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Frequent fire reorganizes fungal communities and slows decomposition across a heterogeneous pine savanna landscape

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Summary

• Pyrogenic savannas with a tree–grassland 'matrix' experience frequent fires (i.e. every 1–3 yr). Aboveground responses to frequent fires have been well studied, but responses of fungal litter decomposers, which directly affect fuels, remain poorly known. We hypothesized that each fire reorganizes belowground communities and slows litter decomposition, thereby influencing savanna fuel dynamics.

• In a pine savanna, we established patches near and away from pines that were either burned or unburned in that year. Within patches, we assessed fungal communities and microbial decomposition of newly deposited litter. Soil variables and plant communities were also assessed as proximate drivers of fungal communities.

• Fungal communities, but not soil variables or vegetation, differed substantially between burned and unburned patches. Saprotrophic fungi dominated in unburned patches but decreased in richness and relative abundance after fire. Differences in fungal communities with fire were greater in litter than in soils, but unaffected by pine proximity. Litter decomposed more slowly in burned than in unburned patches.

• Fires drive shifts between fire-adapted and sensitive fungal taxa in pine savannas. Slower fuel decomposition in accordance with saprotroph declines should enhance fuel accumulation and could impact future fire characteristics. Thus, fire reorganization of fungal communities may enhance persistence of these fire-adapted ecosystems.

Introduction

Recurrent fires maintain and structure many terrestrial ecosystems, including prairies, chaparral, savannas, and some coniferous forests. In many of these 'pyrogenic' systems, dominance of flammable grasses in the ground layer promotes the spread of fire (Beckage *et al.*, 2011; Staver *et al.*, 2011; Cardoso *et al.*, 2018), which prevents ecosystem transition to a different state (Beckage *et al.*, 2009; Callaham *et al.*, 2012; Dantas *et al.*, 2016; Pausas & Bond, 2019). Unlike high-profile, destructive wildfires (Chen, 2006; Hood *et al.*, 2018), frequent low-intensity fires are essential for maintaining biotic and abiotic components of 'pyrogenic' biomes (Fill *et al.*, 2015; He & Lamont, 2018) that include some of the most diverse plant communities on Earth (Bond *et al.*, 2005; Noss *et al.*, 2015; Peet *et al.*, 2018). Frequent prescribed burning is currently used to mimic natural fire regimes and help conserve many pyrogenic ecosystems and their biodiversity (Peet *et al.*, 2018).

Recurrent fires create characteristic landscapes in some pyrogenic systems. Pine savannas of the North American Coastal Plain are a key example characterized by discontinuous tree cover within a continuous ground-layer vegetation. This pine–grassland 'matrix' supports a diverse, highly endemic flora and fauna (Platt, 1999; Noss *et al.*, 2015; Peet *et al.*, 2018) and structures the spatial distribution of fuels, soil properties, and plants that, in turn, influence fire characteristics (Ellair & Platt, 2013; Platt *et al.*, 2015, 2016; Varner *et al.*, 2015; Veldman *et al.*, 2015). For example, fuel loads from overstory pines (*Pinus palustris*) directly influence fire intensity (Platt *et al.*, 2016), and dominance of pines defines the chemical composition of litter, soil pH and nutrient concentrations, loads of photosynthetic carbon (C), and vegetation demand for water and nutrients (Priha *et al.*, 1999; Osono *et al.*, 2014; Deng *et al.*, 2015; Stoppe *et al.*, 2016). Fire, the pine–grassland matrix, and associated variability in edaphic factors are all anticipated to affect microbial communities below ground (Prober *et al.*, 2015). Little research, however, has explored these effects in pyrogenic systems like pine savannas.

Fungi are a foundational component of terrestrial ecosystems, including in pine savannas. Saprotrophic fungi drive litter decomposition (Rayner & Boddy, 1988), and mycorrhizal taxa affect the diversity and productivity of plant communities (van der Heijden *et al.*, 1998). Fungi tend to be more susceptible to fire than bacteria are (Dooley & Treseder, 2012), but their

responses to fire vary among taxa and ecological guilds (Dumontet *et al.*, 1996; Mabuhay *et al.*, 2003). Most fire–fungus studies have assessed the effects of high-intensity wildfires, which cause large declines in fungal biomass and result in strong compositional shifts in fungal communities (Treseder *et al.*, 2004; Dooley & Treseder, 2012; Holden *et al.*, 2013; Prendergast-Miller *et al.*, 2017). Although fire responses may vary among biomes, fungal community shifts in managed pyrogenic ecosystems may differ from wildfire effects because prescribed fires often have very different characteristics (e.g. frequent, low intensity).

Fungi in pyrogenic ecosystems may be adapted to frequent fires. Some fungi produce heat- and smoke-activated spores (Carpenter & Trappe, 1985), whereas others may benefit from postfire ash deposits (Hart *et al.*, 2005; Dean *et al.*, 2015), altered soil hydrophobicity (Certini, 2005), or reduced competition from other species (Wicklow & Hirschfield, 1979). Recurrent fires consume less fuel and produce less heat, which does not penetrate into soil as deeply as during high-intensity fires (Smith *et al.*, 2016). Accordingly, fungal community shifts in pyrogenic ecosystems may be 'relatively modest' (Johnson & Miyanishi, 1995; Choromanska & DeLuca, 2001; Korb *et al.*, 2004) and primarily be driven by indirect fire-induced changes in soil properties (e.g. pH shifts) and plant communities (e.g. Hart *et al.*, 2005; Ponder *et al.*, 2009; Oliver *et al.*, 2015). The pine–grassland matrix created by recurrent fires (*sensu* Platt *et al.*, 2016) leads to spatial variation in fuel loads, plant communities, and substrates, all of which could structure fungal communities and determine their responses to fire. We propose that prescribed fires select for fire-adapted fungal species, with fungal community responses to fire depending on location within the pine–grassland matrix.

