McMullan-Fisher S, Morgan JW *et al.* Fire regime, not time-since-fire, affects soil fungal community diversity and composition in temperate grasslands. *FEMS Microbiol Lett* 2016;**363**, DOI: 10.1093/femsle/fnw196.

Ficken CD, Wright JP. Effects of fire frequency on litter decomposition as mediated by changes to litter chemistry and soil environmental conditions. *PloS one* 2017;**12**:e0186292.

Ford PL. Grasslands and Savannas. *Encylopedia of Life Support Systems* 2009;**III**:10.

Gelman A. Analysis of variance: why it is more important than ever. *Annals of Statistics* 2005;**33**:1–53.

Glassman SI, Levine CR, DiRocco AM *et al.* Ectomycorrhizal fungal spore bank recovery after a severe forest fire: some like it hot. *The ISME Journal* 2016;**10**:1228–39.

Goberna M, García C, Insam H *et al.* Burning Fire-Prone Mediterranean Shrublands: Immediate Changes in Soil Microbial Community Structure and Ecosystem Functions. *Microbial Ecology* 2012;**64**:242–55.

Hamman ST, Burke IC, Stromberger ME. Relationships between microbial community structure and soil environmental conditions in a recently burned system. *Soil Biology and Biochemistry* 2007;**39**:1703–11.

Hansen K, Perry BA, Dranginis AW *et al.* A phylogeny of the highly diverse cup-fungus family Pyronemataceae (Pezizomycetes, Ascomycota) clarifies relationships and evolution of selected life history traits. *Molecular Phylogenetics and Evolution* 2013;**67**:311–35.

Hansen PM, Semenova-Nelsen TA, Platt WJ *et al.* Recurrent fires do not affect the abundance of soil fungi in a frequently burned pine savanna | Elsevier Enhanced Reader. *Fungal Ecology* 2019;**42**:1–7.

Hanula JL, Ulyshen MD, Wade DD. Impacts of Prescribed Fire Frequency on Coarse Woody Debris Volume, Decomposition and Termite Activity in the Longleaf Pine Flatwoods of Florida. *Forests* 2012;**3**:317–31.

Hardison JR. Fire and Flame for Plant Disease Control. *Annual Review of Phytopathology* 1976;**14**:355–79.

Hart SC, DeLuca TH, Newman GS *et al.* Post-fire vegetative dynamics as drivers of microbial community structure and function in forest soils. *Forest Ecology and Management* 2005;**220**:166–84.

Harvey AE, Jurgensen MF, Larsen MJ. Seasonal Distribution of Ectomycorrhizae in a Mature Douglas-fir/Larch Forest Soil in Western Montana. *for sci* 1978;**24**:203–8.

Holden SR, Gutierrez A, Treseder KK. Changes in Soil Fungal Communities, Extracellular Enzyme Activities, and Litter Decomposition Across a Fire Chronosequence in Alaskan Boreal Forests. *Ecosystems* 2013;**16**:34–46.

Holm L. Studies in the Lophiostomataceae with emphasis on the Swedish species. *Symb bot ups* 1988;**28**:1–50.

Hopkins J, Huffman JM, Platt WJ *et al.* Frequent fire slows microbial decomposition of newly deposited fine fuels in a pyrophilic ecosystem. *Oecologia* 2020;**193**:631–43.

Horton TR, Cázares E, Bruns TD. Ectomycorrhizal, vesicular-arbuscular and dark septate fungal colonization of bishop pine (Pinus muricata) seedlings in the first 5 months of growth after wildire. *Mycorrhiza* 1998;**8**:11–8.

Huffman DM. *Mushrooms and Other Fungi of the Midcontinental United States*. University of Iowa Press, 2008.

Hughes KW, Matheny PB, Miller AN *et al.* Pyrophilous fungi detected after wildfires in the Great Smoky Mountains National Park expand known species ranges and biodiversity estimates. *Mycologia* 2020;**112**:677–98.

Iverson LR, Hutchinson TF. Soil Temperature and Moisture Fluctuations During and After Prescribed Fire in Mixed-Oak Forests, USA. *Natural Areas Journal* 2002;**22**:296–304.

Johnson DW, Curtis PS. Effects of forest management on soil C and N storage: meta analysis. *Forest Ecology and Management* 2001;**140**:227–238.

