Fire and fungi: fungal ecology in pyrophilic ecosystems

By
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Fire and fungi: fungal ecology in pyrophilic ecosystems

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Abstract

Fire is a global phenomenon that annually burns approximately 3% of Earth's terrestrial surface. While fire is an important component of many terrestrial ecosystems, climate change and anthropogenic influence are expected to alter global fire regimes (particularly fire frequency and intensity). This makes understanding fire's role under a changing climate critical to preserving threatened ecosystems. Despite the need to protect these ecosystems, the manipulation of fire regimes is dangerous and often impossible in most systems. Pyrophilic, or fire recurrent ecosystems, however, may offer a useful model due to their long-term adaptation to recurrent fires. The fire regimes of pyrophilic ecosystems are maintained by feedbacks between fire and plant fuels, which has led to a historical focus on the role of plants in pyrophilic systems. This approach has largely ignored the role of soil microbes in these systems, despite their ability to modify plant fuel loads through saprotrophic, mutualistic, and pathogenic interactions. By improving our knowledge of the microbial processes that underly pyrophilic ecosystems, we may be able to better respond to future changes in the frequency and intensity of global fire regimes. In this dissertation, I assessed microbial roles in pyrophilic ecosystems by testing four primary questions 1) Does fire drive similar shifts to microbial community structure and seasonal trajectories across pyrophilic systems, 2-3) Do fire regime components (e.g. fire frequency and intensity) alter the microbial mediation of plant fuel loads via decomposition, and 4) Does fire modify microbial and abiotic soil components in ways that influence plant fuel production? I hypothesized that fire would modify microbial communities and their function (e.g. decomposition, mutualism, and pathogenic effects) in ways that modified plant fuel dynamics. I used four complementary experiments that manipulated fire regime components and combined molecular, field, and greenhouse techniques to develop a holistic understanding of microbial roles in pyrophilic ecosystems. Fire had similar

effects on fungal community structure and seasonal trajectories across pyrophilic ecosystems. Furthermore, as the frequency and intensity of fires increased, microbial functions like decomposition slowed, and microbial interactions with plant fuel production were altered. This indicates that fire alters microbial community structure, seasonal dynamics, and function in ways that modify plant fuel loads. Since fire-microbial interactions influence plant fuel dynamics in ways that lead to fuel accumulation, this could drive positive feedbacks on future fires, and suggests that soil microbes play integral roles in maintaining the fire regimes of pyrophilic ecosystems. By understanding the processes that govern the fire regimes of pyrophilic ecosystems, we can better respond to and preserve terrestrial systems against future increases in fire frequency and intensity due to climate change and anthropogenic influence.

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Introduction

Disturbances are important determinants of biological communities; however, they are often left out of assembly models as they are viewed as rare, pre-successional events. Disturbances are relatively common however, and often play high level roles in determining the structure of biological communities (Grime 1973; Horn 1975; Beckage *et al.* 2009; He *et al.* 2019). Many communities have long evolutionary histories with disturbances, which act as selective forces on traits and function. Case in point, many of Earth's ecosystems are considered disturbance mediated (e.g. intertidal zones, flood plains, and pyrophilic ecosystems), as they possess suites of species adapted to surviving and taking advantage of recurrent disturbances. Amongst the best studied of disturbances is fire, which produces easily recognizable changes in ecosystems and species pools, and has had a global influence on Earth since the evolution of terrestrial plants (Archibald *et al.* 2018).

Fire is a widespread phenomenon that annually burns approximately 3% of Earth's terrestrial surface. While fires are often considered large-scale, highly destructive disturbances, many systems are actually maintained by relatively frequent (1-3yr fire return intervals), low intensity fires (Mutch 1970; Bowman *et al.* 2009; Archibald *et al.* 2013; Nerlekar & Veldman 2020). Since global fire regimes, particularly their frequencies and intensities, are expected to change due to climate change (Liu & Wimberly 2016) and anthropogenic influence (Balch *et al.* 2017), understanding fire's role in ecosystems is crucial to the preservation of terrestrial systems. Despite the importance of fire research under a changing climate, direct manipulation of fire regimes is often extremely dangerous or impossible. Pyrophilic, or fire recurrent ecosystems, however, may provide useful models for testing fire based questions due to their long-term adaptation to recurrent fires.

Pyrophilic ecosystems like grasslands, savannas, and shrublands, are subjected to frequent (in some cases annual) fires that are necessary for their maintenance and preservation (Platt 1999; Mistry *et al.* 2005; Towne 2012). The reliance on frequent fires in these systems means that experimental manipulation of fire regime components like frequency and intensity is relatively easy, less damaging to the environment, and can be done in a safer, controlled manner. Furthermore, pyrophilic system's long-term adaptation to recurrent fires implies that they may provide a suitable model for estimating the long-term effects of altered fire regimes in less fire tolerant systems. Despite early differences in fire intensities between pyrophilic and less-fire tolerant systems, it is likely that fire intensity will decrease as fires become more frequent and fuel loads are reduced (Archibald *et al.* 2013; Kalies & Yocom Kent 2016), and that fires will become more comparable or begin to approximate to those in pyrophilic systems. Since fires have well known effects on ecological processes in pyrophilic ecosystems (Certini 2005; Ficken & Wright 2017; Butler *et al.* 2019), understanding the processes that are central to pyrophilic systems may help us forecast the effects of fire in less fire tolerant ecosystems under changing climate and fire regime conditions.

In pyrophilic ecosystems, interactions between fire and plants (i.e. fuels) engineer fire regimes (Birk & Bridges 1989; Beckage & Ellingwood 2008; Beckage *et al.* 2009, 2011; Platt *et al.* 2016) and determine the characteristics of individual fires (e.g. temperature, duration, and severity; Platt *et al.* 2016). The fire regimes produced by interactions between fire and plant fuels select for fire adapted or tolerant plant taxa (Platt 1999; Keeley & Fotheringham 2000; Gagnon *et al.* 2010; Harms *et al.* 2017; Pyke 2017), which possess traits such as rapid post-fire resprouting and growth that can drive the rapid accumulation of new plant fuels (Birk & Bridges 1989; Brewer & Platt 1994; Whelan 1995; Bond & Keeley 2005; Beckage *et al.* 2011; Tiribelli *et al.* 2018). The

quick build-up in plant fuels in turn favors recurrent fires of varying intensity and duration. The close connection between fire and plant fuels implies that processes that modify plant fuel loads, may also drive feedbacks on future fires (Beckage *et al.* 2011). Due to their clear importance in the fire regimes of pyrophilic systems, plant communities have historically received the most attention from fire ecologists. This has largely left other ecological processes with the potential to modify plant fuels, such as those dominated by fungi (e.g. decomposition, pathogenicity, and mutualisms), unconsidered despite their potential to modify fire characteristics through their interactions with plant fuel loads.

Fungi play foundational roles in ecosystems and are affected by fire in ways that alter their community structure and function. Fire restructures fungal communities by suppressing dominant taxa (Brown *et al.* 2013; Pressler *et al.* 2019; Semenova-Nelsen *et al.* 2019), favoring species that can endure the passage of fire (Peay *et al.* 2009; Glassman *et al.* 2016a; Owen *et al.* 2019), and selecting for taxa that can survive in harsh, post-fire environments (Sharma 1981; Hamman *et al.* 2007; Hansen *et al.* 2013). Since fungi directly modify plant fuel loads through saprotrophic, mutualistic, and pathogenic interactions, fires that disproportionally effect certain taxa or functional groups could drive feedbacks on future fire characteristics. For example, if fire largely removes surface associated decomposer and plant pathogen species, while leaving mycorrhizal mutualists unharmed, then this could favor the build-up of plant-fuels and plant fuel production. Furthermore, many fungal taxa follow seasonal trends in abundance (Harvey *et al.* 1978; Santos-Gonzalez *et al.* 2007; Averill *et al.* 2019), and are intrinsically connected to ecological processes like nutrient cycles (Hanula *et al.* 2012; Fultz *et al.* 2016; Butler *et al.* 2019). Since fungi are highly associated with their local environment (Tedersoo *et al.* 2014; Bahram *et al.* 2018; Delgado-

Baquerizo *et al.* 2018; Averill *et al.* 2019), fire effects on microbes may also interact with seasonal trends and fire driven changes to ecosystems.

The tight associations between fungi and their local environment imply that fire driven changes to nutrient cycles and abiotic conditions may indirectly modify fungal effects on plant fuel loads across time. Early after fire, when fire related mortality effects on fungal communities are strongest (Muñoz-Rojas *et al.* 2016; Smith *et al.* 2016; Semenova-Nelsen *et al.* 2019), the post-fire nutrient flush (e.g. C, N, and P; Neary *et al.* 1999; Johnson & Curtis 2001) and suppression of non-fire tolerant fungal taxa may govern microbial community structure and function. However, with increasing time after fire, fire implicated nutrient loss due to erosion, wind, and leaching (Bell & Binkley 1989; Knelman *et al.* 2019), as well as inhospitable soil environments (e.g. hydrophobic and dry conditions; Iverson & Hutchinson 2002; MacDonald & Huffman 2004) may limit fungal function. The lack of available nutrients and inhospitable conditions could slow fungal activities like decomposition due to high C:N ratios and low moisture conditions, which are less favorable for fungal function (Orchard & Cook 1983; Manzoni *et al.* 2010; Cornelissen *et al.* 2017). Despite strong early effects on fungal communities and their function, fire effects on fungi may attenuate with time as fungi recover, recolonize, or disperse into burned areas, and plant fuel loads are replenished (Treseder *et al.* 2004; Hart *et al.* 2005; Bastias *et al.* 2006; Holden *et al.* 2013). In summary, understanding how fire effects fungi and their roles in pyrophilic ecosystems is central to understanding the processes that engineer the fire regimes of pyrophilic ecosystems.

I used four complementary experiments to elucidate fungal roles in the fire-fuel feedback processes that engineer pyrophilic ecosystems. These experiments answered four primary questions: 1) Does fire have similar effects on fungal taxa and their seasonal trajectories across pyrophilic ecosystems (Fig.1 path 1), 2-3) Do fire regime components like fire history and intensity alter fungal functions like decomposition (Fig.1 path 2,3), and 4) Does fire modify biotic and abiotic conditions in ways that alter plant fuel production (Fig.1 path 4)? We hypothesized that fire would shift fungal communities and their associated functions (e.g. decomposition, mutualisms, and pathogenic effects) in ways that modified plant fuel loads. We specifically predicted that the recurrent, lower intensity fires common in pyrophilic ecosystems would favor similar fungal taxa and functional groups across pyrophilic ecosystems and alter fungal seasonal trajectories from nearby unburned sites. We also predicted that as fires increased in frequency and intensity, that fungal functions like decomposition and involvement in plant biomass production would decrease. I evaluated these questions by combining molecular, field, and greenhouse techniques to create a comprehensive picture of fungal roles in pyrophilic ecosystems. My findings suggest that fungi are foundational members of pyrophilic ecosystems, and that their long term adaptation to recurrent fire helps promote the fire regimes that underlie pyrophilic systems (Fig.1 "Feedbacks to future fires" path).

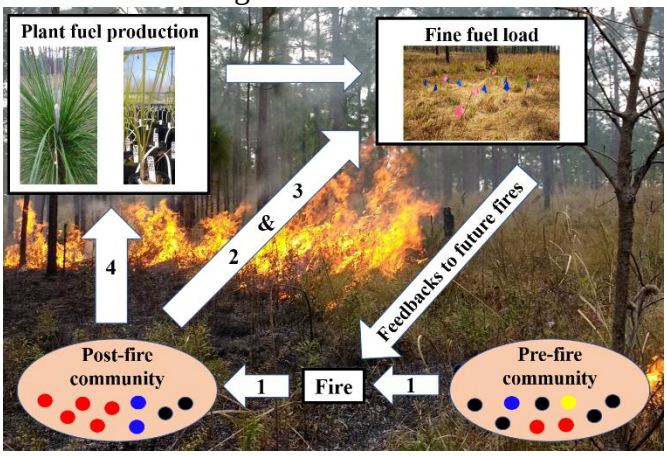

Figure 1: Fire-plant-fungi feedback model based on R-A-M model for pyrophilic plants (Platt 1999).

Chapter 1 - Fungal community structure and seasonal trajectories respond similarly to fire across pyrophilic ecosystems*

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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 *et al.* 2019; Semenova-Nelsen *et al.* 2019). As predicted, anthropogenic influence (Balch *et al.* 2017) and climate change (Liu & 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 *et al.* 2007; Dooley & Treseder 2012), suppressing dominant taxa (Brown *et al.* 2013; Pressler *et al.* 2019; Semenova-Nelsen *et al.* 2019), and selecting for species that can withstand extreme fire temperatures and survive in harsh, post-fire environments (Hamman *et al.* 2007; Peay *et al.* 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 *et al.* 1978; Santos-Gonzalez *et al.* 2007), and are strongly impacted by fire (Klopatek *et al.* 1988; Dhillion & Anderson 1993; Taudière *et al.* 2017). Fire is well known to decrease the richness and *in situ* colonization of AM and EM fungal communities (Dove & 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 & 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 *et al.* 2004; Hart *et al.* 2005; Bastias *et al.* 2006a; Holden *et al.* 2013). These dynamic fire effects on fungal communities likely overlay, and interact with, seasonal changes in fungal communities (Averill *et al.* 2019; Šťursová *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 & 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.* 2016a; 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_2O 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 & 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 & 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, taxonomic and ecological data are mentioned in the results and provided in the Ch.1 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 *et al.* 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 *et al.* 2013), Chaetothyriales (Sterflinger *et al.* 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 & Gadgil 2009), *Myrothecium* (Chen 2016), and Mycosphaerellaceae (Taylor *et al.* 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 & 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 & Gadgil 2009), *Phoma* (Kirk *et al.* 2008), *Zymoseptoria* (Quaedvlieg *et al.* 2011), and Mycosphaerellaceae (Taylor *et al.* 2003), as well as saprotrophic taxa like *Cryptococcus* (May *et al.* 2016), Phaeosphaeriaceae (Cannon & Kirk 2007), *Periconia* (Markovskaja & Kačergius 2014), and *Bullera* (Nakase & 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 *et al.* 2009), Loblolly pine forests (Brown *et al.* 2013), and Ponderosa pine forests (Stendell *et al.* 1999; Hamman *et al.* 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 & Hart 2017; Day *et al.* 2019), where rarer, high intensity fires often drive fungal mortality and declines in species richness (Treseder *et al.* 2004; Glassman *et al.* 2016; Dove & 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.* 2006a; 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 *et al.* 2004; Hart *et al.* 2005; Bastias *et al.* 2006a; Bárcenas-Moreno *et al.* 2011; Holden *et al.* 2013). Since burned and unburned communities remained distinct throughout the study (~1 yr.), this may suggest that post-fire priority effects (Kennedy & 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.* 2006a; Cairney & 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 & 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 & 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 *et al.* 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 *et al.* 1988; Horton

et al. 1998; Baar *et al.* 1999; Glassman *et al.* 2016; Owen *et al.* 2019). If low-intensity 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 & 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 & 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 & 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.