Frequent fires that alter fungal communities may modify ecosystem functions that, in turn, influence future fires. Fire intensity in pine savannas is directly related to accumulation of fine fuels produced by trees – shed pine needles, cones, and bark (Platt *et al.*, 2016; Peet *et al.*, 2018). These fine fuels are decomposed primarily by microbial saprotrophs, so fire-induced changes to functioning of these organisms should influence decomposition of new litter. Proposed fire effects on litter decomposition include those mediated by altered soil parameters (pH, changes in nitrogen (N) concentration, or soil moisture) or plant composition (Allison *et al.*, 2013; Ficken & Wright, 2017), whereas the contribution of fungi that play a major role in decomposition (Kjøller & Struwe, 1992) has received lesser attention. Well-known postfire increases in ascomycetes (Cairney & Bastias, 2007), many of which are saprotrophic, might temporarily increase fuel decomposition and reduce available fuels for future fires. Alternatively, fire suppression of basidiomycetes (Cairney & Bastias, 2007), many of which are also important saprotrophs, might promote fuel accumulation and potentially increase spread of future fires. The net effects of these fire responses are important for predicting microbial feedbacks to fuels in fire-frequented ecosystems.

In this study, we assessed the interactive effects of fire and the pine–grassland matrix on fungal community composition and microbial decomposition of litter in a pine savanna ecosystem. We characterized fungal communities in replicated patches that were burned or unburned in the most recent fire (mid-June 2014), near and away from overstory pines. We sampled litter and the uppermost soil layer and then characterized fungal communities by amplicon sequencing of the internal transcribed spacer 2 (ITS2) region (Illumina MiSeq; Illumina, San Diego, CA, USA). We collected additional data on soil properties and plant community composition to relate variation in fungal community structure to other ecosystem components. In the same plots, litter decomposition rates were assessed over a 9-month period. We hypothesized that:

(**1**) Fire would reorganize fungal communities, but effects would differ depending on substrate and pine proximity. Locations near or away from pines and in litter or soil substrates may result in compositionally different fungal communities that, in turn, are differentially reorganized by fire. For example, fire intensity increases with greater density of pine needles (Ellair & Platt, 2013; Platt *et al.*, 2016), so fungal communities in the vicinity of pines may be altered by fire more than communities away from pines are.

(**2**) Postfire shifts in fungal communities should be tied to fire-induced shifts in soil properties and vegetation, as suggested by prior studies in similar systems (Hart *et al.*, 2005; Ponder *et al.*, 2009; Oliver *et al.*, 2015). For example, fungal taxa associated with flammable plants favoured by fire (*sensu* Gagnon *et al.*, 2010) might respond positively to fire, whereas fungi associated with less-resistant plant hosts may decline (*sensu* Platt *et al.*, 2016). Fungal taxa adapted to alkaline or high-nutrient conditions might also be favoured by postfire shifts in soil properties, such as increased soil pH, N, phosphorus (P), and C associated with low-intensity fires (Raison & McGarity, 1980; Certini, 2005; Ponder *et al.*, 2009).

(**3**) Fine-fuel decomposition should be governed by both fire and proximity to pine. Pine needles should decompose slower than grass litter, leading to reduced decomposition below pines (Platt *et al.*, 2016). Fire effects on decomposition, however, might be tied to fungal taxa that fire enhances or suppresses. Depending on which group of fungi – fire sensitive or fire adapted – contain more efficient saprotrophs, we anticipate corresponding effects on litter decomposition.

Materials and Methods

Study site

We conducted our study in an old-growth longleaf pine savanna at the Wade Tract, Thomas County, GA, USA (30°45′N, 84°00′W). This 80 ha site, on moderately dissected terrain 25–50 m above sea level, is characterized by a growing season of 10–11 months and average precipitation of *c.* 1350 mm. Surficial soils are Pliocene-aged, acidic, fine-textured sands with A horizons 50–100 cm deep over a clay hardpan (Typic and Arenic Kandiudults; Carr *et al.*, 2009; Levi *et al.*, 2010). From the early 1800s, the site was annually–biennially burned with low-intensity fires (Platt *et al.*, 1988), resulting in an open savanna landscape with discontinuous canopy of pines (*P. palustris*) and shrub

seedlings (*Quercus* spp.), and continuous ground-layer vegetation dominated by warm-season grasses (Platt *et al.*, 1988; Gilliam & Platt, 1999). Managed by Tall Timbers Research Station since 1978, the site has been burned annually–biennially using prescribed fires conducted between April and August, using drip torches, 1–2 wk after rain at relative humidity of 50–60% and winds 10–20 km h^{-1}. Flame heights commonly approach 1–2 m and result in 60–90% removal of accumulated fuels.

Study plots

Study plots were established in mid-June 2014, right after prescribed fires. Thirty patches unburned in 2014, at least 5 m^2 in area, were randomly selected in upland pine savanna, such that 15 were near (< 5 m) and 15 away (> 10 m) from overstory pines. Given the ubiquity of fire in this system, even 'unburned' patches had burned in the last 1–3 yr. Next, similar-sized patches that burned in 2014 were selected as randomly as possible within 5–10 m of the unburned patches. This resulted in 30 pairs of burned and unburned patches located next to each other, and arrayed across upland regions of the easement (Fig. 1). The paired design reduced variation in overstory conditions, plant community composition, and soil properties to isolate fire effects on fungal community composition. Although pairs had similar environmental conditions, across patches there was a wide range of plant communities and soil properties. All 60 patches were selected such that they did not contain large amounts of woody debris, such as fallen trees or large branches. A sampling plot (1 × 1 m^2) was randomly located within each of the 60 patches, at least 1 m from the patch border.

Plant community composition

We used quadrats to assess plant community composition within each 1 m^2 plot in mid-July 2014 as a potential driver of fungal community composition and decomposition. Nomenclature followed Weakley (2015). Voucher specimens of each plant species included in the study were collected outside plots and deposited in the herbarium at Tall Timbers Research Station. Specimens are in the Florida State University online herbarium (https://herbarium.bio.fsu.edu).

Soil chemical analyses

For each plot, soil chemical and physical properties were assessed as potential drivers of fungal community composition and decomposition. Soils within plots were subsampled from those used to assess fungal communities (see next section 'Sampling of fungal communities'). Chemical and physical analyses were conducted at the Kansas State University Soil Testing Laboratory, unless otherwise noted. P content was measured using the Mehlich-3 method on a Lachat Quickchem 8000 (Lachet Instruments, Loveland, CO, USA). Total C and N were analysed on a Leco TruSpec CN Carbon/Nitrogen combustion analyser (Leco Corporation, St Joseph, MO, USA). Particle size (sand, silt, clay) was estimated by a modified hydrometer method (Bouyoucos, 1962). Soil pH was measured using 1 : 2 (v/v) soil : double-distilled H_2O (ddH_2O) solution using a pH meter (Mettler Toledo, Columbus, OH, USA). Gravimetric soil moisture was measured as weight loss after drying a 10 g soil subsample at 60°C for 48 h; soil organic content was then measured by sample ignition at 550°C for 1 h.