Kalies EL, Yocom Kent LL. Are fuel treatments effective at achieving ecological and social objectives? A systematic review. *Forest Ecology and Management* 2016;**375**:84–95.

Katan J. Physical and cultural methods for the management of soil-borne pathogens - ScienceDirect. *Crop Protection* 2000;**19**:725–31.

Kennedy PG, Bruns TD. Priority effects determine the outcome of ectomycorrhizal competition between two Rhizopogon species colonizing Pinus muricata seedlings. *New Phytologist* 2005;**166**:631–8.

Kirk PM, Cannon PF, Minter DW *et al. Dictionary of the Fungi*. 10th ed. Wallingford, UK: CAB International, 2008.

Klopatek C, Debano L, Klopatek J. Effects of simulated fire on vesicular-arbuscular mycorrhizae in pinyon-juniper woodland soil. *Plant and soil* 1988;**109**:245–9.

Knelman JE, Graham EB, Trahan NA *et al.* Fire severity shapes plant colonization effects on bacterial community structure, microbial biomass, and soil enzyme activity in secondary succession of a burned forest. *Soil Biology and Biochemistry* 2015;**90**:161–8.

Knelman JE, Schmidt SK, Garayburu-Caruso V *et al.* Multiple, Compounding Disturbances in a Forest Ecosystem: Fire Increases Susceptibility of Soil Edaphic Properties, Bacterial Community Structure, and Function to Change with Extreme Precipitation Event. *Soil Systems* 2019;**3**:1–16.

Kolden CA. We're Not Doing Enough Prescribed Fire in the Western United States to Mitigate Wildfire Risk. *Fire* 2019;**2**:30.

König S, Köhnke MC, Anouk-Letizia F *et al.* Disturbance Size Can Be Compensated for by Spatial Fragmentation in Soil Microbial Ecosystems. *Frontiers in Ecology and Evolution* 2019;**7**:1–11.

Lenth RV. Emmeans: Estimated marginal means, aka least-squares means. *Journal of Statistical Software* 2018;**69**:1–33.

Levi MR, Shaw JN, Wood CW *et al.* Land Management Effects on Near-Surface Soil Properties of Southeastern U.S. Coastal Plain Kandiudults. *Soil Science Society of America Journal* 2010;**74**:258.

Liu Z, Wimberly MC. Direct and indirect effects of climate change on projected future fire regimes in the western United States. *Science of The Total Environment* 2016;**542**:65–75.

MacDonald LH, Huffman EL. Post-fire Soil Water Repellency: persistence and soil moisture thresholds. *Soil Science Society of America Journal* 2004;**68**:1729–34.

Markovskaja S, Kačergius A. Morphological and molecular characterisation of Periconia pseudobyssoides sp. nov. and closely related P. byssoides. *Mycol Progress* 2014;**13**:291–302.

May RC, Stone NRH, Wiesner DL *et al.* Cryptococcus : from environmental saprophyte to global pathogen. *Nat Rev Microbiol* 2016;**14**:106–17.

McGee PA, Markovina A-L, Jeong GCE *et al.* Trichocomaceae in bark survive high temperatures and fire. *FEMS Microbiol Ecol* 2006;**56**:365–71.

McMullan-Fisher SJM, May TW, Robinson RM *et al.* Fungi and fire in Australian ecosystems: a review of current knowledge, management implications and future directions. *Aust J Bot* 2011;**59**:70.

Muñoz-Rojas M, Erickson TE, Martini D *et al.* Soil physicochemical and microbiological indicators of short, medium and long term post-fire recovery in semi-arid ecosystems. *Ecological Indicators* 2016;**63**:14–22.

Nakase T, Suzuki M. Bullera derxii sp. nov. and Bullera pseudoalba sp. nov. isolated from dead leaves of Oryza sativa and Miscanthus sinensis. *Journal of general and applied microbiology* 1986.

Neary DG, Klopatek CC, DeBano LF *et al.* Fire effects on belowground sustainability: a review and synthesis. *Forest ecology and management* 1999;**122**:51–71.

Nguyen HDT, Nickerson NL, Seifert KA. Basidioascus and Geminibasidium: a new lineage of heat-resistant and xerotolerant basidiomycetes. *Mycologia* 2013;**105**:1231–50.

Oksanen J, Blanchet FG, Kindt R *et al.* Package 'vegan.' *Community ecology package, version* 2013;**2**.