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Conflicts of Interest

Authors have no conflicts of interest to declare.

Chapter 1 - Tables

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

* ≤ 0.1, ** ≤ 0.05, *** ≤ 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

* ≤ 0.1, ** ≤ 0.05, *** ≤ 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
OTU Richness	*fire treatment*	1	19.19	1.325	0.2638
	time	6	2.97	1.891	0.3226
	pine proximity	1	19.18	1.9	0.1839
	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
Inverse Simpson Index	*fire treatment*	1	19.2	1.854	0.1891
	time	6	5.58	1.733	0.2685
	pine proximity	1	19.16	2.108	0.1627
	*fire * time*	6	47.89	1.432	0.2221
	*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

* ≤ 0.1, ** ≤ 0.05, *** ≤ 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

Chapter 1 - Figures

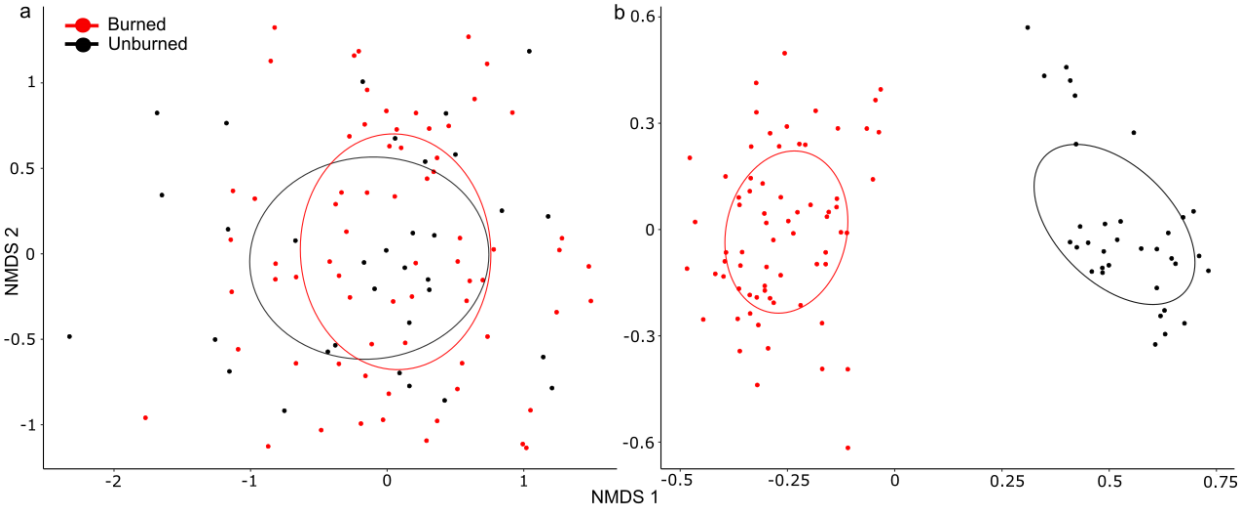

Figure 2: Non-metric multidimensional ordinations for burned and non-burned fungal communities. Ellipses represent the standard deviation of each burn treatment group (black = no burn, red = burned). a) Longleaf pine savanna fungal communities. b) Tallgrass prairie fungal communities.

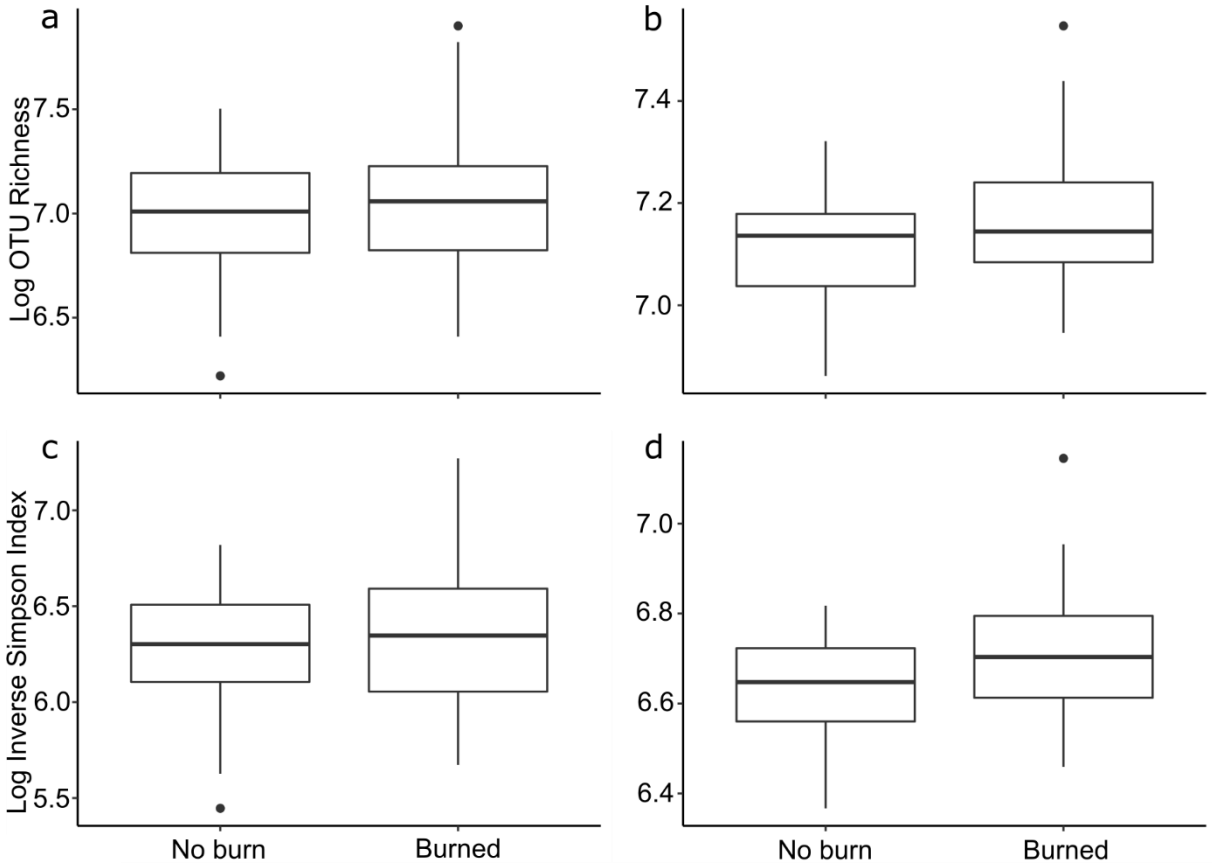

Figure 3: OTU richness and Inverse Simpson Index values for Longleaf pine savanna and tallgrass prairie fungal communities. Box charts display the mean, lower & upper quartiles, and extremes. Outliers are denoted with solid black points. a) OTU richness of Longleaf pine savanna and b) tallgrass prairie 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.

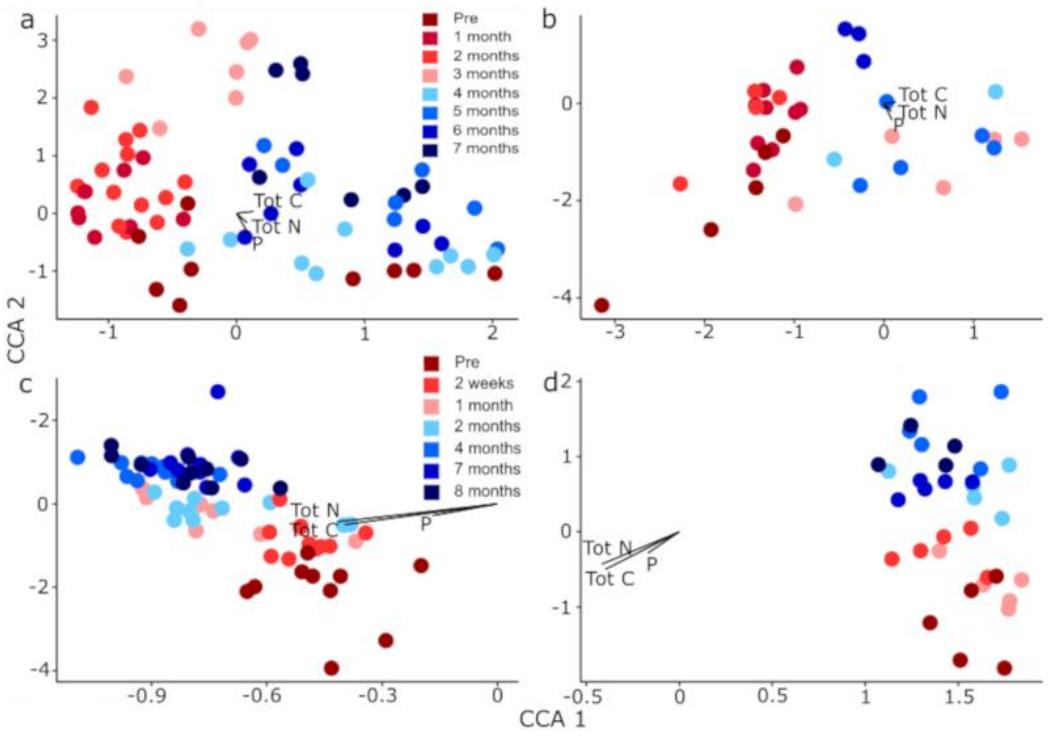

Figure 4: Canonical correspondence analysis ordinations for burned and non-burned fungal community composition across time. Prescribed fires occurred between March and April of 2017, and sampling times reflect time since fire. Total Carbon, total nitrogen, and P were projected onto ordinations using 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.

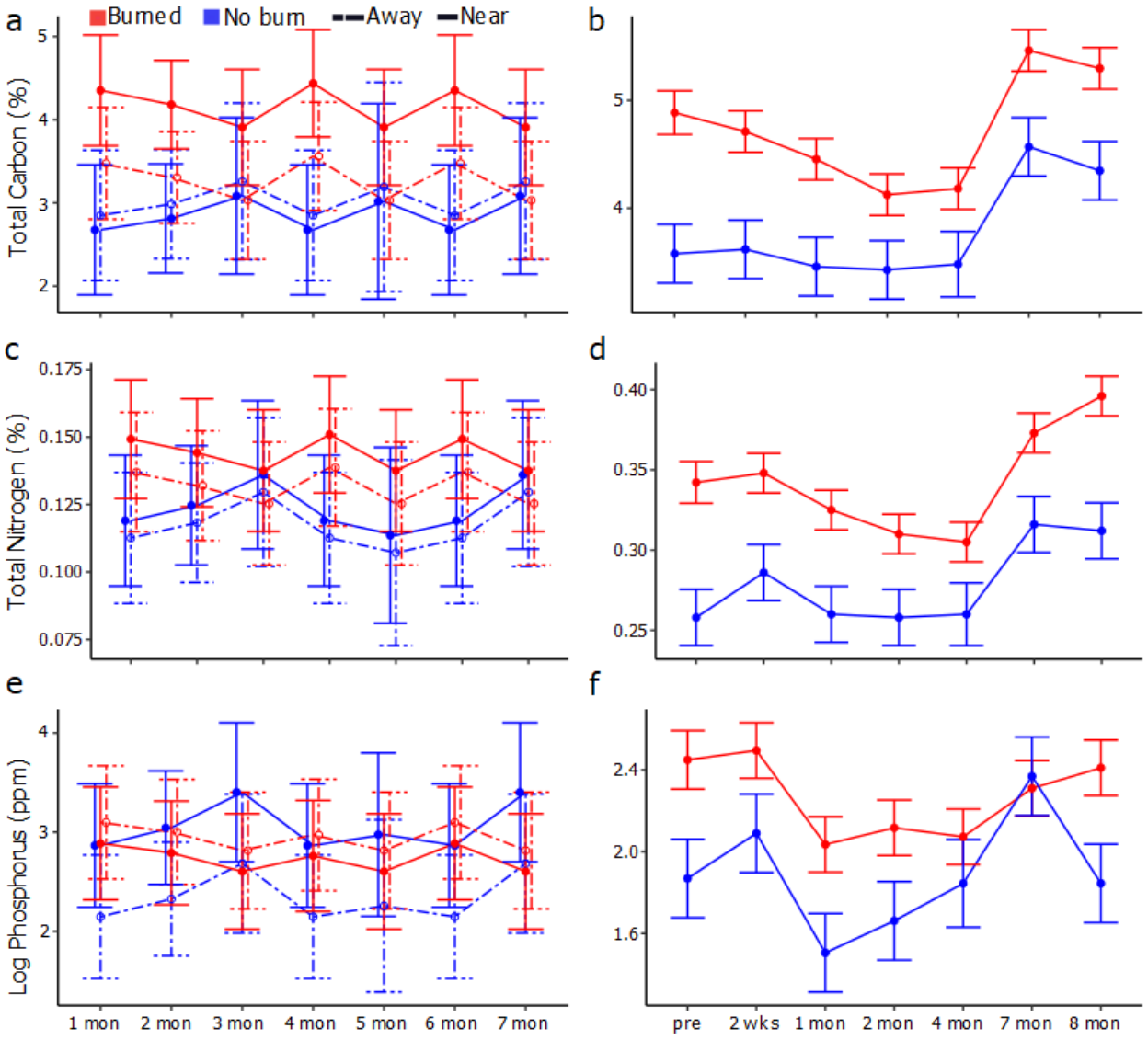

Figure 5: Carbon, nitrogen, and phosphorus levels following prescribed burns. Points represent mean C, N, and P at each sampling time, solid lines and points correspond with near pines sites, dashed lines and open points correspond with away from pines sites. Error bars represent the mean +/- one standard 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.