Sampling of fungal communities

Litter and the uppermost soil layer inside each plot were sampled in mid-July 2014 (14 July 2014). Samples were carefully collected from three randomly selected 9 × 9 cm^2 areas in each plot. First, litter was collected manually from the soil surface, ensuring that no recently fallen material (postfire) was collected. In burned plots, litter was often charred, but was not entirely consumed in the most recent fire; in unburned plots, litter had been present for some time, typically since the previous fall. After litter was collected, surficial soils to a depth of 1.5 cm were collected. The litter and soil collected from each plot were pooled in separate plastic bags ($n = 120$). To avoid cross-contamination, all sampling equipment was sterilized with 10% bleach and 90% isopropyl alcohol between plots. Collected soil and litter samples were kept in a cooler with freezer packs in the field, frozen at −20°C within 4 h, and then shipped overnight to the University of Kansas, where they were kept at −80°C until further analysed. Before downstream applications, soils and litter were thawed and thoroughly homogenized within sealed collection bags. A 100 g subsample was taken for soil chemical analyses and a *c.* 2 g subsample of soil and litter material was used for further molecular work.

DNA extraction and PCR amplification

A DNA metabarcoding approach was used to assess fungal community composition. DNA extraction of soil and litter samples was carried out using the MoBio PowerSoil Kit (MoBio, Carlsbad, CA, USA). Litter samples were homogenized before DNA extraction: 2 g of litter was frozen in liquid N and turned into fine powder in a sterile porcelain mortar. DNA extraction was carried out according to the manufacturer's protocol, using 0.25 g of material for both soils and litter. The forward primer fITS7 (Ihrmark *et al.*, 2012) and the reverse primer ITS4 (White *et al.*, 1990) were used to amplify the ITS2 region of the ribosome encoding operon. This region is a universal barcode for fungi (Schoch *et al.*, 2012) and is particularly suited for short paired-end Illumina MiSeq sequencing (Oliver *et al.*, 2015). Two replicated PCR reactions were done for each sample. Template DNA (5 ng) was used for the two-step PCR process, as recommended by Berry *et al.* (2011). The PCR mix contained 5 μg μl^{-1} DNA, 5 μl 5× Q5 buffer (New England Biosystems, Ipswich, MA, USA), 0.625 μl dNTPs (10 mM), 1.25 μl each of forward and reverse primers (10 μM), 0.125 μl Q5 proof-reading polymerase (New England Biosystems), and ddH_2O to adjust the reaction volume to 25 μl. The reaction scheme was one cycle at 98°C for 30 s, 25 cycles of 98°C for 10 s, 57°C for 30 s, and 72°C for 30 s, followed by one cycle at 72°C for 2 min and 10 min hold at 4°C. PCR products were checked on agarose gels to ensure successful amplification and were cleaned using

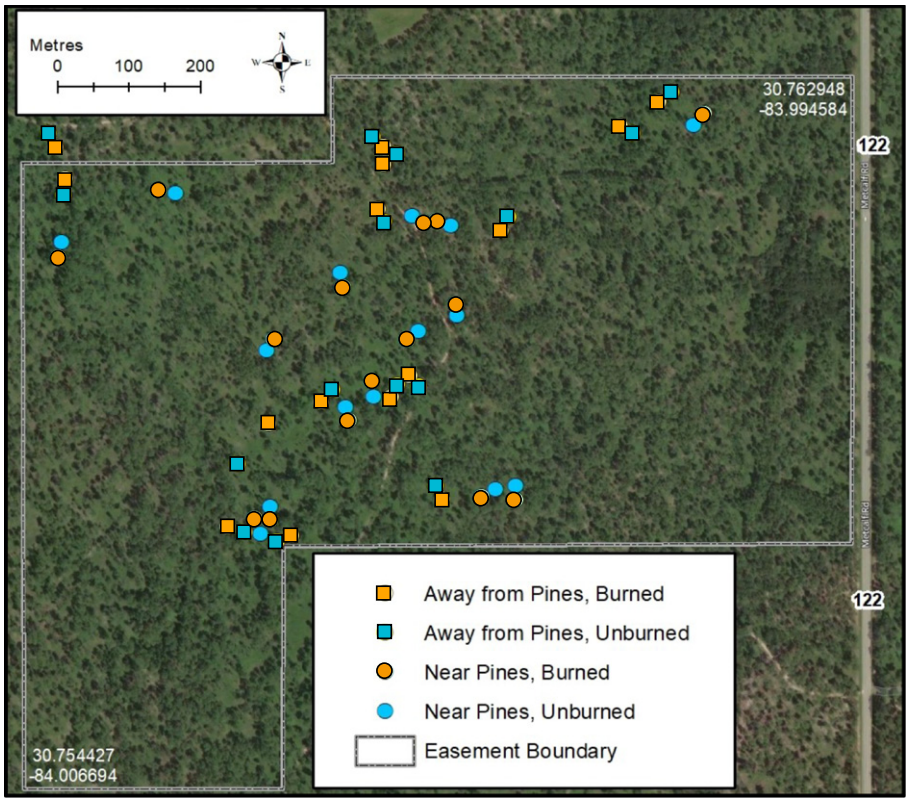

Fig. 1 Location of the experimental plots on the Wade Tract easement. The 60 plots represented four treatment combinations: away from pines (squares) and near pines (circles), unburned (blue), or burned (orange) in 2014.

Agencourt AMPure XP magnetic beads (Beckman Coulter, Indianapolis, IN, USA).