Owen SM, Patterson AM, Gehring CA *et al.* Large, high-severity burn patches limit fungal recovery 13 years after wildfire in a ponderosa pine forest. *Soil Biology and Biochemistry* 2019;**139**:1–12.

Pausas JG. Alternative fire-driven vegetation states. *Journal of Vegetation Science* 2015;**26**:4–6.

Peay KG, Garbelotto M, Bruns TD. Spore heat resistance plays an important role in disturbance-mediated assemblage shift of ectomycorrhizal fungi colonizing Pinus muricata seedlings. *Journal of Ecology* 2009;**97**:537–47.

Peiris D, Dunn WB, Brown M *et al.* Metabolite profiles of interacting mycelial fronts differ for pairings of the wood decay basidiomycete fungus, Stereum hirsutum with its competitors Coprinus micaceus and Coprinus disseminatus. *Metabolomics* 2007;**4**:52.

Persiani AM, Maggi O. Species-abundance distribution patterns of soil fungi: contribution to the ecological understanding of their response to experimental fire in Mediterranean maquis (southern Italy). *Mycologia* 2013;**105**:260–76.

Platt WJ. Southeastern pine savannas. *Savannas, Barrens, and Rock Outcrop Plant Communities of North America*. Cambridge, England: Cambridge University Press, 1999, 23–51.

Platt WJ, Ellair DP, Huffman JM *et al.* Pyrogenic fuels produced by savanna trees can engineer humid savannas. *Ecological Monographs* 2016;**86**:352–372.

Ponder F, Tadros M, Loewenstein EF. Microbial properties and litter and soil nutrients after two prescribed fires in developing savannas in an upland Missouri Ozark Forest. *Forest Ecology and Management* 2009;**257**:755–63.

Pressler Y, Moore JC, Cotrufo MF. Belowground community responses to fire: meta-analysis reveals contrasting responses of soil microorganisms and mesofauna. *Oikos* 2019;**128**:309–27.

Quaedvlieg W, Kema GHJ, Groenewald JZ *et al.* Zymoseptoria gen. nov.: a new genus to accommodate Septoria-like species occurring on graminicolous hosts. *Persoonia: Molecular Phylogeny and Evolution of Fungi* 2011;**26**:57.

Raison RJ. Modification of the soil environment by vegetation fires, with particular reference to nitrogen transformations: a review. *Plant and soil* 1979;**51**:73–108.

Reazin C, Morris S, Smith JE *et al.* Fires of differing intensities rapidly select distinct soil fungal communities in a Northwest US ponderosa pine forest ecosystem. *Forest Ecology and Management* 2016;**377**:118–27.

Rossman AY. *The Tubeufiaceae and Similar Loculoascomycetes*. London, UK: CAB International Mycological Institute, 1987.

Santos-Gonzalez JC, Finlay RD, Tehler A. Seasonal Dynamics of Arbuscular Mycorrhizal Fungal Communities in Roots in a Seminatural Grassland. *Applied and Environmental Microbiology* 2007;**73**:5613–23.

Schoennagel T, Balch JK, Brenkert-Smith H *et al.* Adapt to more wildfire in western North American forests as climate changes. *Proc Natl Acad Sci USA* 2017;**114**:4582–90.

Semenova-Nelsen TA, Platt WJ, Patterson TR *et al.* Frequent fire reorganizes fungal communities and slows decomposition across a heterogeneous pine savanna landscape. *New Phytologist* 2019;**224**:916–27.

Sharma GD. Effect of fire on soil microorganisms in a Meghalaya pine forest. *Folia Microbiologica* 1981;**26**:321–7.

Smith JE, Cowan AD, Reazin C *et al.* Rapid response of soil fungal communities to low and high intensity fire. *Geophysical Research Abstracts* 2016;**18**:EPSC2016-8665.

Stendell ER, Horton TR, Bruns TD. Early effects of prescribed fire on the structure of the ectomycorrhizal fungus community in a Sierra Nevada ponderosa pine forest. *Mycological Research* 1999;**103**:1353–1359.

Sterflinger K, De Hoog GS, Haase G. Phylogeny and ecology of meristematic ascomycetes. *Studies in mycology* 1999:5–22.