Chapter 2 - Frequent fire slows microbial decomposition of newly deposited fine fuels in a pyrophilic ecosystem*

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Abstract

Frequent fires maintain nearly 50% of terrestrial ecosystems, and drive ecosystem changes that govern future fires. Since fires are dependent on available plant or fine fuels, ecosystem processes that alter fine fuel loads like microbial decomposition are particularly important and could modify future fires. We hypothesized that variation in short-term fire history would influence fuel dynamics in such ecosystems. We predicted that frequent fires within a short-time period would slow microbial decomposition of new fine fuels. We expected that fire effects would differ based on dominant substrates and that fire history would also alter soil nutrient availability, indirectly slowing decomposition. We measured decomposition of newly deposited fine fuels in a Longleaf pine savanna, comparing plots that burned 0, 1, 2, or 3 times between 2014 & 2016, and which were located in either close proximity to or away from overstory pines (Longleaf pine, *Pinus palustris*). Microbial decomposition was slower in plots near longleaf pines and, as the numbers of fires increased, decomposition slowed. We then used structural equation modeling to assess pathways for these effects (number of fires, 2016 fuel/fire characteristics, and soil chemistry). Increased fire frequency was directly associated with decreased microbial decomposition. While

increased fires decreased nutrient availability, changes in nutrients were not associated with decomposition. Our findings indicate that increasing numbers of fires over short time intervals can slow microbial decomposition of newly deposited fine fuels. This could favor the fine fuel accumulation and drive positive feedbacks on future fires.

Introduction

Fire is a consistent disturbance in terrestrial ecosystems that profoundly changes biological and biogeochemical processes. Although often thought of as rare, catastrophic events (Bowman et al. 2009), frequent fires are necessary to maintain nearly 50% of terrestrial ecosystems including grasslands, savannas, and many Mediterranean-type ecosystems (Archibald et al. 2018). Since wildfire frequency is expected to increase due to human influence (Balch et al. 2017) and climate change (Liu and Wimberly 2016, Schoennagel et al. 2017), understanding how ecosystems respond to frequent fire is important for their preservation and maintenance. While directly manipulating aspects of fire regime is impossible in many systems, prescribed fire in naturally fire-frequented ecosystems may represent a conservative model to predict the pathways through which increased fire frequencies can impact systems that otherwise rarely experience fire.

Frequent fires in grasslands and savannas alter organisms and their environment in ways that can impact subsequent fires. The fine fuels produced by fire-adapted plants (i.e. their litter) are key examples, as frequent fires favor plant species that rapidly recover following fire and produce biomass that fuels future fires (Whitlock et al. 2003, Beckage et al. 2009, Cornelissen et al. 2017). By favoring fire adapted plant species, characteristics of previous fires can create a feedback, through the rapid production of fine fuels that control the spread and intensity of new fires (i.e. short-term fire history; Neary et al. 1999, Ficken and Wright 2017). Fine fuel

accumulation, however, is also governed by other processes like microbial decomposition, which may also produce feedbacks based on fire history (Butler et al. 2019). Quantifying the pathways by which fire history impacts decomposition is critical for the maintenance of fire-frequented ecosystems and predicting potential mechanisms by which frequent fires impact other systems.

Repeated fires directly govern microbial decomposition by altering fine fuel loads and shaping the microbial communities that control fuel decay. The strength of fire's effect on decomposition is constrained by fire regime components like fire history, which can determine the quantity of available fine fuels and the intensity of future fires. For example, longer fire return intervals result in increased fuel loads (Archibald et al. 2013, Harris et al. 2016) and longer recovery times for microbes, while shorter fire return intervals, or frequent fires, can decrease plant fuel loads and microbial recovery times due to repeated combustion (Platt et al. 2016). When combined with natural variations in fuel load production (i.e. tree-grassland matrices of savannas; Platt et al. 2016), this can produce fires of varying frequencies and intensities that drive differential mortality of microbial decomposers and slow decomposition depending on location. Since fire can cause the mortality of microbial decomposers and filter communities for particular functional groups (Dooley and Treseder 2012, Ferrenberg et al. 2013, Brown et al. 2013), understanding how fire-history and intensity alters microbial decomposition can clarify the dynamics of fire-microbe-plant interactions in pyrophilic ecosystems. If microbial decomposition is strongly altered by fire, this could directly shift new fine fuel accumulation rates and affect the intensity and likelihood of future fires.

Fire regimes also influence the substrates and nutrients available for microbial decomposition, which may create indirect pathways for fire feedbacks. Fine fuel traits (e.g. carbon to nitrogen ratio and lignin content) directly govern decomposition (Manzoni et al. 2010), and also

determine the duration and intensity of fires (Demirbaş 2001). For example, the larger quantities of needles near longleaf pines can increase local fire intensities (Platt et al. 2016), and are also harder to decompose than grass and forb litter. As such, fire histories that change the composition of new fine fuels may change both the substrates available to microbial decomposers, and fire's direct effects on them. Fire history can also govern stoichiometry through fire effects on limiting nutrients like nitrogen (N) and phosphorus (P) (Raison 1979, Butler et al. 2018). Rapid post-fire decomposition may be favored by N and P mineralization if enzyme production and microbial growth would be otherwise limited. Longer intervals between fires can allow fuels to build-up, and increase fire intensity to the point where temperature-sensitive elements like N are volatilized (Raison 1979). N availability then may vary with fire due to interactions between fire history and intensity (i.e. maximum temperature and duration). Low N availability may a) slow decomposition if N-limited microbes cannot make enzymes or b) accelerate decomposition if microbes can make enzymes, and rapidly decompose new fuels to acquire N and other limiting nutrients lost with fire (Parnas 1975). Apart from individual fire intensity, repeated fires decrease nutrient availability (Bell and Binkley 1989), and drive leaching that could slow microbial decomposition.

These combined effects of short-term fire history on substrates and nutrients likely combine with direct fire effects to determine microbial decomposition of fuels. While single fires can slow decomposition and promote fuel accumulation (Semenova-Nelsen et al. 2019), increasing the number of fires within a short period could produce synergistic effects that further slow decomposition. These effects could result from both the direct and indirect effects of fire on microbial decomposition mentioned above. We hypothesized that increasingly frequent fires would slow decomposition, and that fire would impact decomposition through mechanisms related to fire characteristics and nutrient availability. We also hypothesized that natural variations in the

type and quantity of fine fuels would slow decomposition independently of fire history due to substrate differences (Taylor et al 1989).

We manipulated short-term fire history in an old-growth Longleaf pine savanna to evaluate the effect of fire history on the microbial decomposition of fine fuels. Pine savannas offer ideal systems for testing our hypotheses because: organisms there have long co-evolutionary histories with fire (Noss et al. 2015), fire history can be experimentally manipulated, and the spatial heterogeneity of the savanna produces variation in dominant vegetation and the fuels microbes decompose. We used mesh litter bags to measure microbial decomposition of new, post-fire fine fuels. Decomposition was assessed both near and away from pines, to reflect differences in fine fuel substrates (Ellair and Platt 2013, Platt et al. 2016) and microbial communities (Semenova-Nelsen et al. 2019). We first assessed the impact of fire history and pine proximity on microbial decomposition rate constants (k) during the year following 2016 fires. We then used structural equation modeling (SEM) to assess the relative importance of direct and indirect mechanisms on decomposition following prescribed fires. In addition to fire history, edaphic properties, fire characteristics, and fine fuel traits in 2016 were all analyzed as potential drivers of microbial decomposition. The resulting SEM model allowed us to identify the primary pathways through which fire history altered microbial decomposition of fine fuels.

Methods

Field Site: We conducted our study on the Wade Tract (30º 45' N; 84º 00' W; Thomas County, Georgia, USA). Situated on moderately dissected terrain 25-50 m above sea level in the Red Hills region of northern Florida-southern Georgia, the 80 ha preserve is characterized by a warm-temperature climate, with a growing season of 10-11 months, a mean annual temperature of

19.6°C, and average precipitation of ca. 1,350 mm that tends to bimodally distributed during the summer and winter months. Surficial soils are 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). Natural fires in this site tended to occur every 1-3 years, generally during a fire season that spanned dry springs to wet summers, when annual thunderstorms first occurred (Platt et al. 2015, Rother et al. 2018). Historical "open-woods burning" and more recently prescribed fires, have maintained the open savanna/woodland physiognomy (Platt et al. 1988, Gilliam and Platt 1999, Mugnani et al. 2019). The ground layer vegetation and litter on the site has burned annually-biennially (return intervals averaging 1.5 years) during prescribed fires between March and June using drip torches, 1-2 weeks after rain at relative humidity of 50-60% and winds 10-20 km/hr. Flame heights during burns can reach 1-2 m, and generally result in 60-90% removal of accumulated fine fuels.

2014 Field Plots: We established experimental plots in mid-June 2014, following 2014 prescribed fires. These fires produced large unburned patches in a matrix of burned vegetation. We randomly selected 24 unburned patches, 12 in each of two fire management units. Within each fire management unit, 6 patches were near (<5 m) and 6 patches were away (>10 m) from overstory pines. We then randomly selected 24 similar sized burned patches (12 near pines, and 12 away from pines), such that each burned plot was near an unburned patch. Thus, 24 unburned and 24 burned patches were evenly distributed across two fire blocks and relative to overstory pines (Table 7). Each patch was at least 5 m in diameter to minimize fire-edge effects, and did not contain large amounts of woody debris such as fallen trees or large branches. Within each patch, we established randomly located, 1x1 m sampling plots for downstream measurements. Note that these plots were same as used in Semenova-Nelsen et al. 2019. This allowed us to test both the

effects of increased fire frequency, as well as the presence/absence of fire on microbial decomposition.

Short-term fire regimes: We generated differences in short-term fire histories (2014-2016) by manipulating fire regimes. The different fire histories are depicted in Table 7. In 2014, unburned and burned plots were selected based on patchiness of prescribed fires conducted that year. In 2015, we manipulated prescribed fires by burning only one fire block, so that half of the experimental plots burned. Then, in 2016 all plots burned during prescribed fires. We thus generated replicated plots with patterns of 1, 2, and 3 fires; six plots with each fire history were located near and away from pines. Following the 2016 fires, we used fire maps to identify patches that did not burn in 2014, 2015, or 2016; we randomly selected 12 of these patches, 6 near pines and 6 away from pines, and established an additional plot in each. This generated a total of five short-term fire histories that involved 0 (0-0-0), 1 (0-0-1), 2 (1-0-1, 0-1-1) and 3 (1-1-1) fires over the three-year study period (Table 7).

We conducted prescribed fires similarly from 2014-2016. All were ignited and occurred under similar conditions. In all three years, head and flanking fires were ignited in the two fire management units between mid-March and early May under Keetch-Byram Drought Indices of 60-250 using drip torches. Fine fuel consumption in burned patches was estimated each year as 60-80%. Because fires were conducted under similar weather conditions and times of the year, short-term fire histories in Table 7 were considered to differ mainly in the numbers of fires.

In 2016 we explored fuel-fire relationships and measured characteristics of fires in the plots. First, we measured fine fuels, pre- and post-fire, in the 48 plots using procedures outlined in Platt et al. 2016. We established pairs of 30 x 30 cm subplots adjacent to each of the $1m^2$ plots. For each plot, we randomly selected one subplot and collected above ground fuels 1-2 days prior

to fires, then sorted those fuels into fine fuels using two categories: pine needles and non-pine fuels (graminoid, forb, shrub, and other non-woody fuels). Additionally, we recorded the total amount of fine fuels and proportion of fuel loads that were Longleaf pine needles. The fine fuels were air-dried and weighed. One day after 2016 fires, we collected the fine fuels from the other subplot. Remaining fine fuels were weighed to estimate fine fuel combustion. Average mass of woody fuels in plots was similar before and after fires, so we did not examine woody fuel effects on fire characteristics.

We assessed fire characteristics using temperatures recorded at the surface and in the soil during the prescribed fires. We placed two thermocouples in the center of each plot. One was placed 2-3 mm above the ground surface, not contacting litter or soil; the second was placed 1 cm in the soil, close to the surface thermocouple. Thermocouples recorded temperatures every second from the time of activation until 5-6 hours after prescribed fires. The temperature data were used to estimate 1) *maximum surface & soil temperature increase* - the largest instantaneous rise in temperature recorded and 2) *duration of heating* - the time (in seconds) that the temperature at the soil surface remained >60°C (Platt et al. 2016).

Quantifying Microbial Decomposition: We measured microbial decomposition of recently deposited litter experimentally in 2016. In October 2016, we collected recently deposited, intact plant material (dead pine needles, grass culms, forbs, and oak leaves) from outside the 4 m^2 sample plots. Litter collected from patches of the same type (i.e. near and away from pines) was pooled, then shipped to the University of Kansas where it was stored at -20°C until processing. Near and away litter was separated to account for inherent differences in litter chemistry (i.e. C:N ratios and lignin content) and composition (i.e. more pine needles near pines) between litter types. Plant litter was dried at 65˚C for 72 hours, ground using a Model 4 Wiley Mill (Thomas Scientific,

Swedesboro, USA) with a 6mm opening, and sterilized via gamma irradiation to ~32 kGy at the Penn State Radiation Science & Engineering Center. Within a biological safety cabinet, we placed the sterilized plant litter in 15 x 15 cm, 30 µM nylon mesh bags, following (Robertson and Paul 2000). This mesh excludes non-microbes and isolates microbial decomposition of plant litter (Bradford et al. 2002). Each bag was filled with 5 g of plant litter collected either near or away from pines. Initial bag masses were recorded, and bags were stored sterilely until deployment.

Bags were deployed in June 2016, 2-3 months after experimental fires. Four decomposition bags with litter corresponding to pine proximity (i.e. near or away from pines), were selected and randomly placed on the soil surface in each plot. The small mesh size used in bag construction prevented photo degradation of bag contents. Bags were anchored along margins with sod-staples so that one surface of the bag contacted litter and soil. One bag from each plot was collected 2, 4, 6, and 8 months after deployment. Any soil or litter on the bag surface was cleared, and then bags were placed in sterile plastic bags. Bags were shipped overnight to the University of Kansas. Litter contents were then removed, dried at 65˚C for 72 hours, and weighed to determine mass loss. Decomposition rate constants (k) were determined by fitting decomposition from 2 - 8 months in each experimental plot to a negative exponential curve using the following equation:

$$\frac{M_t}{M_0} = e^{-k*t}$$

where the M_0 = starting mass, M_t = mass at time of collection, and t is the number of months the bag was deployed in the field. A negative exponential curve was used to estimate k, as decomposition is well known to follow an exponential decay function when measured over time (Olson 1963; Karberg *et al.* 2008). This produced a decomposition rate constant (k) for each experimental plot during the year following 2016 prescribed fires.