Library preparation and sequencing

DNA libraries from fungal communities were created using a Nextera protocol, and then sequenced using Illumina MiSeq. In a secondary PCR reaction, unique 12 bp sequence barcodes (Nextera indices; Illumina) were ligated to each sample. The PCR was run under similar conditions, except 5 µl of the primary PCR amplicon was used instead of the original DNA template, and number of cycles was set to eight. Secondary PCR amplicons were purified with Agencourt AMPure XP magnetic beads, and DNA concentrations were assessed by Qubit 2.0 (LifeTechnologies, Carlsbad, CA, USA). Samples were pooled in equimolar concentration to a single library. Fungal sequences were generated using an Illumina MiSeq (Illumina) at the Kansas State Integrated Genomics Center. The entire bioproject, including raw sequencing data (fastq files), is available in the National Center for Biotechnology Information Sequence Read Archive, accession no. PRJNA350328.

Bioinformatics

Sequencing data were analysed following Caporaso *et al.* (2010) using QIIME v.1.9.0, the complete bioinformatics pipeline is available upon request. The sequencing was paired end reads. Quality and barcode filtering resulted in 6322 437 reads with an average Phred score ≥ 30 and median length of 268 bp. Open-reference operational taxonomic unit (OTU) picking using Usearch 6.1 and the UNITE fungal ITS reference database v.7.2 (Nilsson *et al.*, 2018) was used to cluster OTUs at 97% similarity. All chimeric sequences and OTUs with fewer than five reads were removed to eliminate potential PCR/sequencing artefacts, as recommended by Lindahl *et al.* (2013). Data were then subsampled to 10 000 high-quality sequences per sample; six samples were removed due to low read number. Taxonomic identification used the Ribosomal Database Project Classifier 2.2 (Wang *et al.*, 2007) matched to the UNITE database. Using taxonomic identities, fungal ecological groups (animal pathogenic, arbuscular mycorrhizal, ectomycorrhizal, lichenicolous, lichenized, mycoparasitic, plant pathogenic, saprotrophic – all probable or highly probable) were identified using FUNGuild (Nguyen *et al.*, 2015).

Assessment of microbial decomposition rate

Fresh litter was used to assess microbial decomposition in plots. Approx. 5 kg of recently deposited and intact dead plant material was collected outside plots in unburned patches in mid-June 2014. This new litter included pine needles, oak leaves, and grass culms produced in the previous year; partially decomposed litter on the ground surface was not included. New litter collected from unburned patches both near and away from pines was kept separate. All collected new litter was shipped to the University of Kansas, where it was kept at −20°C for < 2 wk until it was processed. In Kansas, field-collected litter was thawed, dried at 60°C for 2 d, and

then ground using a Wiley Mill #4 (Thomas Scientific, Swedesboro, NJ, USA) with a 6 mm opening. Grinding increased the surface area, mimicking size transformations normally produced by macrofauna and invertebrates excluded from our litter bags (by using 30 μm nylon mesh, see below). New, ground litter, separated for sources near or away from pines, was placed in plastic containers, shipped to the Radiation Science & Engineering Center at Pennsylvania State University, where the litter was sterilized via gamma-irradiation to *c.* 32 kGy, and then returned to the University of Kansas. To assess decomposition, *c.* 5 g of sterilized new litter and a sterilized, internal identification tag were placed in 14 × 14 cm^2 mesh bags created out of 30 μm nylon mesh (Amazon LLC, Seattle, WA, USA). This mesh size allowed entry only of microbes, including fungi, bacteria, and some protists (Bradford *et al.*, 2002). Bags were weighed before and after litter addition to measure the exact initial mass of the sterilized litter. Mesh bags were then heat-sealed, placed in sterile plastic bags, and frozen at −80°C until deployment. All litter work was done in a sterile hood. In mid-July of 2014, triplicate bags were placed within the experimental plots. Litter bags were placed in plots that matched their origin, to avoid home-field advantage (Chomel *et al.*, 2015) on the ground surface among vegetation, and anchored with 5 cm sod-staples, so that one side contacted existing litter and any exposed soil. Over time, litter deposited naturally often was present on top of the bags (especially after 9 months), but did not form a continuous mat over the bags. Bags were collected 3, 6 and 9 months after fire. Upon collection, bags were placed into separate sterile plastic bags and shipped overnight to Kansas. Contents were dried at 60°C for 3 d and reweighed to determine percentage mass loss during incubation in the field.

Statistical analyses

Variation in plant community composition and soil variables caused by spatial location, proximity to pines and fire was analysed by PERMANOVA, using the PRIMER 7+ software (Anderson *et al.*, 2008). Variability in the data cloud was assessed according to sets of predictor variables: plot coordinates were set as 'Geo factors', soil variables were set as 'environmental (Env) factors'. Environmental data were transformed ($\log_e(X+1)$) as recommended by software to obtain a resemblance matrix. For species data, the Bray–Curtis similarity index was used. Distribution in predictor variables was checked before running the analysis, to make sure they were not highly collinear or redundant. Plot coordinates were included in the model as first factor, as we expected plot location to impact soil variables and species diversity, and not vice versa. The best possible rank-order for other factors was suggested by software to maximize Spearman rank correlation. The analysis provided pseudo-*F*-statistics, percentage variation explained by factors in the plant community composition, and a *P*-value for statistical significance. We also quantified the effect of variation in soil variables on plant community composition (presence–absence data). Similarity in plant communities were calculated as Bray–Curtis indexes (presence–absence) and visualized using nonmetric multidimensional scaling (NMDS) with 500 iterations in PC-ORD (McCune & Mefford, 2011). Soil variables (C, P, N, pH, soil moisture, etc.) were assessed as predictors of plant community compositions, using Pearson's correlation analysis, in PC-ORD. Indicator species analysis (Dufrêne & Legendre, 1997) was then used to identify plant taxa indicative of burned or unburned conditions, in PC-ORD.

Variations in fungal communities were assessed using PERMANOVA, but also in relation to plant community data in addition to soil variables. Within the PERMANOVA, we assessed the effects of soil parameters (Env factor as above) and plant community structure (Species data factor) on fungal community composition (OTU abundance by plots). As with plants, we then visualized differences in fungal community structure using NMDS in PC-ORD and carried out indicator species analysis to reveal fungal indicators of burned and unburned communities. Pearson correlation tests included individual plant species and soil variables to reveal potential correlations with fungal community structure.