Štursová M, Kohout P, Rainer Human Z *et al.* Production of Fungal Mycelia in a Temperate Coniferous Forest Shows Distinct Seasonal Patterns. *Journal of Fungi* 2020;**6**:1–14.

Taudière A, Richard F, Carcaillet C. Review on fire effects on ectomycorrhizal symbiosis, an unachieved work for a scalding topic. *Forest Ecology and Management* 2017;**391**:446–57.

Taylor JE, Groenewald JZE, Crous PW. A phylogenetic analysis of Mycosphaerellaceae leaf spot pathogens of Proteaceae. *Mycological Research* 2003;**107**:653–8.

Treseder KK, Mack MC, Cross A. Relationships among fires, fungi, and soil dynamics in Alaskan boreal forests. *Ecological Applications* 2004;**14**:1826–1838.

Villaseñor CR. Isolation and characterization of melanized, slow-growing fungi from semiarid rock surfaces of central Spain and Mallorca. *Dissertationi - Universidad Autónoma de Madrid* 2004.

Wang Q, Garrity GM, Tiedje JM *et al.* Naive Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. *Applied and Environmental Microbiology* 2007;**73**:5261–7.
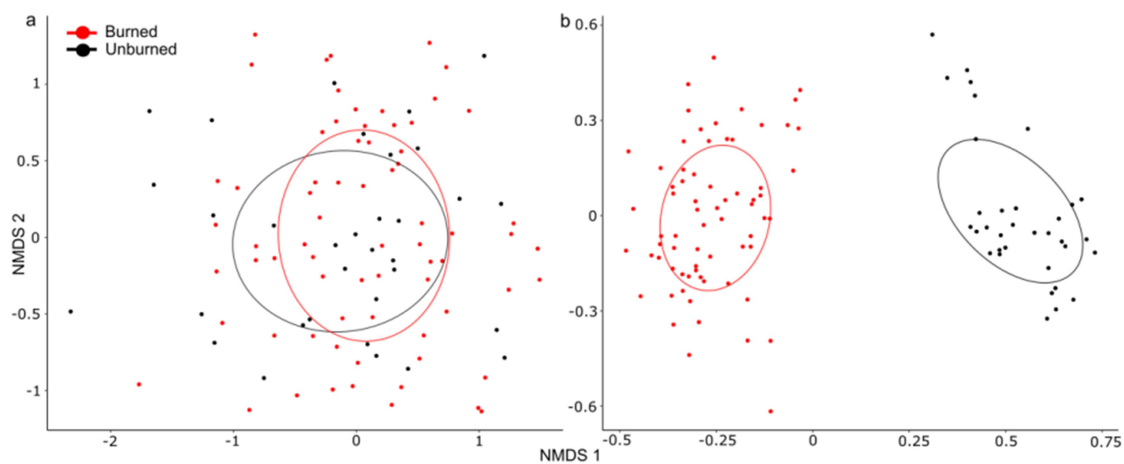