Soil analysis: Soil samples were collected from all plots in June 2016 to measure post-fire nutrient flux. We collected soil at three randomly located points, avoiding ground layer plants. We collected the upper 1.5 cm of soil within a 9 x 9 cm quadrat (i.e., depth potentially affected by increasing fire temperatures; Mehlich 1984, Gagnon et al. 2015). Soil samples from each plot were combined, and kept cool until frozen at -20°C within 6 hours of sampling. Samples were overnighted to the University of Kansas, thawed, and homogenized by hand, before subsampling.

A 100 g subsample was sent to the Kansas State University Soil Testing Lab for analysis. Soil phosphorus was measured using the Mehlich-3 method (Mehlich 1984) on a Lachat Quickchem 8000 (Lachat Instruments, Loveland, USA). Total soil nitrogen and carbon were measured on a LECO TruSpec CN Carbon/Nitrogen combustion analyzer (LECO Corporation, St. Joseph, USA). Carbon to nitrogen ratio was also calculated. NH_4^+ and NO_3^- were extracted using 1 M KCl on 2 g of soil, then Cadmium reduction for nitrate and colorimetric procedures were used, followed by flow analysis for ion quantification (Brown 1998).

Data Analysis: All analyses were conducted in R version 3.5.1 (R Core Team 2013). Analyses of variance (ANOVAs) assessed the effect of short-term fire history and proximity to pines on microbial decomposition rate constants (k). Differences in decomposition, fine fuels, nutrients, and fire characteristics between short-term fire history and pine proximity treatments were first assessed using Type III analysis of variance (ANOVA) using the "Emmeans" package (Lenth 2018). Note that pine proximity treatments were considered in these analyses to account for inherent differences in litter chemistry, fuel traits, and flammability, between near pines fuels vs. away from pines fuels. Following ANOVAs, apriori contrasts regarding differences in decomposition based on the frequency of fires were assessed using the contrast function.

We then developed a structural equation model to assess the causal pathways by which fires impacted plot-level microbial decomposition rate constants (k). Based on existing literature, we hypothesized three specific pathways between fire history and microbial decomposition (Figure 1). These pathways included both direct fire history effects and indirect effects through 2016 fire characteristics and initial changes to soil properties. Chapter 1 Appendix Tables S1 and S1.5 describe variables and justifications for model pathways included in the SEM analysis. We also hypothesized that fuel characteristics play an independent role in determining both fire characteristics and decomposition. We hypothesized that frequent fires would 1) reduce microbial decomposition rates (Figure 6; Path A; Ficken and Wright 2017), 2) reduce the severity of individual fires thereby increasing decomposition rates (Figure 6; Path B; Ficken and Wright 2017, Ellair and Platt 2013, and 3) modify the initial flux of nutrients mineralized by fire and slow decomposition (Figure 6; Path C; Bell and Binkley 1989, Czimczik et al. 2005, Butler et al. 2018). Distinct from short-term fire history effects, locational effects due to larger fine fuel loads and larger amounts of pine needles near pines, should 4) increase fire intensity and slow decomposition (Figure 6; Path D; Ellair and Platt 2013). Our SEM contained categorical, continuous, and ratio variables. All continuous variables were transformed and scaled prior to analysis (Chapter 1 appendix Table S1). After developing an initial model based on these hypotheses, the R Package: "lavaan" (Rosseel 2012) was used to evaluate the preliminary SEM for convergence. Upon convergence, fit measures and parsimony were used to assess the modification of model parameters. Further models were then evaluated per Hooper et al. 2008.

Results

Fine Fuels: Pre-fire fine fuel loads varied based on proximity to overstory longleaf pines and short-term fire history treatment. The largest differences were between pine needle fuels, with near pines sites having larger amounts ($F_{1,59} = 33.4$, $p < 0.001$; Ch.2 App. Table S2, Fig.S1) and proportions ($F_{1,59} = 22.3$, $p < 0.001$; Ch.2 App. Table S2, Fig.S1) of Longleaf pine needles. Total fine fuels ($F_{4,59} = 7.34$, $p < 0.001$; Ch.2 App. Table S2, Fig.S1) and non-pine fuels ($F_{4,59} = 7.37$, $p < 0.001$; Ch.2 App. Table S2, Fig.S1) also differed between short-term fire history treatments, with sites experiencing two fires in the final two years having lower amounts of both. In summary, near pines sites had larger amounts of Longleaf pine needles, and more frequently burned sites had smaller fine fuel loads and amounts of non-pine fuels.

Soil Nutrients: Post-fire nutrients levels varied based on the short-term fire history. As the number of fires increased, total soil nitrogen ($F_{4,59} = 5.17$, $p = 0.001$; Ch.2 App. Table S3, Fig.S2), inorganic phosphorus ($F_{4,60} = 4.49$, $p = 0.003$; Ch.2 App. Table S3, Fig.S2), ammonium ($F_{4,60} = 10.6$, $p < 0.001$; Ch.2 App. Table S3, Fig.S2), and nitrate ($F_{4,60} = 3.39$, $p = 0.02$; Ch.2 App. Table S3, Fig.S2) levels decreased. While nitrate levels decreased when there were two fires in the final two years, it is worth noting that sites burned in only the final year (0_0_1) or the first and final year (1_0_1) actually saw an increase in nitrate levels. Total soil carbon however did not vary between experimental treatments ($F_{4,60} = 1.02$, $p = 0.4$; Ch.2 App. Table S3, Fig.S2). While soil carbon did not vary between short-term fire history treatments in this analysis, C:N ratios increased as fires became more frequent ($F_{4,60} = 10.4$, $p < 0.001$; Ch.2 App. Table S3, Fig.S2). Frequent fires were associated with lower amounts of soil nutrients, and changed nutrient levels in ways that shifted stoichiometric ratios of carbon and nitrogen.

Fire Characteristics: 2016 prescribed burn characteristics differed between short-term fire history treatments; however, these differences were primarily related to the presence or absence of fire. While there was some natural variation between maximum surface ($F_{4,51} = 105.1$, $p < 0.001$; Ch.2 App. Table S4, Fig.S3) and soil temperature ($F_{4,51} = 3.75$, $p = 0.009$; Ch.2 App. Table S4, Fig.S3) increases, surface fire duration >60°C ($F_{4,51} = 25.3$, $p < 0.001$; Ch.2 App. Table S4, Fig.S3), and percent fine fuel combustion ($F_{4,51} = 72.6$, $p < 0.001$; Ch.2 App. Table S4, Fig.S3), between burned sites, prescribed fires generally did not very in intensity between our experimental treatments.

Microbial decomposition: Short-term fire history and pine proximity independently affected microbial decomposition rate. As fires increased in frequency, decomposition rates decreased ($F_{4,48} = 3.971$, $p = 0.007$; Ch.2 App. Table S5, Fig.7a & S4) with contrasts revealing that burning at least once during the 3-year period was associated with slower decomposition than not burning ($P = 0.01$; Ch.2 App. Table S5). Additionally, decomposition rates were lower in sites that burned at least two times as compared to sites that only burned once ($P = 0.009$; Ch.2 App. Table S5). There were no overall differences in decomposition rates between sites that burned two times and sites that burned 3 times ($P = 0.4$; Ch.2 App. Table S5).

Proximity to pines also altered microbial decomposition. During the year following 2016 prescribed fires, litter bags located near pines had slower decomposition rates than bags placed away from pines ($F_{1,48} = 3.921$, $P = 0.05$; Ch.2 App. Table S5, Fig.7b). In summary, increasing the number of fires during the study period and close proximity to pines slowed microbial decomposition.

Structural equation modeling of causal pathways for fire history effects: We initially began with a highly saturated SEM based on our hypothesized pathways (Appendix; Table S1.5 and SEM model fitting section). The first model converged, but was poorly supported ($X^2 = 102.392$, D.F. = 28, P

< 0.001). Through four iterations, poorly supported paths in the model were successively pruned to improve model fit using an increasingly conservative threshold for relationships (e.g. P > 0.5, P > 0.2). Model support was checked after each pruning step (support for each included in appendix table S6) with fit statistics assessed according to Hooper et al. 2008. The final model was well supported (X^2 = 21.795, D.F. = 23, P = 0.533; Table 8), and further removal of unsupported pathways did not improve overall fit. Final SEM pathways and coefficients, along with literature support for these pathways are presented in Table 7.

SEM results: The final SEM model supported our hypotheses that short-term fire history altered the microbial decomposition of fine fuels. We used our initial hypotheses (Figure 6) to construct pathways for relationships in our SEM model (Figure 8). In this way, we could distinguish the underlying mechanisms through which fire history was postulated to modify microbial decomposition in the final model. Numbers in parentheses are the standardized regression coefficients (Table 8). These values indicate the direction (+/-) and strength of relationships between variables, and allow for direct comparisons between model pathways.

SEM-Direct Impact of short-term fire history: In line with our causal model (Figure 6; path A), short-term fire history was linked to microbial decomposition rate (-0.517; Figure 8). Specifically, as the number of fires a plot experienced increased, the decomposition rate constants (k) decreased, paralleling the ANOVA analyses above.

SEM-Modification of Edaphic Factors: While short-term fire history directly modified edaphic pathways, changes to nutrient availability did not alter microbial decomposition rates (Fig. 8). Increased numbers of fires during the study period were associated with decreases in ammonium (-0.77), nitrate (-0.37), phosphorus (-0.58), and total nitrogen (-0.55), and marginally significant decreases in soil carbon (p = 0.08, -0.25). Overall, increasingly frequent fires were associated with

decreased nutrient availability, but these changes were not associated with decomposition rates during the year following 2016 prescribed fires.

SEM-Fire Characteristics: As hypothesized in our causal model (Fig. 6; Path B), short-term fire history was associated with 2016 fire characteristics, but changes in fire characteristics were not associated with microbial decomposition. More fires during the study period corresponded with greater maximum surface temperature increases (0.55), although this was largely driven by the presence vs. absence (0-0-0) of fire in the final year. Additionally, increased numbers of fires during the study were associated with shorter fire durations (-0.22). Greater surface fire temperature increases were also correlated with increased surface fire durations (1.03) and larger soil temperature increases (0.65). Surface fire temperature increases and durations also altered edaphic properties as fires became hotter and longer. Hotter surface temperatures were associated with decreased nitrate (-0.69) and phosphorus (-0.49). Longer fire durations, however, were associated with increased phosphorus (0.86), ammonium (0.2), and nitrate (1.1), and decreased carbon (-0.28). In summary, increasing the number of fires shifted 2016 fire characteristics, which were associated with altered edaphic properties, but not microbial decomposition.

SEM-Fuel traits: Fuel traits were directly linked to microbial decomposition and 2016 fire characteristics (Figure 6; path D). As shown in previous work (Ellair and Platt 2013), sites located near pines had more pine needles (0.53), which were directly linked with greater increases in maximum surface temperature (0.34) and indirectly linked to longer fire durations (0.34) and higher soil temperatures (0.22) through changes to surface temperatures. Fuel traits also had indirect effects on nutrient availability through their modification of fire characteristics (Figure 3). Additionally, near pine sites had lower decomposition rate constants (k) than those located away

from pines (-0.27). Taken together, fuel traits modified the intensity of 2016 fire characteristics, and slowed decomposition in sites located near pines.

Discussion

Microbial decomposition of new fine fuels was slower in frequently burned sites during the year following 2016 prescribed fires. These fire driven changes are consistent with studies that show repeated fires shift microbial community structure, cause the loss of key functional groups (Hart et al. 2005, Ferrenberg et al. 2013, Brown et al. 2013), and are associated with slower decomposition (Ficken and Wright 2017, Butler et al. 2019). While fire history in our system did not suppress total fungal abundance (Hansen et al 2019), it likely impacted microbial community structure (Semenova-Nelsen et al. 2019) in ways that slowed decomposition. This demonstrates that short-term variations in fire history are as important as single fires (Ficken and Wright 2017, Semenova-Nelsen et al. 2019) or long-term fire regime differences (Butler et al. 2019), in determining ecological functions like decomposition. Moreover, decomposition differences arose quickly (i.e. within 3 years) in this pyrophilic ecosystem, so rarely burned systems, which lack fire-adapted organisms, may respond more strongly to repeated fires. It is important to note however, that the pathway linking fire history to microbial decomposition includes other unmeasured processes besides direct fire effects on microbial decomposers.

Although short term fire history impacted nutrients, these effects were not linked to shifts in microbial decomposition. Our study confirms well known impacts of fire on the availability of soil carbon and nutrients (Raison 1979, Neary et al. 1999, Certini 2005). While nutrient availability influences decomposition (Manzoni et al. 2010), significant fire-driven changes to carbon, nitrogen (NO3 and NH4), and phosphorus did not slow decomposition rates during the

year following 2016 prescribed fires. Two key factors may explain the absence of this relationship. First, short term nutrient effects directly after fire may have been obscured when evaluated on decomposition rate constants (k) that integrate seasonal variation. Stoichiometric controls on decomposition vary seasonally (Schmidt et al. 2007), and fire-induced differences in nutrient availability may have had decomposition effects that were balanced out at other time points. For example, frequent burning that reduced nutrients for decomposition directly after the 2016 spring fire, also likely reduced plant production throughout the year perhaps leaving greater soil nutrients for fall microbial decomposition. Second, the high frequency of fires at this site (return interval of ~1 year) may cause long term nutrient limitations (Knicker 2007; Toberman *et al.* 2014; Butler *et al.* 2019), which could mask the effect of short term changes in nutrient levels following single fires. Despite immediate C, N, and P losses in our study, the associations between microbial decomposition and fire history were stronger than associations between decomposition and altered nutrient availability. The long term adaptation to frequent fires and low nutrient availability at this site likely had a stronger effect on decomposition rates (Butler *et al.* 2019) than short term nutrient effects following the most recent fire. Other unmeasured factors like soil moisture, pH, and temperature, are also shifted by fire and can modify microbial decomposition, but past studies have not shown these factors were linked to microbial communities or decomposition at this site (Semenova-Nelsen et al. 2019). In summary, short term fire history altered nutrient availability, yet these changes were not linked to variation in microbial decomposition.