Differences in microbial decomposition at three time points (3, 6, and 9 months) were assessed, in response to fire and pine proximity. Variation in decomposition based on these factors was calculated using a generalized linear model, which tested for differences in proportional mass loss of litter between burned and unburned plots, near and away from pines. For all analyses, R code is available upon request. In addition, decomposition data were included in ordination analyses (NMDS see earlier) to assess whether plant or fungal community structure or soil variables were correlated with decomposition rates.

To understand how individual factors differed with fire, we used paired *t*-tests (burned and unburned) for means of individual soil variables, richness and abundance in fungal ecological guilds, and taxonomic genera using R (R Core Team, 2013). We assessed fire effect on richness and abundance in 40 fungal genera that either had richness of > 20 OTUs, or abundance of > 0.5% of fungal reads obtained. Fungal taxa and ecological groups located near or away from pines in litter or soil (resulting in four community types) were analysed separately to account for initial variation in community structure that could impact revealed responses to fire. Among the ecological groups, arbuscular mycorrhizal, lichenicolous, and lichenized fungi were omitted from the analysis due to low number of OTUs. Fire effect (i.e. percentage increase or decline in variable) was calculated for soil parameters, and both richness and abundance of fungal genera/or ecological guilds, along with corresponding *P*-values and *t*-statistics. To account for multiple tests, the Šidák correction (Šidák, 1967) was used to assess statistical significance of differences in these variables between burned and unburned plots.

To visualize taxonomic/ecological ranking of the fungal communities among litter and soil substrates, pie charts were generated to show relative richness and abundance of taxa/or ecological guilds.

Results

Effects of fire and pine proximity on soil properties and plant communities

Soil properties varied widely across plots and were only weakly related to fire or pine proximity, as revealed by PERMANOVA.

Table 1 Comparison of soil variables between burned and unburned plot as revealed by two-sample pairwise *t*-test.

	Statistics	
Soil variable	t_{df}	*P*-value
Phosphorus	$t_{14} = -0.383$	0.707
Nitrogen (N)	$t_{14} = 0.997$	0.336
Carbon (C)	$t_{14} = -0.095$	0.926
C : N ratio	$t_{14} = -2.19$	0.046
Sand	$t_{14} = -0.22$	0.827
Silt	$t_{14} = 0.56$	0.581
Clay	$t_{14} = -1.0$	0.334
Moisture (gravimetric water content)	$t_{14} = 1.23$	0.238
Organic matter	$t_{14} = 0.096$	0.925
pH	$t_{14} = 0.309$	0.770

None of the variables was significantly different ($P < 0.0051$; Šidák correction for multiple tests).

Spatial location of the plot was the strongest predictor of soil properties, explaining *c.* 19% of the variation (Supporting Information Table S1). Proximity to pines also had a significant effect on soil properties ($P < 0.001$) but explained less variation (*c.* 4.5-%) than spatial location. Soil properties were not different between burned and unburned plots (Table 1).

Across the experimental plots, we identified 178 plant species belonging to 41 taxonomic families (Table S2). These species represented about one-third of the total number of vascular plant species recorded in the flora of the Wade Tract (W.J. Platt, unpublished data). Plant community composition varied widely across the experimental plots but was more related to soil properties than to either fire or pine proximity. As indicated in Table S3, plant community composition was largely explained by soil properties (PERMANOVA: 22% of the variation), especially soil P (8% of the variation), and spatial location (10% of the variation). Pine proximity and fire had lesser effects on plant community composition; each explained *c.* 3% of plant compositional variation (Table S3). Plant communities in burned and unburned plots were not strongly differentiated by NMDS ordination (Fig. S1). Indicator species analysis revealed four herbaceous plants associated with fire: *Euphorbia discoidalis* ($P = 0.005$), *Mimosa quadrivalvis* ($P = 0.01$), *Symphotrichum adnatum* ($P = 0.018$), and *Asclepias verticillata* ($P = 0.027$). Three herbaceous plant species were indicators of unburned communities: *Ageratina aromatica* ($P = 0.038$), *Andropogon gerardi* ($P = 0.047$), and *Saccharum alopecurioides* ($P = 0.049$).

The effects of pine proximity and fire on fungal community structure

A taxonomic description of fungal communities of litter and soil is presented in Figs S2–S4 and Table S4. The measured predictors of fungal community composition explained a major fraction (*c.* 66%) of the observed variation in fungal community structure. Contrary to our expectation, there was no strong effect of plot spatial location on fungal communities (Table 2). Plant community composition was the strongest predictor of fungal community structure, explaining about one-third of variation

Table 2 Factors affecting community structure of savanna fungi.

Variable	Pseudo-*F* statistics	*P*-value	Variation explained in fungal community composition (%)
Plot spatial location	1.24	0.08	
Fire	7.5	**0.0001**	6.69.3, litter fungi, $P < 0.00013.9$, soil fungi, $P = 0.0002$
Substrate	15.5	**0.0001**	11.9
Pine proximity	2.25	**0.0001**	1.7
Soil variables (overall)	1.5	**0.0001**	8.8
Phosphorus		**0.0027**	1.8
Nitrogen (N)		**0.0014**	1.9
Carbon (C)		**0.0019**	1.9
C : N ratio		0.247	
Particle size		0.163	
Moisture		0.344	
Organic matter		0.495	
pH		0.724	
Overall plant community composition	1.3	**0.0001**	34.5
Total variation explained			65.8

Statistically significant factors are shown in bold alongside the percentage of variation they explained in fungal community composition.

(Table 2). Other strong predictors included substrate (i.e. litter vs soil; *c.* 12%), followed by soil parameters (*c.* 9%) and fire (*c.* 7%). When fire effect was assessed for litter and soil communities separately, the effect on litter fungi (*c.* 9%) was double that of soil fungi (*c.* 4%). The effect of pine proximity on fungal community structure, although significant ($P < 0.001$), was relatively weak (*c.* 2%).

Although plant community composition and soil variables best explained overall fungal community composition (Table 2), no individual plant species or soil variable was a strong predictor (Fig. 2). Of the plant species recorded in experimental plots and soil variables measured in our study, none correlated strongly with fungal community composition ($r^2 > 0.25$). Weaker correlations ($r^2 > 0.15$) were found with three plant species: *Galium hispidulum*, *E. discoidalis*, and *M. quadrivalvis* (Fig. 2).