Figure 1: Non-metric multidimensional ordinations for burned and non-burned fungal communities. Ellipses represent the standard deviation of each burn treatment group (black = no burn, red = burned). a) Longleaf pine savanna fungal communities. b) Tallgrass prairie fungal communities.
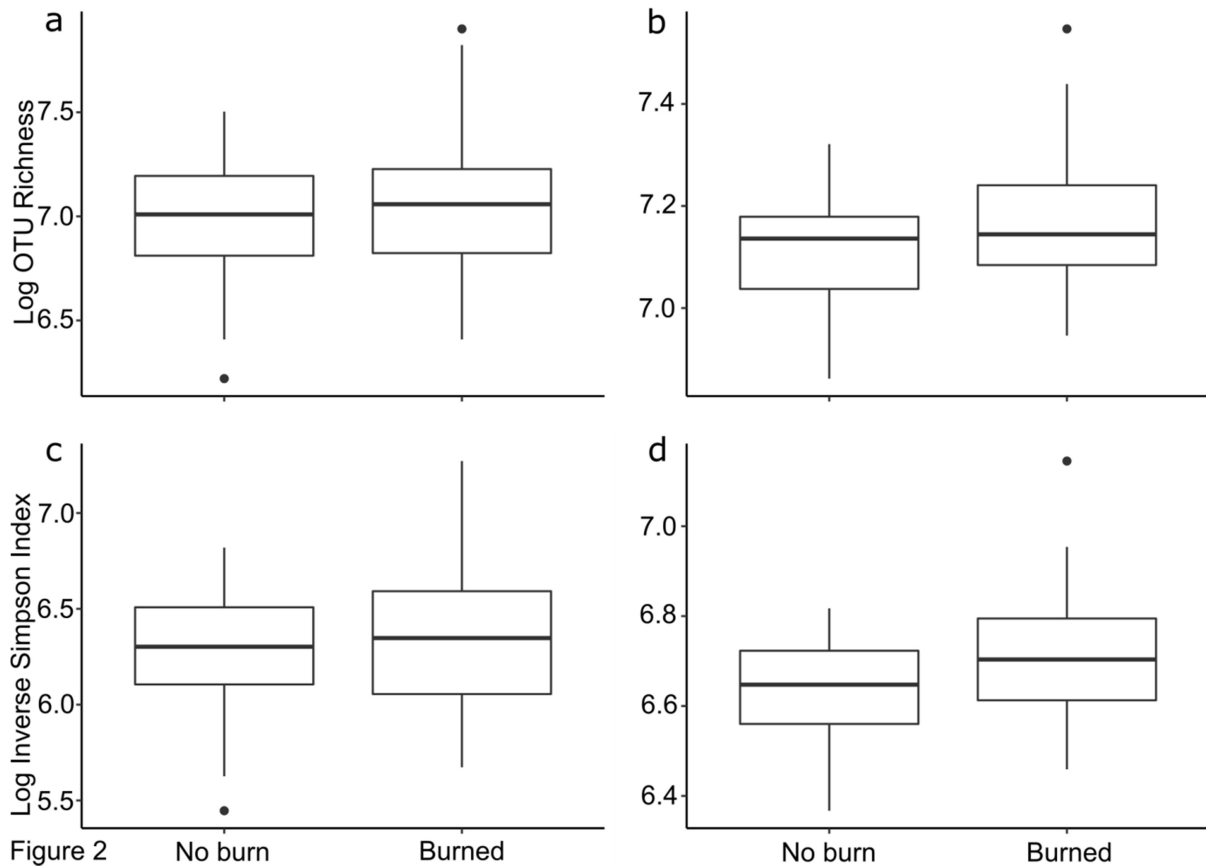
Figure 2: OTU richness and Inverse Simpson Index values for Longleaf pine savanna and tallgrass prairie fungal communities. Box charts display the mean, lower & upper quartiles, and extremes. Outliers are denoted with solid black points. a) OTU richness of Longleaf pine savanna and b) tallgrass prairie fungal communities did not differ between burned and unburned sites. c) Average Inverse Simpson Index value for Longleaf pine savanna fungal communities did not vary significantly between burned and unburned sites, however, d) Inverse Simpson Index values were marginally higher for fungal communities in burned tallgrass prairie sites relative to unburned sites.
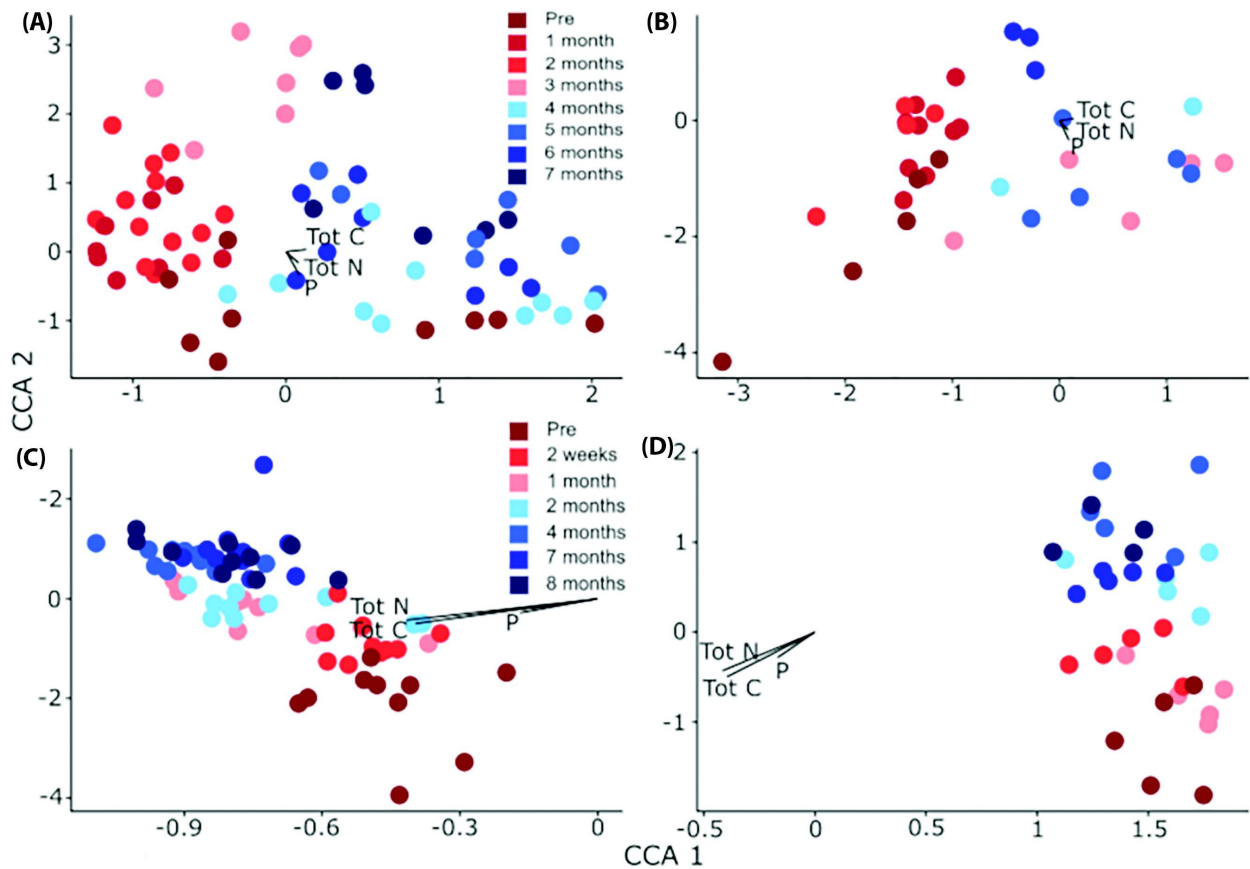
Figure 3: Canonical correspondence analysis ordinations for burned and non-burned fungal community composition across time. Prescribed fires occurred between March and April