Short-term fire history also modified the intensity of 2016 fires, but this was not strongly linked to microbial decomposition rates. Larger temperature increases and longer burn durations are expected to kill more microbes (Bárcenas-Moreno and Bååth 2009, Dooley and Treseder 2012), and alter microbial decomposition rates due to microbial mortality. However, even in

"long" unburned plots, prescribed 2016 fires at this site may not have reached sufficient intensity to cause significant microbial mortality (Hansen *et al.* 2019). As with nutrient effects, it is also possible that fire intensity related effects on microbial decomposition are strongest immediately after fires, and dissipate with time (or are even offset) as microbial communities recover (Bárcenas-Moreno *et al.* 2011a). Consistent with this interpretation, while fire characteristics did not impact microbial decomposition, they did have strong effects on nutrient availability. High intensity fires can increase nutrient volatilization (Neary et al. 1999), while longer, low intensity fires (i.e. < 200°C) may favor the release of nutrients from fine fuels (Certini 2005). These were born out by our data, as hotter fires (i.e. higher surface temperatures) were associated with decreased N and P, while longer fires (i.e. longer durations above 60°C) were associated with increased N and P. Fire characteristics, including intensity and duration, may play a larger role in decomposition after wildfires, since wildfire intensity commonly surpasses that of prescribed fires (Certini 2005). Overall, short-term fire history modified fire characteristics and nutrient availability, but these changes were not associated with shifts in microbial decomposition rates.

The types of fuels present determined both fire characteristics and postfire decomposition. While location and fuel composition covary, we show that microbial decomposition rates were slower in near pines sites. The direct link between pine proximity and decomposition suggests that the high lignin and C:N content of near pines fuels (Wardle et al. 2002) and location based differences in microbial communities result in slower decomposition. While larger amounts of Longleaf pine needles increased the intensity of 2016 prescribed fires, this did not affect microbial decomposition rates following 2016 fires. Since microbial decomposition rates are slower near pines, the greater suppression of decomposition following fire may contribute to natural fuel accumulation that alters the likelihood or spread of future fires. At our study site, fires commonly

consume more than 60% of fuels (see appendix section “fuels”), and are primarily reliant on fuel accumulated in the last year. This may be a key difference, for example, from fire suppressed forests in the Western US, where the buildup of coarse woody debris (Brown 1983, Kalies and Yocom Kent 2016), can create fires so severe that upper soil horizons are completely lost or sterilized. Fire-driven decomposition differences then may depend on the fuels accumulated since the last fire, and in fire-frequented systems fuel loads may have a strong seasonal relationship.

Linking fire regime and microbial function elucidates the largely unconsidered, but important roles that microbes play in pyrophilic ecosystems. Historically, fire ecology has focused on the interaction of fire with above ground communities (i.e. plants) and biogeochemistry (Archibald et al. 2018), while rarely exploring microbial functions like decomposition. Our study identified pathways through which fire history governs microbial decomposition of fuels, fire characteristics, and soil nutrient availability. Short-term fire history's effects on microbial decomposition should modify fine fuel loads, which could ultimately impact future fires. Other microbial functions, however, may also contribute to (or mitigate) fire feedbacks. Fire regime impacts on microbial mutualists, (i.e. mycorrhizae), could alter their benefits for post-fire plant survival and fuel production (Peay et al. 2009, Glassman et al. 2016, Carson et al. 2019). Microbial pathogen responses to fire history may also be important due to their role in plant productivity (Schnitzer et al. 2011), with pathogen suppression by fire potentially allowing for greater post-fire plant survival and faster fuel production. The indirect impact of microbe-plant symbioses on fuels may counterbalance, or even exacerbate the positive fire feedbacks from microbial decomposition. Future work can explore how fire-microbe interactions shape fire feedbacks through fuel load alterations (as seen here) and plant-microbe interactions.

In conclusion, we demonstrated that short-term fire history and microbial decomposition are closely connected through direct fire and fuel related pathways. Furthermore, we identified a feedback mechanism through which increased numbers of fires may increase fine fuel accumulation and the intensity of future fires. Understanding how different fire histories impact microbial decomposers and associated fine fuels is critical to our knowledge and maintenance of pyrophilic ecosystems, many of which are endangered (Bowman et al. 2009). Furthermore, our study system may provide a conservative model for predicting the effects of increasing fire frequencies in other ecosystems. Fire helps maintain more than 50% of terrestrial ecosystems, and its occurrence is becoming increasingly frequent due to anthropogenic change (Archibald et al. 2018). Including foundational microbial processes like decomposition in fire models can improve our understanding and management of fire-dependent and non-fire dependent ecosystems alike.

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Breazeale Nuclear Reactor for assistance with gamma sterilization. As Rumi said, life is a balance of holding on and letting go.

Chapter 2 - Tables

Table 7: Experimental field design for short-term fire history treatments (2014-2016) in plots located near and away from pines. For each year, 1 indicates groups of plots that were burned and 0 indicates groups of plots that were not burned. The design produced five short-term fire history treatments that involved 0-3 fires, both near & away from pines. Of the total of 60 plots, 48 were established in 2014. Fire maps were used to establish an additional 12 plots (marked with *) in 2016 that did not burn in the three previous years.

		Near Pines					Away from Pines				
Short-Term Fire History	2014	1	1	0	0	0	1	1	0	0	0
	2015	1	0	1	0	0	1	0	1	0	0
	2016	1	1	1	1	0*	1	1	1	1	0*
Number of Plots		6	6	6	6	6	6	6	6	6	6
Number of Fires		3	2	2	1	0	3	2	2	1	0

Table 8: Final SEM pathway coefficients and justifications. For each pathway in the model, the table identifies the response variable(s), explanatory variable(s), standardized estimate (effect size), standard errors, P-values for significance, R-squared estimate for model pathway, and justification for inclusion in the final model.

Response Variable	Explanatory Variable	Stand. Estim.	Stand. Err.	P-value	R^2	Justification
Microbial Decomposition Rate Constant (k)	# of Fires	-0.517	0.123	<0.001***	0.325	Ficken and Wright 2017 pine fuels are more recalcitrant than non-pine fuels Peay et al. 2009
	Pine Proximity	-0.268	0.236	0.021**		
	Surf. Dur. > 60	0.169	0.129	0.181		
Maximum Instant Surface Temp. Increase (°C)	# of Fires	0.546	0.097	<0.001***	0.441	presence of fire = hotter temperatures Ellair and Platt 2013
	Pine Needle Fuels	0.335	0.11	0.002**		
Maximum Instant Soil Temp. Increase (°C)	Max Inst. Surf. Inc.	0.645	0.091	<0.001***	0.416	Peay et al. 2009
Surface Fire Duration > 60°C (sec)	# of Fires	-0.223	0.067	0.001***	0.843	smaller fuel loads w/ increased fire frequencies Bárcenas-Moreno and Bååth 2009
	Max Inst. Surf. Inc.	1.027	0.073	<0.001***		

Total Soil Carbon (%)	# of Fires	-0.252	0.13	0.08*		Czimczik et al. 2005
	Max Inst. Surf. Inc.	0.241	0.146	0.102	0.06	Johnson & Curtis 2000
	Surf. Dur. > 60	-0.279	0.121	0.032**		Johnson & Curtis 2000
Total Soil Nitrogen (%)	# of Fires	-0.551	0.111	<0.001***	0.304	Christensen 1977
Inorganic Soil Phosphorus (ppm)	# of Fires	-0.58	0.132	<0.001***		Butler et al. 2018
	Max Inst. Surf. Inc.	-0.494	0.304	0.071*	0.519	Butler et al. 2018
	Surf. Dur. > 60	0.861	0.252	<0.001***		Butler et al. 2018
NO_3^- (ppm)	# of Fires	-0.368	0.15	0.008**		Christensen 1977
	Max Inst. Surf. Inc.	-0.686	0.348	0.02**	0.447	Raison 1979
	Surf. Dur. > 60	1.094	0.289	<0.001***		longer, low intensity fires release more N
NH_4^+ (ppm)	# of Fires	-0.774	0.1	<0.001***	0.526	Christensen 1977
	Surf. Dur. > 60	0.195	0.096	0.041**		longer, low intensity fires release more N
Pine Needle Fuels (g)	Pine Proximity	0.53	0.217	<0.001***	0.281	more pine needles near pines

Chapter 2 - Figures

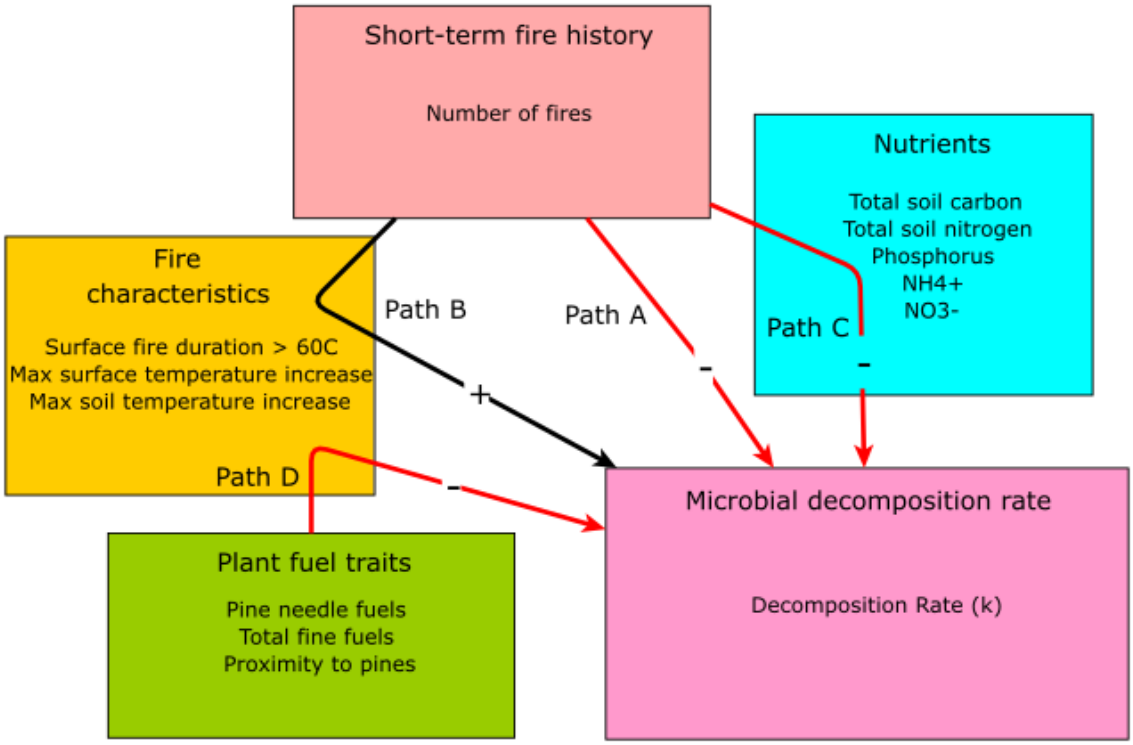

Figure 6: Hypothesized pathways by which short-term fire history modifies microbial decomposition of fine fuels. Fire History shown in light red, nutrients in blue, fire characteristics in orange, fuel traits in green, and decomposition in pink. Path A: Increasing recurrence of fire should slow decomposition through repeated negative effects on microbes. Path B: Frequent fires should lessen fire severity characteristics and the negative effect of fire on decomposition. Path C: Frequent fires alter nutrient availability, which could lead to nutrient loss and slow decomposition. Path D: Distinct from fires, increasing amounts of fine fuels should increase fire severity characteristics and slow decomposition. citations for hypothesized pathways are detailed in Ch.1 appendix table S1.5.

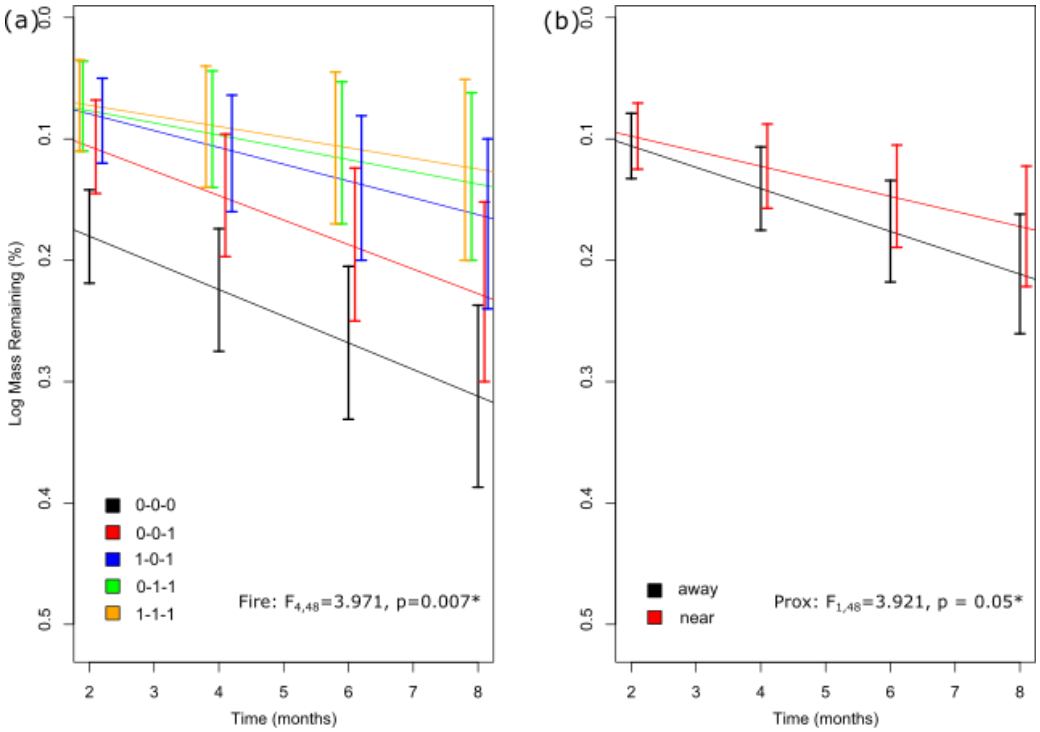

Figure 7: Short-term fire history and pines proximity effects on microbial decomposition. Trend lines represent microbial decomposition rate constants (k) calculated by fitting decomposition measurements in each plot to a negative exponential decay function. Error bars and points represent 95% confidence intervals and means for microbial decomposition rate constants (k). ANOVA results are annotated in the figures, fire = fire history and prox = pine proximity. a) As fires became more frequent, decomposition rate constants (k) were lower, and larger amounts of plant fuels remained at the end of the experiment. b) Experimental sites located near pines had lower decomposition rate constants (k) than sites located away from pines. Note that * $p \leq 0.05$.