Fire had a significant effect on fungal communities, in contrast to weak fire effects on plant communities and soil variables. Unburned and burned fungal assemblages were distinctly clustered in the NMDS ordination (Fig. 2), as were soil and litter communities.

Decomposition data

Litter bags were often colonized by fungal mycelia within 3 months of placement in the field (personal observations). In unburned patches, fungi were often noted as having colonized litter bags, and mycelia often covered the bag undersides. The amount of decomposed litter did not change with pine proximity, as revealed in generalized linear model analyses ($P = 0.292$). Mass loss was, however, significantly greater in unburned plots (all $P < 0.0001$ for each sampling time), as indicated in Fig. 3. The difference in litter mass lost between burned and unburned

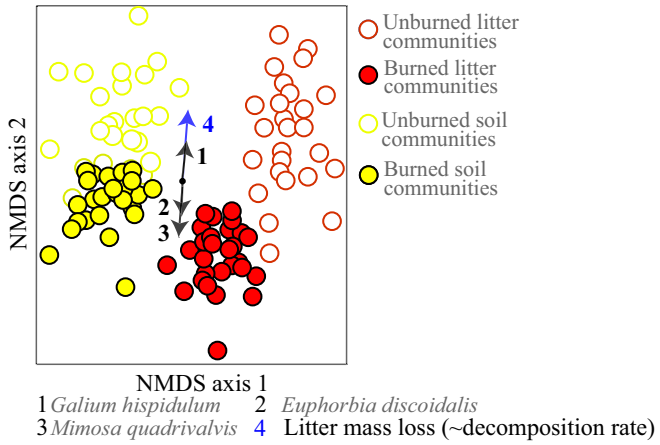

Fig. 2 Nonmetric multidimensional scaling (NMDS) ordination of fungal communities across plots and substrate types based on Bray–Curtis distances. The best ordination solution was two-dimensional, with final stress of 17.82. Litter communities are indicated with red circles and soil communities with yellow circles. Communities in burned plots are closed symbols, and those in unburned plots are open symbols. Arrows indicate significant Pearson's correlations with Axis2, which strongly reflects fungal community differences between burned and unburned plots (see the Results section). Arrows 1–3 reflect community correlations with individual plant species, whereas arrow 4 is the correlation with microbial decomposition.

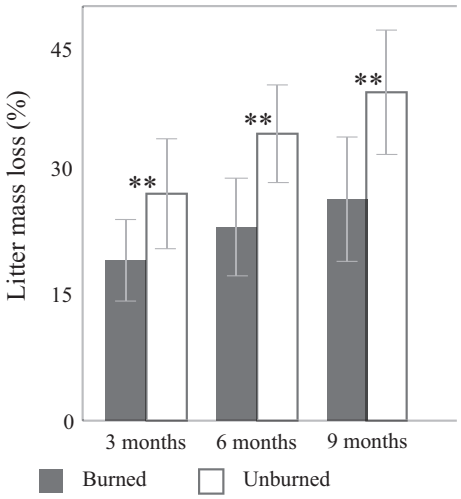

Fig. 3 Percentage litter mass loss (mean ± SD) in decomposition bags at 3, 6, and 9 months placed in either recently burned (closed bars) or unburned (open bars) experimental plots. Asterisks indicate significant differences (all $P < 0.0001$) in decomposition rates observed between the unburned and burned fungal communities for a given time period.

bags was *c.* 6.5% at 3 months (20.6 ± 6.7% vs 27.1 ± 9.9%), and this difference increased slightly over time: 6 months, *c.* 11% (23.4 ± 7.7% vs 34.2 ± 8.1%); 9 months, *c.* 13% (26.5 ± 9.9% vs 39.3.6 ± 9.7%). Moreover, the amount of mass lost to microbial decomposition was strongly correlated with fungal community structure ($R = 0.496$), as depicted in the NMDS plot (vector in Fig. 2). Mass lost was only weakly correlated with total N ($R = 0.267$), total C ($R = 0.269$), and organic matter ($R = 0.196$). However, none of these soil variables was strongly affected by fire, and, therefore, unlikely could explain the differences in decomposition rates between burned and unburned plots.

Responses of fungal ecological groups to fire

We analysed ecological group responses to fire independently for communities of litter and soil, located near and away from pines. Saprotrophic fungi were the most sensitive to fire and declined in richness (18–34%) in all litter and near pine soil communities. A decline in abundance (49%) of saprotrophs was also observed in litter away from pine (Table 3). Within saprotrophic fungi, the relative abundance of ascomycetes in litter increased with fire from *c.* 65% to 88% ($t_{26} = 4.7$; $P < 0.001$), whereas the relative proportion of basidiomycetes declined from *c.* 34% to 12% ($t_{26} = 4.7$; $P < 0.001$). Ectomycorrhizal fungi were negatively affected by fire, particularly near pine communities, but these differences were not statistically significant after correction for multiple comparisons. Animal pathogenic fungi declined in richness and relative abundance in response to fire (Table 3). In contrast to these three groups, mycoparasites had higher abundance (by 500–1200%) in burned litter communities near pine.

Fungal taxonomic responses to fire

Fire caused major declines in richness and abundance in many fungal genera, but some genera thrived following recent fire. As with ecological groups, we first divided samples into four community types (pine proximity, substrate) to account for clear community differences. The strongest positive response to fire was observed for the genus *Periconia*, which increased 2–10 times in richness and abundance in burned litter communities (Table S4). In some community types, increases in the richness or abundance following recent fire was observed for the plant pathogenic genus *Teratosphaeria*, and ectomycorrhizal *Cenococcum*. By contrast, strong declines were observed for saprotrophic genera, including *Acremonium*, *Mycena*, *Trichoderma*, and *Phialophora*.

Indicator species analysis revealed many different fungal OTUs clearly associated with burned or unburned communities. Overall, 1004 OTUs were identified as indicators of unburned communities and 566 OTUs were indicators of recently burned communities (see Table S5 for details).