of 2017, and sampling times reflect time since fire. Total Carbon, total nitrogen, and P were projected onto ordinations using Pearson correlations. In Longleaf pine savanna sites a) burned fungal communities differed between successive sampling times, while c) non-burned fungal communities only showed longer term differences in composition. In tallgrass prairie sites c) burned communities shifted between successive sampling times, where d) non-burned communities differed primarily across longer intervals of time.
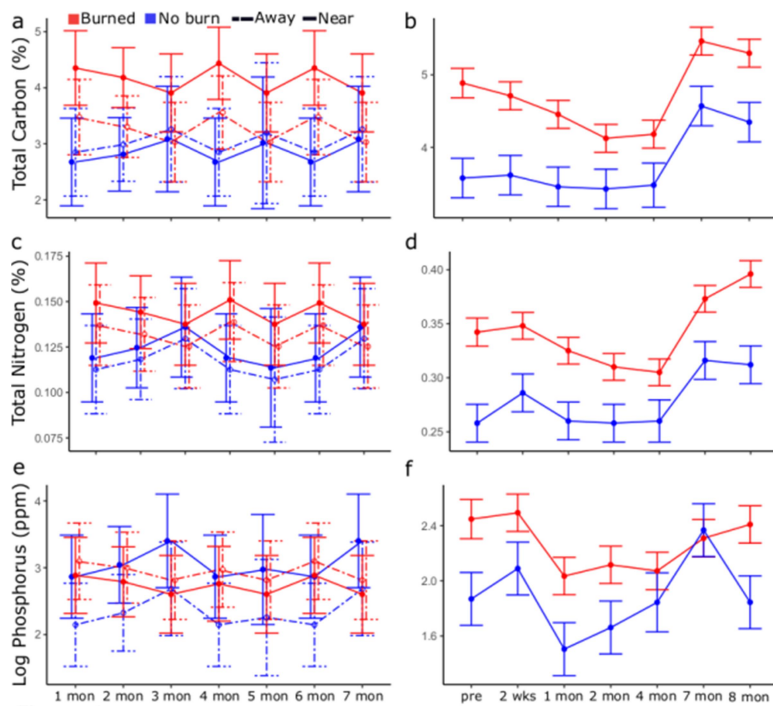
Figure 4: Carbon, nitrogen, and phosphorus levels following prescribed burns. Points represent mean C, N, and P at each sampling time, solid lines and points correspond with near pines sites, dashed lines and open points correspond with away from pines sites. Error bars represent the mean +/- 1 standard error. a) Total soil carbon (%) in Longleaf pine savanna and in b) tallgrass prairie sites. c) Total soil nitrogen (%) in Longleaf pine savanna and d) tallgrass prairie sites. e) Natural log inorganic phosphorus (ppm) in Longleaf pine savanna and f) tallgrass prairie sites.