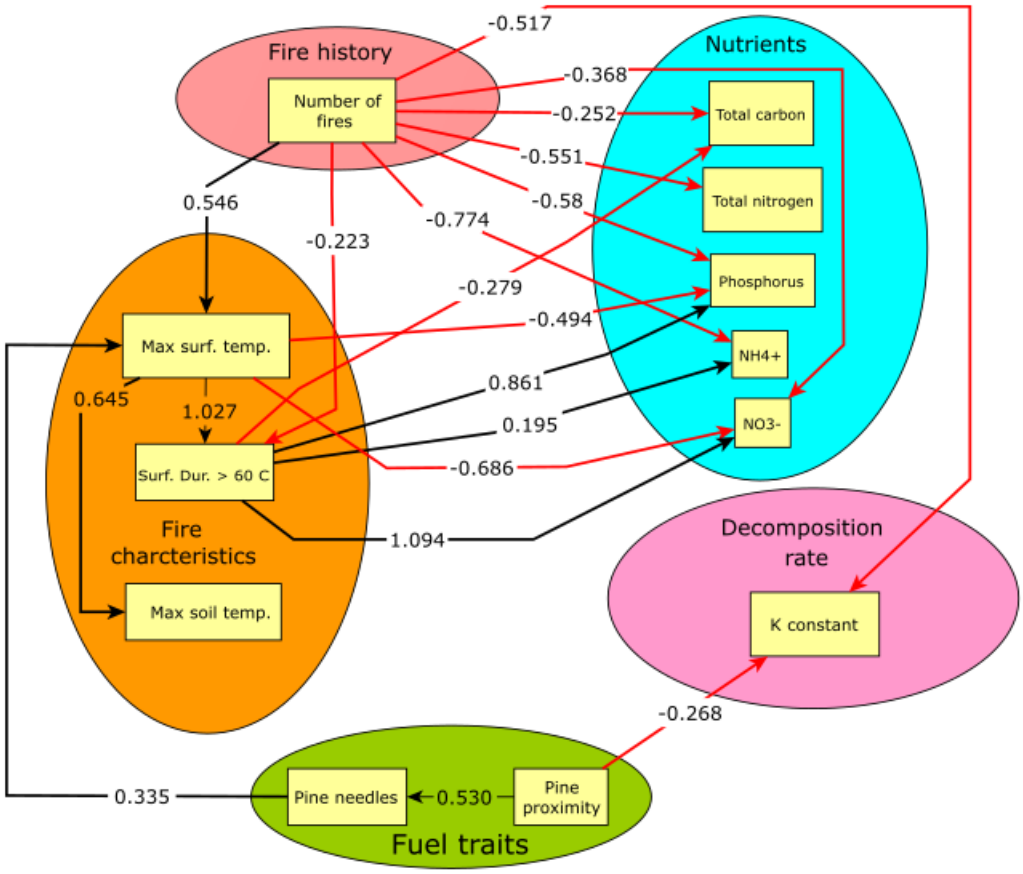

Figure 8: SEM model for short-term fire history's effect on microbial decomposition. Components are group by color as in Figure 1. Coefficients are standardized regression coefficients. Red and black paths denote negative and positive associations between linked variables respectively. Increasingly frequent fires were associated with lower microbial decomposition rates, however fire history related effects on fire characteristics and nutrients did not affect decomposition. Additionally, sites located near pines had lower decomposition rates than sites located away from pines.

Chapter 3 - Fire temperature and duration determine microbial decomposition in a fire frequented ecosystem

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Abstract

Fire is a common disturbance in more than 40% of Earth's terrestrial ecosystems that alters biotic processes and the local environment. Since climate change models predict an increase in the intensity of future fires, understanding how fire intensity modifies ecosystems is crucial for predicting climate driven shifts to the ecological dynamics of systems. The intensity of fire is primarily governed by plant fuel loads, therefore fire intensity related effects on plant communities and processes that directly modify plant fuel loads may drive feedbacks on future fires. One such process is microbial decomposition, which directly controls the amount of above ground plant fuels and is likely affected by fire intensity. We hypothesized that increasingly intense fires would slow the microbial decomposition of new plant fuels and increase fuel build-up for a given fire return interval. We designed six fuel treatments that mimicked the effect of increasingly intense fires, and measured above ground microbial decomposition the year following fires. Increasing the intensity of fire (e.g. temperature and duration) had a negative impact on above ground microbial decomposition, however, it did not appear that this was due to differential effects of fire on microbial groups (i.e. bacteria and fungi). We then used structural equation models (SEM) to observe fire energy release and microbial decomposition in a broader context which included nutrient availability and plant fuel type. Microbial decomposition responded negatively to longer

and hotter fires, and positively to nutrient availability, however, there was no relationship between fire energy release and nutrient availability. Additionally, sites near longleaf pine trees (*Pinus palustris*) had higher mass loss soon after fires and lower mass loss later relative to sites located away from longleaf pines. This suggests that intense fires can slow microbial decomposition and cause post-fire plant fuels to accumulate faster. Increased plant fuel loads in turn could increase the intensity of future fires and establish a positive feedback mechanism that favors recurrent fires in ecosystems.

Introduction

Fire modifies biological communities and biogeochemical processes in 20% of Earth's terrestrial ecosystems (Archibald *et al.* 2018). Although often considered cataclysmic events like wildfires in the Western United states and Australia, recurrent, low intensity fires are common in approximately 23% of terrestrial ecosystems (e.g. grasslands and savannas; Ford 2009). Across ecosystems, climate change models (Liu & Wimberly 2016; Schoennagel *et al.* 2017) and human influence (Balch *et al.* 2017) predict an increase in the frequency and intensity of future fires, which could have profound effects on ecological processes like plant fuel production and nutrient cycling. While manipulating fire intensity is dangerous and often impossible in most ecosystems, fire recurrent, pyrophilic grasslands and savannas may provide a model (Semenova-Nelsen *et al.* 2019; Hopkins *et al.* 2020; Hopkins *et al.* 2021) for testing fire intensity related questions due to their long term adaptation to fire (Bowman *et al.* 2009; Pausas 2015; Archibald *et al.* 2018).

In pyrophilic grasslands and savannas, the intensity of individual fires modifies biological communities and their environment in ways that can affect future fires. The relationship between fire intensity and plant communities is particularly important as the intensity of future fires may

be affected if the amount or type of plant fuels is changed (Ellair & Platt 2013; Platt *et al.* 2016; Cornelissen *et al.* 2017). For example, if fire favors species that can endure large heat fluxes like the longleaf pine (*Pinus palustris*), then this could increase the amount of flammable pine needle fuels and raise the intensity of future fires (Ellair & Platt 2013; Platt *et al.* 2016; Pausas 2017). Additionally, fire intensity is closely linked to the combustion of plant fuels, which can directly determine the intensity of future fires through residual fuel loads (Shinneman & Baker 1997; Barker & Price 2018). Plant fuel loads are also governed by other biotic processes like microbial decomposition however, which could be both affected by and drive feedbacks on fire intensity depending on how decomposers alter the quantity of new and residual plant fuels (Cornelissen *et al.* 2017). Therefore, understanding fire intensity's role in ecosystems requires consideration of plant fuel dynamics and microbial function.

Microbial decomposition influences plant fuel loads through the decay of plant matter, and is likely governed by fire characteristics related to intensity. Fire characteristics generally associated with intensity (e.g. heat flux and burn duration) are known to increase microbial mortality (Bárcenas-Moreno & Bååth 2009; Glassman *et al.* 2016), which could hinder microbial decomposition of new plant fuels if surviving litter and soil microbes are less efficient decomposers. Additionally, fire has differential effects on decomposer groups, which could further slow decomposition since fungi, the dominant decomposers in most ecosystems, are more susceptible to large heat fluxes than bacteria (Hamman *et al.* 2007; Bárcenas-Moreno & Bååth 2009). While it is difficult to determine the strength of decomposition effects in relation to fire intensity, Hopkins *et al.* 2020 showed that larger heat fluxes and longer durations above 60°C slowed the decomposition of plant fuels following prescribed burns. This suggests that fire

characteristics associated with fire intensity govern microbial decomposition, and that these fire characteristics may drive feedbacks on future fires.

Soil nutrient availability is closely linked to microbial decomposition and fire characteristics associated with intensity. Apart from direct effects of fire characteristics on microbial decomposers, fire can indirectly effect microbial decomposition by changing the availability of carbon (C) and nitrogen (N) (Raison 1979; Johnson & Curtis 2001). Low intensity fires can increase above and belowground C and N availability, favoring microbial decomposition and potentially decreasing the intensity of future fires due to increased decomposition of residual and new plant fuels. However, high intensity fires can lead to nutrient loss through oxidation, volatilization, ash transport, and leaching, which may favor higher intensity future fires due to decreased microbial decomposition of new, post-fire plant fuels (Certini 2005). Furthermore, microbial decomposition is linked to stoichiometric nutrient ratios like C:N, with lower C:N ratios favoring decomposition (Manzoni *et al.* 2010). Depending on the intensity of fires, decomposition could potentially increase at low intensities due to nitrogen mineralization (low C:N), or decrease at higher intensities as nitrogen volatilizes relatively easily around 200˚C (higher C:N; Raison 1979). Since fire intensity, nutrient availability, and microbial decomposition are likely linked, understanding how they interact is crucial to comprehending the mechanisms that govern pyrophilic ecosystems and fire intensity.

Fire intensity likely has immediate and long-term impacts on microbial decomposition and nutrient availability. The length of time that microbial decomposition is affected following fire may be a function of fire induced microbial mortality and post-fire microbial recovery rates (Hedo *et al.* 2015; Muñoz-Rojas *et al.* 2016), with higher intensity fires affecting greater mortality and slower recovery than less severe fires (Dooley & Treseder 2012). Increasingly intense fires may

also slow microbial decomposition for long periods following fire (i.e. 2+ months) due to nutrient loss (Butler *et al.* 2019). While nutrient availability increases immediately following fires, leaching, surface erosion, and nutrient uptake by plants may decrease nutrient availability with time. Due to the inherent complexity of interactions between fire and microbial decomposition, and their potential to change with time, quantifying the relationship between fire intensity and microbial decomposition requires a holistic approach capable of accounting for parallel and interacting ecological processes.

We manipulated fire characteristics associated with intensity in an old-growth Longleaf pine savanna to evaluate the effect of fire intensity on the microbial decomposition of post-fire plant fuels. We hypothesized that as fire temperature and duration increased, fires would slow microbial decomposition through mechanisms related to fire characteristics, nutrient availability, and natural variations in the type of plant fuels. We also hypothesized that fire intensity treatments would affect microbial decomposer groups differently, and alter their ability to decompose new plant fuels. We used mesh litter bags to measure microbial decomposition of new, post-fire fine plant fuels, and also applied three antibiotic treatments (anti-fungal, anti-bacterial, and water control) to explore differential responses of microbial decomposer groups to fire characteristics. Decomposition was assessed both near and away from overstory *Pinus palustris* trees to reflect differences in plant fuels and microbial communities. We first assessed the effect of fire intensity on microbial decomposition following prescribed fires in 2017. Then structural equation modeling (SEM) was used to observe the relationship between intensity and decomposition from an ecological perspective, which took nutrient availability, proximity to overstory *P. palustris*, and time since fire into account. This allowed us to identify mechanisms through which intensity

related fire characteristics shifted above ground microbial decomposition and altered plant fuel loads.

Methods

Field Site: We conducted our study in old-growth pine savanna on the Wade Tract (30˚ 45' N; 84˚ 00' W; Thomas County, Georgia, USA). Situated on moderately dissected terrain 25-50 m above sea level in the headwaters of the St. Marks River in the Red Hills region of northern Florida-southern Georgia, the 85 ha Pliocene-aged site contains Ultisol soils characterized by surface sands underlain by a clay-rich horizon (Typic and Arenic Kandiudults; Carr *et al.* 2009; Levi *et al.* 2010). The open savanna/woodland physiognomy is characterized by overstory pines and diverse herbaceous-dominated ground layer vegetation.

Management actions over the past century have maintained an old-growth population of longleaf pine (*Pinus palustris*) on the Wade Tract. Prescribed fire management has 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 & Brueckheimer 2012b). Records indicate 25 fires in each of the two burn units encompassing the site during the 3.5 decades between 1982 and 2016. Return intervals within the two fire management units averaged 1.5 years, with 90% occurring between mid-March and late June. Since protection by a perpetual conservation easement in 1978, vehicular traffic has been kept out of the area.

2017 Field Plots: The study was initiated following 2017 prescribed fires. The two fire management units were burned on March 23rd (Keetch-Byram Drought Index = 150) and April 12th (Keetch-Byram Drought Index = 105) using drip torches along a central access road. Fires

were ignited late morning, with winds of 11.3-27.4 km/hr and relative humidities of 37-83%. Flaming lengths were typically in the range of 0.5-1.5 m high. Fine fuel consumption in burned patches was estimated to be 59-66%.

Using GPS maps, 8 experimental patches of ground layer vegetation that were >5m^2 were chosen. Four of the experimental patches were located within 10 m of overstory pines, and the other four were located at least 10 m away from the nearest overstory pine. Within each of the eight experimental patches, twelve 4 m^2 plots were established and received 1 of 6 fuel manipulation treatments described in the next section. After plot establishment, we GPS mapped each plot and marked corners with flags and aluminum id tags for future relocation. This resulted in 96 total plots, with 48 located both near and away from overstory pines.

Fire Intensity Treatments: Fire intensity treatments were designed for "near pines" and "away from pines" plots by measuring the amount of pine needle fuels, non-pine needle fuels, and total fine fuels for 20 near and 20 away fuel test plots (Ch.3 Appendix 1). The weights were then averaged and used to create 6 fuel manipulation treatments (listed in order of decreasing intensity): 2x pine needle addition, 1x pine needle addition, fuel swap (proportions of pine needle and non-pine fuels were swapped between near and away plots), a reference treatment (average amount of pine needles and non-pine fuels), fuel removal (all pine needle fuels removed), and unburned (did not burn during 2017 prescribed burns). The fuel manipulation treatments were evenly applied to plots 1-2 days before prescribed burns for each fire management unit to minimize the impact of precipitation. Note that in the structural equation model, fire intensity treatment was coded as an ordinal variable ranked in terms of increasing energy release. Specifically, unburned as 1, fuel removal as 2, reference as 3, fuel swap as 4, 1x pine needle addition as 5, and 2x pine needle

addition as 6. This variable was used in early SEM model fitting steps to verify that the fuel manipulation treatments altered fire characteristics associated with intensity appropriately.