Discussion

Fire reorganized fungal communities and slowed litter decomposition by fungi and other microbes. Fungal assemblages of burned plots were dominated by postfire specialist fungi but lacked key fungal saprotrophs compared with unburned plots. Fire-sensitive fungi, including key decomposers, likely replace fire-adapted species over time and restore decomposition to pre-burn levels. Based on our comparison of recently burned and unburned plots, we expect that significant postfire reorganization (*sensu* Schmidt *et al.*, 2007) of fungal communities occurs within 1–2 yr after fire in pine savannas. By contrast, wildfire studies indicate that transitions of fungal communities from 'burned' to 'unburned' states can take more than a decade (Dooley & Treseder, 2012; Holden

Table 3 Differences in richness and relative abundance of fungal ecological groups between paired burned and unburned samples.

	Abundance differences			Richness differences		
Functional group/community location	Effect	Statistics		Effect	Statistics	
Saprotrophic fungi						
Litter away from pine	↓49.4%	**$P = 0.0007$**	$t_{12} = 4.1$	↓34.0%	**$P = 0.0003$**	$t_{12} = 4.5$
Litter near pine		$P = 0.022$	$t_{13} = 2.2$	↓34.0%	**$P = 3\times10^{-5}$**	$t_{13} = 5.8$
Soil away from pine		$P = 0.047$	$t_{12} = 2.2$		$P = 0.007$	$t_{12} = 2.8$
Soil near pine		$P = 0.017$	$t_{13} = 2.4$	↓18.4%	**$P = 5\times10^{-5}$**	$t_{13} = 5.6$
Ectomycorrhizal fungi						
Litter away from pine		$P = 0.420$	$t_{12} = 0.2$		$P = 0.047$	$t_{12} = 1.8$
Litter near pine		$P = 0.014$	$t_{13} = 2.5$		$P = 0.005$	$t_{13} = 2.9$
Soil away from pine		$P = 0.421$	$t_{12} = 0.2$		$P = 0.016$	$t_{12} = 2.4$
Soil near pine		$P = 0.009$	$t_{13} = 2.7$		$P = 0.004$	$t_{13} = 3.1$
Plant pathogens						
Litter away from pine		$P = 0.466$	$t_{12} = 0.09$		$P = 0.421$	$t_{12} = 0.42$
Litter near pine		$P = 0.055$	$t_{13} = 1.72$		$P = 0.451$	$t_{13} = 0.12$
Soil away from pine		$P = 0.078$	$t_{12} = 1.51$		$P = 0.445$	$t_{12} = 0.14$
Soil near pine		$P = 0.314$	$t_{13} = 0.49$		$P = 0.321$	$t_{13} = 0.47$
Animal pathogens						
Litter away from pine	↓87.8%	**$P = 0.0003$**	$t_{12} = 4.6$	↓83.0%	**$P = 0.0003$**	$t_{12} = 4.6$
Litter near pine		$P = 0.105$	$t_{13} = 1.3$		$P = 0.04$	$t_{13} = 1.9$
Soil away from pine		$P = 0.07$	$t_{12} = 1.57$		$P = 0.017$	$t_{12} = 2.4$
Soil near pine		$P = 0.04$	$t_{13} = 1.9$	↓82.2%	**$P = 0.001$**	$t_{13} = 3.7$
Mycoparasites						
Litter away from pine		$P = 0.011$	$t_{12} = 2.6$		$P = 0.0035$	$t_{12} = 3.2$
Litter near pine	↑*c.* 500%	**$P = 0.0003$**	$t_{13} = 4.6$		$P = 0.08$	$t_{13} = 1.5$
Soil away from pine		$P = 0.278$	$t_{12} = 0.6$		$P = 0.5$	$t_{12} = 0$
Soil near pine		$P = 0.155$	$t_{13} = 1.1$		$P = 0.1$	$t_{13} = 1.4$

Statistically significant responses, following Šidák correction for multiple testing ($P < 0.0013$) are in bold and include effect size and direction (increase shown with upward arrows and decline shown with downward arrows). The effect (%) was calculated based on obtained mean read counts (for abundance) or number of operational taxonomic units (OTUs, richness). Arbuscular mycorrhizal, lichenicolous, and lichenized fungi were not analysed owing to low OTU richness.

et al., 2013; Oliver *et al.*, 2015). Decomposition data here indicate that fire effects on microbial decomposition may be important, albeit transient, compared with fire regime impacts through litter stoichiometry (Ficken & Wright, 2017; Butler *et al.*, 2019). These differences suggest that variation in fire return intervals and/or fire intensities may have large effects on microbial community dynamics and function.

Plant community composition was a strong predictor of fungal community structure in the pine savanna; however, the observed responses to fire in fungi were not due to plant community shifts. No strong effects of fire were observed for plant communities, whereas fungal community composition changed significantly. Pyrogenic plants have underground organs that result in rapid resprouting after top-kill by fire (Collins & Gibson, 1990; Olson & Platt, 1995; Bellingham & Sparrow, 2000; Lamont *et al.*, 2011), although resprouting may be sensitive to fire intensity (Drewa *et al.*, 2006). Pine savannas have experienced low-intensity fires for many millennia, filtering ground-layer plant communities for species with such key adaptations to frequent fire (Platt, 1999; Peet *et al.*, 2018). By contrast, although experiencing the same historical fire regime as plants, fungal communities showed significant reorganization: we noted > 1000 fungi characteristic to unburned communities and > 500 fungi characteristic to burned communities. We anticipate that fire-sensitive fungi, removed from litter and upper soil layers by fire, regrow from deeper soils or perhaps recolonize from unburned patches or new litter inputs (Ashkannejhad & Horton, 2006; Palmero *et al.*, 2011). Such highly dynamic fungal communities, which reorganize rapidly after each fire and then transition to distinctly different assemblages over 1–2 yr, only to be reorganized again after the next fire, thus appear to differ markedly from the more-persistent plant communities in pyrogenic ecosystems. Of three plant species identified as fire indicators, only two (*E. discoidalis* and *M. quadrivalvis*) were weakly correlated with fungal community structure and could contribute to fire-driven changes in fungal communities.