Table 1: Longleaf pine savanna prescribed fire and sampling time effects on fungal communities PERMANOVA table. All tests used 999 permutations.

Factor	d.f.	Pseudo-F	P-value	% Variance Explained
fire treatment	1	2.1304	0.001***	1.9
time	7	2.0751	0.001***	12.7
pine proximity	1	1.3461	0.078*	1.2
side	1	2.4268	0.001***	2.1
fire x time	6	0.97346	0.61	5.1
fire x pine	1	1.6375	0.013**	1.4
time x pine	7	0.89878	0.895	5.5

$* \leq 0.1$, $** \leq 0.05$, $*** \leq 0.001$

Table 2: Longleaf pine savanna fungal community multivariate homogeneity of groups dispersion table. All contrasts used 999 permutations, and p-values were derived from permutations.

Factor	d.f.	F-value	P-value
fire treatment	1	0.013	0.931
time	7	2.9	0.04**
fire x time	14	2.52	0.34

$* \leq 0.1, ** \leq 0.05, *** \leq 0.001$

Table 3: Fire effects on Longleaf pine savanna fungal community OTU richness and Inverse Simpson Index values.

Response	Term	d.f.1	d.f.2	F-ratio	P-value
	fire treatment	1	19.19	1.325	0.2638
	time	6	2.97	1.891	0.3226
OTU	*pine proximity*	1	19.18	1.9	0.1839
Richness	*fire x time*	6	47.86	1.158	0.3444
	fire x pine	1	19.16	1.634	0.2165
	*time * pine*	6	47.86	0.812	0.566
	*fire * time * pine*	6	47.86	0.935	0.4789
	fire	1	19.2	1.854	0.1891
	time	6	5.58	1.733	0.2685
Inverse	*pine proximity*	1	19.16	2.108	0.1627
Simpson	*fire * time*	6	47.89	1.432	0.2221
Index	*fire * pine*	1	19.12	1.246	0.2782
	*time * pine*	6	47.89	0.798	0.5761
	*fire * time * pine*	6	47.89	0.975	0.4524

*: $p < 0.1$, **: $P < 0.05$

Table 4: Tallgrass prairie prescribed fire and sampling time effects on fungal communities PERMANOVA table. All tests used 999 permutations.

Factor	d.f.	Pseudo-F	P-value	% Variance Explained
fire treatment	1	8.6781	0.001***	14.9
time	6	2.1448	0.001***	7.2
plot	14	3.3192	0.001***	26
fire x time	6	1.47	0.001***	5

* ≤ 0.1, ** ≤ 0.05, *** ≤ 0.001

Table 5: Tallgrass prairie fungal community multivariate homogeneity of groups dispersion table. All contrasts used 999 permutations, and p-values were derived from permutations.

Factor	d.f.	F-value	P-value
fire treatment	1	1.9393	0.208
time	6	0.38675	0.911
fire x time	13	1.2126	0.864

$* \leq 0.1, ** \leq 0.05, *** \leq 0.001$

Table 6: Fire effects on tallgrass prairie fungal community OTU richness and Inverse Simpson Index values.

Response	Term	d.f.1	d.f.2	F-ratio	P-value
OTU Richness	*fire treatment*	1	13	2.412	0.1443
	time	6	63	2.744	0.0263**
	*fire * time*	6	63	1.884	0.1096
Inverse Simpson Index	*fire treatment*	1	13	3.655	0.0781*
	time	6	63	2.706	0.0195**
	*fire * time*	6	63	1.02	0.4189

*: p < 0.1, **: P < 0.05