Quantifying Fire Characteristics: Fire characteristics were measured in the 2017 fires based on procedures developed by Ellair & Platt 2013 and Gagnon *et al.* 2015. We placed two thermocouples in the center of each plot on the day before the fire. One was placed just above the ground surface, but not contacting litter or soil; the second was placed 1 cm below the soil surface close to the surface thermocouple. These thermocouples were attached to data loggers by cables, and recorded temperatures every second from the time of activation (several hours prior to ignition of prescribed fires) until collection several hours after passage of flaming fronts during the prescribed fires.

Three variables extracted from temperatures recorded over time by data loggers were used in analyses. Maximum surface and soil temperature increases were calculated as the largest instantaneous temperature increases during the 2017 prescribed fires. Duration of heating was calculated as the time (seconds) that the surface temperature remained >60°C; this temperature is widely used as a threshold for lethal effects on plant tissues (Platt *et al.* 2016).

Microbial Decomposition Bags: We collected approximately 5 kg of recently deposited, intact dead plant material outside the 4 m2 sample areas of each patch prior to 2017 prescribed burns. This new litter included pine needles, grass culms, forbs, and oak leaves produced that year; partially decomposed litter on the ground surface was not included. Additionally, the collected litter was kept separate depending on proximity to longleaf pines. All collected litter was shipped to the University of Kansas where it was stored at 4°C for less than 1 week until processed. The plant litter was dried at 65˚C for 72 hours, ground using a Model 4 Wiley Mill (Thomas Scientific, Swedesboro, NJ) with a 6mm opening, and then sterilized via gamma irradiation to ~32 kGy at the

Penn State Radiation Science & Engineering Center. Using a sterile hood, we then placed sterilized plant litter in 15 x 15 cm, 30 µm nylon mesh bags, following procedures of Robertson & Paul 2000. This mesh size is sufficient to exclude non-microbes, and thus measure only microbial decomposition of plant litter (Bradford *et al.* 2002). Each decomposition bag was filled with approximately four grams of plant litter collected from either near or away from pines. Initial mass for each bag was recorded before and after litter was added; then bags were heat sealed and stored in sterile plastic bags until deployment in plots. Note that decomposition bags serve a dual purpose, as they allow for consideration of the effect of proximity to pines and different plant fuel contents (i.e. pine vs. non-pine) on above ground microbial decomposition.

Antibiotic Treatments: Captan PESTANAL® (99% pure), bronopol PESTANAL™ (≥98% pure), and distilled water (pH 7), were used as the anti-fungal, anti-bacterial, and sterile control treatments respectively based on (Bailey *et al.* 2003). Antibiotics were applied at concentrations of 0.016 g/L for captan and 0.008 g/L bronopol (Shepherd *et al.* 1988; Díaz Dellavalle *et al.* 2011). Bags receiving the anti-fungal treatment were soaked in the Captan solution for 20 minutes to ensure proper saturation and labeled with a green zip tie. Bags receiving the anti-bacterial treatment were soaked in the bronopol solution for 10 minutes and labeled with a white zip tie. Finally, the control set of bags were dipped in DI water for 10 minutes and left unlabeled. After bags were dipped, they were kept at 4˚ Celsius until deployment. At the 2, 4, and 6-month bag collection dates, 25 mL of the appropriate anti-biotic solution was reapplied evenly to the upward facing bag surface without disturbing bag contents.

Decomposition Bag Deployment and Collection: Bags were placed in the plots in May 2017, following the 2017 prescribed burns. Twelve decomposition bags (4 anti-fungal, 4 anti-bacterial, and 4 control), each with litter corresponding to local pine overstory conditions, were randomly

selected, and placed on the soil surface in each plot. Bags were placed among the bases of vegetation in plots so that one flat surface of the bag contacted bare soil. All bags were anchored along margins with 5 cm sod-staples to ensure that they remained in the plot and stayed in contact with litter and soil surfaces. From within each bag treatment group, one bag was collected approximately 2, 4, 6, and 8 months after the 2017 prescribed fires. Any soil or litter on the bags was carefully removed, and then bags were placed in sterile plastic bags. Collected bags were shipped overnight to the University of Kansas to be analyzed. Once received, litter contents were carefully removed, dried at 65˚C for 72 hours, and then weighed to determine percent mass loss.

Soil analysis: Soil samples were collected from all plots in 2017. Soil was collected at three randomly located points just outside each $4m^2$ plot, shifting locations slightly to avoid bases of established ground layer plants. We removed litter carefully, then collected the upper 1.5 cm of soil within a 9 x 9 cm quadrat (soil depth where heating effects begin to decrease during prescribed fires). We removed obvious roots from the samples. All equipment used to collect samples was sterilized with 10% bleach and 90% isopropyl alcohol between sampling of plots. The three soil samples from each plot were combined and kept cool with freezer packs after collection, frozen at -20°C until shipped overnight to the University of Kansas, and stored at -20°C upon arrival. Samples were thawed and homogenized by hand using sterile technique (within sealed bags).

A 100 g subsample was sent to the Kansas State University Soil Testing Lab for analysis. Soil phosphorus content was measured using the Mehlich-3 method on a Lachat Quickchem 8000 (Lachat Instruments, Loveland, USA; Mehlich 1984). Total soil nitrogen and carbon samples were measured on a LECO TruSpec CN Carbon/Nitrogen combustion analyzer (LECO Corporation, St. Joseph, USA). NH4+ and NO3- were extracted using 1 M KCl on 2 g of soil, then Cadmium reduction for nitrate and colorimetric procedures was used, followed by flow analysis for ion

quantification (Brown 1998). C:N ratio as obtained by dividing the amount of total soil carbon by the amount of total soil nitrogen. Soil pH was determined using a 1:1 soil-distilled water slurry, where each slurry was assayed 3 times (probe cleaned between trials), and results were averaged to attain sample pH.

Data Analysis: All analyses were completed in R 4.0.1 (R Core Team 2020). Linear mixed effect models available in the "lme4" (LMERs; Bates *et al.* 2015) were used to assess the effects of fire intensity treatment, location in proximity to pines, and antibiotics, on microbial decomposition. For random effects, we controlled for plot, patch, and fire management unit (side) level differences, with a three level nested structure (plot within patch, patch within fire management unit). Model fitting for LMERs at all time points started with fully factorial models and removed main and random effects until model fit criteria (AIC and BIC) were minimized. Following LMERs, type III ANOVAs with the Satterthwaite Method were used to evaluate main effects, and then estimated marginal means in the "emmeans" (Lenth 2018) package were used to evaluate significant ($\alpha = 0.05$) pairwise differences between experimental treatments.

We then explored possible mechanisms underlying fire driven shifts to microbial decomposition using structural equation modeling (SEM). Based on existing literature, we hypothesized specific pathways relating fire intensity and microbial decomposition that included direct effects of fire on microbes, as well as indirect relationships mediated through fire driven changes to soil properties. We also hypothesized that proximity to overstory longleaf pines might play an independent role in determining decomposition due to differences in plant fuel loads. Initial model pathways are shown in Figure 9. Specifically, we hypothesized that 1) heat flux and burn duration related fire characteristics would govern the energy released from individual fires (Figure 9, path A) 2) increasing fire energy release would be associated with decreased

decomposition (Figure 9, path B), and 3) higher fire energy release would decrease nutrient availability, and slow decomposition (Figure 9, path C). We also hypothesized that 4) proximity to pines, although independent of fire energy release, would be associated with slower decomposition due to the recalcitrant nature of pine needle fuels (Figure 9, path D). Our SEM contained categorical, continuous, and ratio variables, which described fire intensity treatments, 2017 prescribed fire characteristics, fuel treatment data, and plant litter decomposition data within plots. Important to note, antibiotic treatments were not included in the SEM due to inherent complications with non-ordinal factor variables. All continuous variables were scaled and transformed appropriately prior to analysis. For a description of model variables see Table 9. After developing an initial hypothesis for model structure, the R Package: "lavaan" was used to evaluate the preliminary SEM for convergence (Rosseel 2012). Upon convergence, a goodness of fit guided approach was used to assess the further modification of model parameters. Models were then evaluated using test statistics suggested by Hooper *et al.* 2008. See the chapter 3 appendix for full model fitting details.

Results

Effect of fire intensity treatment, proximity to pines, and antibiotics on microbial decomposition: As hypothesized, increasingly intense fires had negative effects on microbial decomposition. Increasing fire intensity slowed microbial decomposition at 2 ($F_{5, 285} = 3.186$, $P = 0.012$; Figure 10a; Tables 10, 11), 4 ($F_{5, 285} = 4.012$, $P = 0.003$; Figure 10c), and 8 ($F_{5, 286} = 2.3855$, $P = 0.0482$; Figure 10d) months. At 2 and 4 months, all intensity treatments had lower mass loss than unburned plots ($P < 0.05$). At 4 months, the 2x addition treatments had lower mass loss than the fuel removal plot as well as the unburned plot ($P < 0.05$). At 8 months, only the 1x and 2x additions had lower

mass loss than the unburned treatment plots ($P < 0.05$). Also at 8 months, the 2x additions had lower mass loss than the reference and fuel swap plots ($P < 0.05$).

The antibiotic treatments had a significant effect on decomposition early in the study. At the 2-month collection point ($F_{2, 285} = 5.213$, $P = 0.006$; Tables 10, 11; Figure 10b), mass loss was higher in the anti-fungal treatments than the anti-bacterial and control treatments ($P < 0.05$). The anti-bacterial and control treatments were not statistically different.

Proximity to pines did not have a significant effect on microbial decomposition throughout the study, and overall model fit was often improved upon its removal.

In summary, fire intensity treatments had the strongest effect on microbial decomposition in the study, with more intense treatments (1x and 2x additions) slowing decomposition the most. Antibiotics altered decomposition at the first collection point, however, later in the study they did not have a discernable effect on the decomposition of plant fuels.

Structural equation modeling of causal pathways for fire intensity effects: Given the scope of our data, we developed a structural equation model to assess the causal pathways by which fire intensity impacted above ground microbial decomposition. Since fire intensity was not directly measured, we modeled it as a "fire energy release" latent variable described by maximum surface and soil temperature increases and surface fire duration > 60˚C. While we were specifically relating fire intensity to above ground microbial decomposition, we included both surface and soil fire characteristics in our fire intensity model. We took this approach, because we expected that larger heat fluxes and longer burn durations would negatively affect microbial decomposers entering decomposition bags from residual fuels and the soil. Initial analysis began with the fire intensity latent variable construction, and included a covariance structure between maximum surface temperature and surface duration > 60˚C. This model converged and was a good fit to the

data (MFTS = 0.013, D.F. = 1, P = 0.911). Following latent variable construction, a highly saturated model containing all variables described in table 9 was created by including mechanisms through which we hypothesized that fire intensity would modify microbial decomposition (Ch.3 App. Table S1). The model fitting approach sought to minimize the MFTS, while achieving a χ^2 p-value greater than 0.05. The initial model converged, but was not well supported (MFTS = 156.382, D.F. = 19, P = 0), so a reductive model approach where non-significant pathways and variables were removed was employed. Following several iterations, a final, well supported model was determined (MFTS = 35.382, D.F. = 26, P = 0.104). A description of this model is available in table 2. Model fit statistics (Ch.3 App. Table S2) were assessed for all models according to (Hooper *et al.* 2008). See the chapter 3 appendix for more details on SEM model fitting.

SEM results: The final SEM model (Table 12, Figure 11) supported our hypotheses that fire intensity governs the microbial decomposition of fine fuels. Furthermore, the model also detailed other mechanisms that govern above ground plant fuel decomposition.

Direct Impact of fire intensity: Fire intensity was associated with microbial decomposition at all time points in the study, as in the LMER analysis above. Specifically, as fire intensity increased, microbial decomposition slowed. The model supported a direct pathway for these effects at 2 (-0.38) and 4 months (-0.18), and indirect pathways at 6 (-0.13) and 8 (-0.15) months. In summary, increasingly intense fires were associated with decreased above ground microbial decomposition throughout the study.

Edaphic and Pine Proximity Effects: While not related to fire intensity, total soil carbon and nitrogen were strong predictors of microbial decomposition. Total soil carbon was an important predictor of increased decomposition across all time points in the study with direct effects at 2 (0.22) and 6 (0.11) months, and weak indirect effects at 4 (0.05) and 8 (0.1) months. Additionally,

soil carbon was positively related to soil nitrogen (0.9), which was related to increased microbial decomposition at 4 months (0.15), and indirectly related to increased microbial decomposition at 6 (0.05) and 8 months (0.05).

Proximity to pines was also associated with microbial decomposition at all time points in the study. Specifically, bags located near pines saw more decomposition at two months (0.22) and slowed decomposition at 6 months (-0.16). Proximity to pines also had weak, but positive effects on decomposition at 4 (0.05) and 8 months (0.01). While not directly related to the fire intensity, increased nutrient availability and proximity to pines were important predictors of microbial decomposition depending on time since fire.

Discussion

Microbial decomposition was slower in intensely burned sites following 2017 prescribed fires and was regulated by soil fungi early after fires. The differences in plant fuel decomposition associated with fire energy release were likely caused by augmented microbial mortality due to larger heat fluxes and longer burn durations (Bárcenas-Moreno & Bååth 2009; Dooley & Treseder 2012). Fire intensity driven effects on microbial communities are consistent with Ficken and Wright 2017, Semenova-Nelsen *et al.* 2019, and Hopkins *et al.* 2020, which found that fire reduced microbes ability to decompose new plant fuels. Independent of fire intensity, antibiotic treatments revealed a suppression of bacterial saprotrophs by fungi early after fires. Despite superior survival of bacteria relative to fungi following intense heating and fires (Hamman *et al.* 2007; Bárcenas-Moreno & Bååth 2009), the higher decomposition in anti-fungal bags suggested that fungi that survive fire can suppress post-fire bacterial function. This suggests a novel mechanism that could alter plant fuel load dynamics following fire if fire resistant fungi suppress decomposition of new

plant fuels. Taken together, this implies that fire intensity is an important driver of microbial function in pyrophilic ecosystems, and that fungi can regulate post-fire plant load dynamics.