Fungal community changes with fire also appear to be weakly related to soil conditions. We observed minimal changes in soil parameters as a result of recent fire. The sandy nature of savanna soils and long history of frequent fires likely explain why there were no lasting shifts in pH, organic matter, or even soil moisture in soils collected the month following fire. Some prior studies in other habitats have shown strong fire-induced shifts in nutrients and soil pH due to ash deposits (Raison & McGarity, 1980; Ponder *et al.*, 2009), but soil structure and chemistry in these systems appear distinct from those in old-growth pine savannas. Given only small changes in soil chemistry and plant community composition, we attribute fire-induced shifts in savanna fungi to direct fire effects (or indirect effects not assessed in this study). Greater heating at ground level, either directly by fire or

indirectly by warming of residual litter postfire, seems the most likely explanation for stronger fire effects on fungal litter communities compared with more-insulated soil fungal communities.

Proximity to pines played a small role in structuring fungal communities and did not alter microbial decomposition or fire effects on communities. We anticipated litter and soil to support different fungal communities with distinct decomposition in plots under pines compared with areas away from pines. Stronger responses to fire near pine communities were also expected, as higher contribution of resinous, lignified pine needles (vs grass leaves) in litter leads to more-severe fires (Ellair & Platt, 2013; Platt *et al.*, 2016). Repeated fires may have created fungal communities in litter and upper soil layers better adapted to fire generally and precluded fungal adaptation with specific regard to overstory pine proximity (Priha *et al.*, 1999; Osono *et al.*, 2014). The trend for ectomycorrhizal fungi near pines to decline with fire is consistent with their presumed sensitivity to fire and may be biologically important.

The loss of saprotrophic fungi with recent fire paralleled declines in microbial decomposition of new litter. Fungal community structure was clearly correlated ($R = 0.495$) with decomposition rates, and fungal saprotrophs were the most fire-sensitive fungi, declining in richness or abundance, across multiple communities (e.g. litter away from pines). Following fire, saprotrophic communities of litter had a significantly higher proportion of ascomycetes and lacked effective basidiomycete decomposers, including those of the genus *Mycena* (Boberg *et al.*, 2011). We expected saprotrophs to recolonize following fire, due to input of new pine needles and plant leaves that both serve as new substrates and contain facultative fungal saprotrophs (e.g. endophytic and phylloplane fungi; Boddy *et al.*, 2007). Nonetheless, no such recovery in decomposition was observed even 9 months after recent fire, suggesting that full recovery of saprotrophic function may be delayed until the second postfire growing season.

Shifts in fungal communities and microbial decomposition are likely to alter fuel loads in ways that may change the intensity and spread of future fires. Fire intensity and spread in pyrogenic systems is related to fine-fuel accumulation, such as long-leaf pine needles and grass litter. The decline in fungal saprotrophs and slower rates of decomposition could, therefore, result in more rapid accumulation of new fine fuels. Greater fuel loads, in turn, will likely increase the intensity and especially spread of new fires across the previously burned savanna (cf. Platt *et al.*, 2015). Although more research is needed, this process may represent a feedback loop, with fires supressing saprotrophic organisms and resulting in higher likelihood of new fires. Future research that integrates fungi and other decomposers into fire ecology and fire regime models would improve our understanding of the maintenance of these threatened ecosystems.

Pine savannas harbour a unique diversity of fire-adapted fungi that are worthy of more study. We identified a high diversity of fungi in the pine savanna, only transiently present in relation to fire. The > 500 fungal species only present immediately after fire could be either fire tolerant, able to recolonize quickly after fire, or benefit from decline in other fire-sensitive species. For example, increase in mycoparasitic fungi following fire was likely related to increased susceptibility of other fungi after heat exposure. Fire-responsive species may represent a hidden diversity (*sensu* Partel, 2014), present only in pyrogenic systems and only within a limited timespan after fire. Few taxa that were favoured by fire in pine savanna have been previously reported as 'fire tolerant' in other studies. For example, some species of *Russula* (Horton *et al.*, 1998) and *Tomentella* (Baar *et al.*, 1999) have both previously been reported to respond positively to wildfire but did not show any significant response to fire in our study. The genus *Periconia* had the strongest positive response to fire in our study, but to our knowledge has not been previously reported as fire tolerant. In addition, many 'pyrophilic' fungal genera identified in other studies, including *Coltricia* (Visser, 1994), *Wilcoxinia* (Fujimura *et al.*, 2005), *Pyronema*, *Morchella* (Smith *et al.*, 2016), and *Thelephora* (Visser, 1994), were not present in our system. Only *Cenococcum* was previously reported as fire resistant (Kipfer *et al.*, 2010) and also increased in richness in our study (in soil communities away from pine).

Changing perspective on pyrogenic ecosystems

Pine savannas harbour a unique and diverse assemblage of fire-adapted fungi. As with plants and animals (Peet *et al.*, 2018), maintenance of this fungal diversity requires a heterogeneous savanna landscape sustained by recurrent fires. We found that fungal communities were much more responsive to recent fire than to plant communities or soil properties. Given the importance of fungi to ecosystem processes such as decomposition, nutrient cycling, and plant productivity (Rayner & Boddy, 1988), it is likely that fungi are important in maintaining such savanna ecosystems. Today, pine savannas represent < 3–4% of their original extent (Outcalt & Sheffield, 1996; Frost, 2006; Noss *et al.*, 2015). As proposed by Peet *et al.* (2018), managing these biomes using frequent prescribed fire that mimics evolutionary fire regimes is essential for conservation of their biota, and such fires may be crucial for microorganisms such as fungi.

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Author contributions

BAS and WJP conceived the study. WJP, BAS, and JH designed the field experiment and collected soil and litter samples. WJP collected plant community data. TRP carried out DNA extraction and library preparation. BAS and TRP conducted bioinformatic analyses. TAS-N and BAS analysed the data. TAS-N, BAS, and WJP wrote the manuscript. All authors read and approved the final manuscript.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Fire effect on plant communities.

Fig. S2 Fungal taxonomic diversity of the longleaf pine savanna.

Fig. S3 Proportions of fungal OTUs belonging to different ecological guilds in the longleaf pine savanna.

Fig. S4 Taxonomic composition of litter and soil fungal communities of the long-leaf pine savanna.

Table S1 The effects of plot spatial location, fire and proximity to pines on soil properties.

Table S2 Vascular plant species recorded in the experimental plots.

Table S3 Predictors of plant community composition in the long-leaf pine savanna.

Table S4 Differences in richness and abundance of fungal genera between burned and unburned plots.

Table S5 Fungal indicators of burned and unburned communities.

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