Surprisingly, fire characteristics related to intensity, microbial decomposition, and nutrient availability did not interact as strongly as predicted. The lack of interaction between fire intensity and nutrient availability is unexpected, but not entirely surprising as fire is known to have differential effects on nutrients in fire frequented, pyrophilic ecosystems (Coates *et al.* 2018). Additionally, fires may not have been intense enough to drive significant carbon and nutrient loss, as commonly occurs following wildfires (Neary *et al.* 1999; Certini 2005). Despite the lack of interaction with fire, higher amounts of nitrogen (N) and carbon (C) were associated with increased decomposition across most of the study, which may be due to increased nutrient availability or C:N ratios favorable for decomposition (Perez-Harguindeguy *et al.* 1999; Manzoni *et al.* 2010). Important to note, it is likely that fire effects on nutrient availability will be stronger in less fire frequented ecosystems, which have larger fuel loads (Shinneman & Baker 1997; Kalies & Yocom Kent 2016) and higher intensity fires that can have highly destructive effects on below ground processes and nutrient availability (Neary *et al.* 1999).

Independent of fire characteristics related to intensity, proximity to pines had differential effects on microbial decomposition depending on time since fire. Higher mass loss near pines as opposed to away from pines sites soon after fires was unexpected, as longleaf pine needles are commonly considered to be resistant to decomposition due to their high lignin content (Wardle *et al.* 2002; Cornelissen *et al.* 2017). The larger amounts of pine needles in plots near longleaf pines may explain this effect though, as pine needle fuels favor higher fuel combustion (Platt *et al.* 2016), which may have caused a larger flux of nutrients following fire that favored decomposition (Neary *et al.* 1999). This effect decreased with time however, likely due to nutrient availability returning

to pre-fire levels (Kutiel & Naveh 1987), and the recalcitrant nature of longleaf pine needles acting as the governing factor of decomposition (Wardle *et al.* 2002). This highlights the importance of considering plant fuel traits in fire intensity and fire-climate models, as the type and quantity of plant fuels are important predictors of microbial decomposition and feedbacks on future fires.

Although fire intensity, nutrient availability, and proximity to pines are important determinants of microbial decomposition, it is apparent that the strength of these effects change with time since fire. Early after fire, the energy released by fire appears to be the dominant factor controlling microbial decomposition, but this effect gradually weakens with time. As the effect of fire intensity weakens, other factors like nutrient availability and proximity to pines take precedence, which is consistent with other studies that found decreasing effects of fire on microbial communities with time since fire (Bárcenas-Moreno *et al.* 2011; Hedo *et al.* 2015). Additionally, the increasing importance of nutrient availability and pine proximity corresponds with the growing season at this site, which is a time of intense competition for nutrients and available substrates (Schmidt *et al.* 2007). By comparing the importance of fire intensity, nutrient availability, and proximity to pines across time, we provide an ecologically relevant model for understanding the short- and longer-term importance of fire intensity in ecosystems.

Linking fire intensity and microbial function expands our understanding of microbial roles in ecosystems. Specifically, we have illustrated a potential mechanism through which energy released during fire alters the microbial decomposition of plant fuels and may shift future fire characteristics due to changes in fine fuel loads. This work builds upon the findings of Semenova-Nelsen *et al.* 2019 and Hopkins *et al.* 2020, which showed the effects of fire and fire history on microbial function, by connecting another component of fire regime, fire intensity, to microbial roles in ecosystem dynamics. In addition to microbial decomposition, fire intensity likely modifies

other microbial related processes like the formation of plant-fungal mutualisms (Klopatek *et al.* 1988; Glassman *et al.* 2016) and plant-pathogen interactions (Hardison 1976). In ecosystems where fuel loads are allowed to build up, increasingly intense fires are known to burn off entire organic matter layers and expose mineral soils (Glassman *et al.* 2016). Fires of this intensity can significantly reduce ectomycorrhizal spore abundance, which could slow plant growth and fuel production. Alternatively, increasingly intense fires may favor plant growth and plant fuel production if microbial pathogen mortality is increased by fire (Hardison 1976). In this case, fire could help plants escape microbial pathogens and produce more fuels, thus increasing the intensity of future fires due to larger fuel loads. Future work must explore microbial responses to other fire regime components (e.g. season and extent of fires) to further our understanding of microbial roles in pyrophilic ecosystems. Additionally, we must acknowledge the potential of fire regime driven feedbacks on future fire characteristics in pyrophilic and non-pyrophilic ecosystems alike.

In summary, we confirmed that fire intensity shifts microbial decomposition, and that the strength of this effect changes across time. Moreover, we identify another potential mechanism through which fire intensity can influence microbial decomposition and future fire characteristics due altered plant fuel loads. This model provides a readily transferable framework, which is applicable to other ecosystems due to its inclusion of fire characteristics, abiotic factors, and time since fire. As mentioned above, there are still other factors to consider where fire regime and microbes are concerned, however, these can be easily included in our model due to the flexibility of SEM. Since fires are expected to increase in number due to climate change effects (Liu & Wimberly 2016; Schoennagel *et al.* 2017), understanding the role of microbes in fire ecology is crucial to predicting and managing future changes to fire mediated ecosystems.

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Chapter 3 - Tables

Table 9: Description of variables included in structural equation model fitting process. Table contains names, means, standard deviations, and descriptions of each variable. Note that means and standard deviations reflect pre-transformed and normalized measurements.

Variable Name	Type	SEM Code	Transformation	Description
Fire energy release	latent	none	none	latent variable described by fire temp. and duration
fire intensity treatment	ordinal	1 = unburned 2 = fuel removal 3 = reference 4 = fuel swap 5 = 1x addition 6 = 2x addition	none	ordinal variable describing fuel manipulation treatments
maximum surface temperature	continuous	none	none	max temp. increase recorded by surface thermocouple
surface duration > 60˚C	continuous	none	natural log	surface temp. duraction (sec) above 60°C
maximum soil temperature	continuous	none	log 10	max. temp. increase recorded by soil thermocouple
total nitrogen	percent	none	natural log	total organic and inorganic nitrogen
total carbon	percent	none	natural log	total soil carbon
month 2 percent mass loss	continuous	none	none	percent mass loss at 2 months
month 4 percent mass loss	continuous	none	none	percent mass loss at 4 months
month 6 percent mass loss	continuous	none	none	percent mass loss at 6 months
month 8 percent mas loss	continuous	none	none	percent mass loss at 8 months
pine proximity	categorical	near - away	none	decomp. bag proximity to overstory pines, and litter type

Table 10: Linear mixed effect model tables for microbial decomposition at each collection point. Tables contain the fixed and random effects for each model. Main effects were determined using Type III sums of squares with Satterthwaite method.

Random effects			Fixed effects			
Term	Variance	S.D.	Term	D.F.	F-value	P-value
			2 months			
plot:patch	1.87	1.368	*intensity treatment*	5	3.186	0.01**
patch	2.662	1.632	*antibiotic*	2	5.2128	0.006**
residual	13.628	3.692	*pine proximity*	1	2.4476	0.15
			4 months			
plot:patch	3.705	1.925	*intensity treatment*	5	4.0119	0.003**
patch	3.797	1.949	*pine proximity*	1	2.6203	0.14
residual	44.411	6.664				
			6 months			
plot:patch:side	18.194	4.265	*intensity treatment*	5	1.6683	0.15
patch:side	1.966	1.402	*pine proximity*	1	0.017	0.90
side	3.98	1.995	*severity * pine*	5	1.7142	0.14
residual	59.154	7.691				
			8 months			
plot:patch:side	3.06E-04	1.75E-02	*intensity treatment*	5	2.3855	0.05**
patch:side	6.53E-04	2.56E-02	*antibiotic*	2	1.0644	0.35
side	1.35E-17	3.67E-09	*pine proximity*	1	0.2939	0.60
residual	1.12E-02	1.06E-01	*intensity*antibiotic*	10	0.8426	0.59
			*intensity*pines*	5	0.4354	0.82
			*antibiotic*pines*	2	0.2876	0.75
			*intensity*anti*pines*	10	0.9578	0.48

Table 11: Pairwise contrasts for linear mixed effect model main effects on microbial decomposition. Tables contain all pairwise contrasts for significant main effects ($\alpha = 0.05$). Pairwise differences were estimated using the Satterthwaite Method. Lowercase letters in the group column denote significance groupings.

Treatment	Estimate	S.E.	D.F.	Group		
2 months						
Intensity treatment						
2x addition	10.64	0.90	77.31	a		
1x addition	11.85	0.90	76.04	a		
fuel swap	11.72	0.90	77.27	a		
reference	12.05	1.04	24.90	a		
fuel removal	12.29	0.90	75.94	a		
unburned	14.81	1.06	75.07			b
Antibiotic treatment						
anti-fungal	13.31	0.54	192.48	a		
anti-bacterial	11.63	0.53	193.32			b
control	12.05	0.65	24.90			b
4 months						
Intensity treatment						
2x addition	19.25	1.53	79.15	a		
1x addition	20.27	1.52	77.65		ab	
fuel swap	21.15	1.29	79.15		ab	
reference	20.84	1.46	26.33		ab	
fuel removal	22.75	1.52	77.54		b	
unburned	26.20	1.71	75.52			c
8 months						
Intensity treatment						
2x addition	29.49	1.83	11.39	a		
1x addition	31.26	1.83	11.39		ab	
fuel swap	34.40	1.83	11.39		bc	
reference	34.30	1.84	11.78		bc	
fuel removal	32.10	1.83	11.39		abc	
unburned	37.05	1.78	10.44			c

Table 12: Final SEM pathway coefficients and justifications. Table contains each pathway in the model with response variables, explanatory variables, effect and standardized effect of each variable, P-values for significance, R-squared estimate for model pathway, and justification for inclusion in final model.

Response Variable	Explanatory Variable(s)	Estimate	Standardized Estimate	P-value	R^2	Justification
Fire energy release	maximum surface temperature	1	0.779	na	0.606	Ellair & Platt (2013) & Platt et al. (2016)
	maximum soil temperature	0.983	0.893	0.001	0.797	Ellair & Platt (2013) & Platt et al. (2016)
	surface duration > 60˚C	1.255	0.898	0.001	0.807	Ellair & Platt (2013) & Platt et al. (2016)
total nitrogen	total carbon	0.698	0.894	0.001	0.799	linked through C:N ratio
month 2 percent mass loss	fire energy release	-0.033	-0.383	0.001	0.265	Bárcenas-Moreno and Bååth 2009
	pines	0.022	0.217	0.001		Hobbie 2000
	total carbon	0.029	0.217	0.001		Taylor et al. 1989
month 4 percent mass loss	fire energy release	-0.024	-0.181	0.001	0.167	Bárcenas-Moreno and Bååth 2009
	month 2 percent mass loss	0.349	0.222	0.001		Voříšková and Baldrian 2013
	total nitrogen	0.041	0.152	0.007		Taylor et al. 1989
month 6 percent mass loss	month 2 percent mass loss	0.246	0.135	0.027	0.164	Voříšková and Baldrian 2013
	month 4 percent mass loss	0.344	0.296	0.001		Voříšková and Baldrian 2013
	pines	-0.029	-0.162	0.005		Hobbie 2000
	total carbon	0.027	0.113	0.054		Taylor et al. 1989
month 8 percent mass loss	month 2 percent mass loss	0.272	0.124	0.028	0.232	Voříšková and Baldrian 2013
	month 4 percent mass loss	0.358	0.255	0.001		Voříšková and Baldrian 2013
	month 6 percent mass loss	0.317	0.262	0.001		Voříšková and Baldrian 2013
			Covariance Structures			
maximum surface temperature & surface duration > 60˚C	na	0.074	0.437	0.061	na	Peay et al. 2009

Chapter 3 - Figures

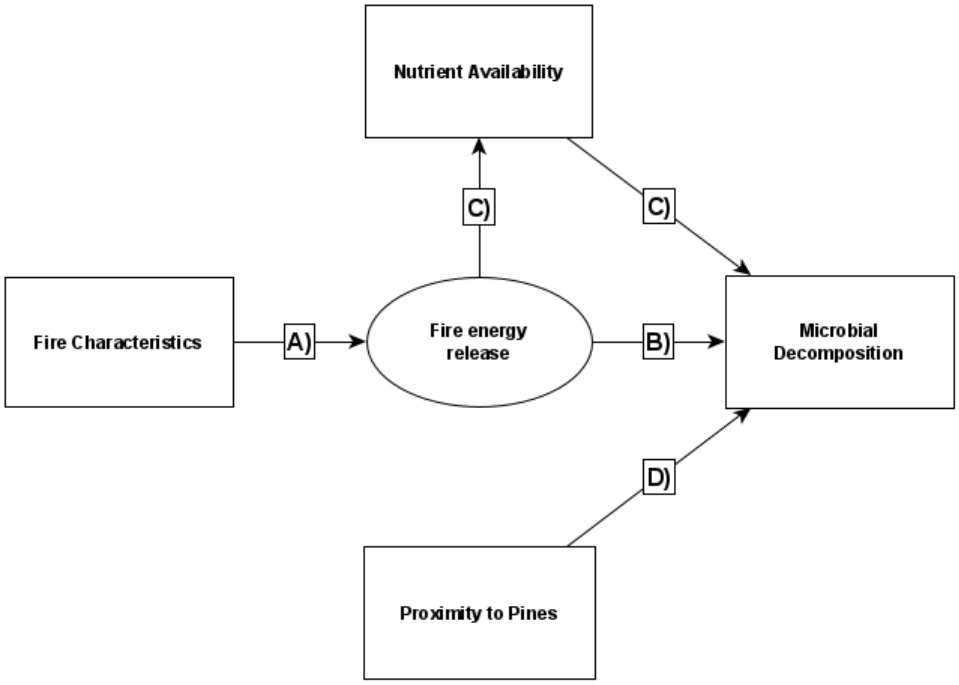

Figure 9: Hypothesized pathways by which energy release associated with fire intensity modifies microbial decomposition of new plant fuels. Path A) increasing the temperature and duration of fires will increase the energy released during fire. Path B) increasing fire energy release will slow microbial decomposition. Path C) fire energy release will alter nutrient availability and impact microbial decomposition. Path D) distinct from fire, pine proximity and litter type will alter microbial decomposition to differences in litter recalcitrance.

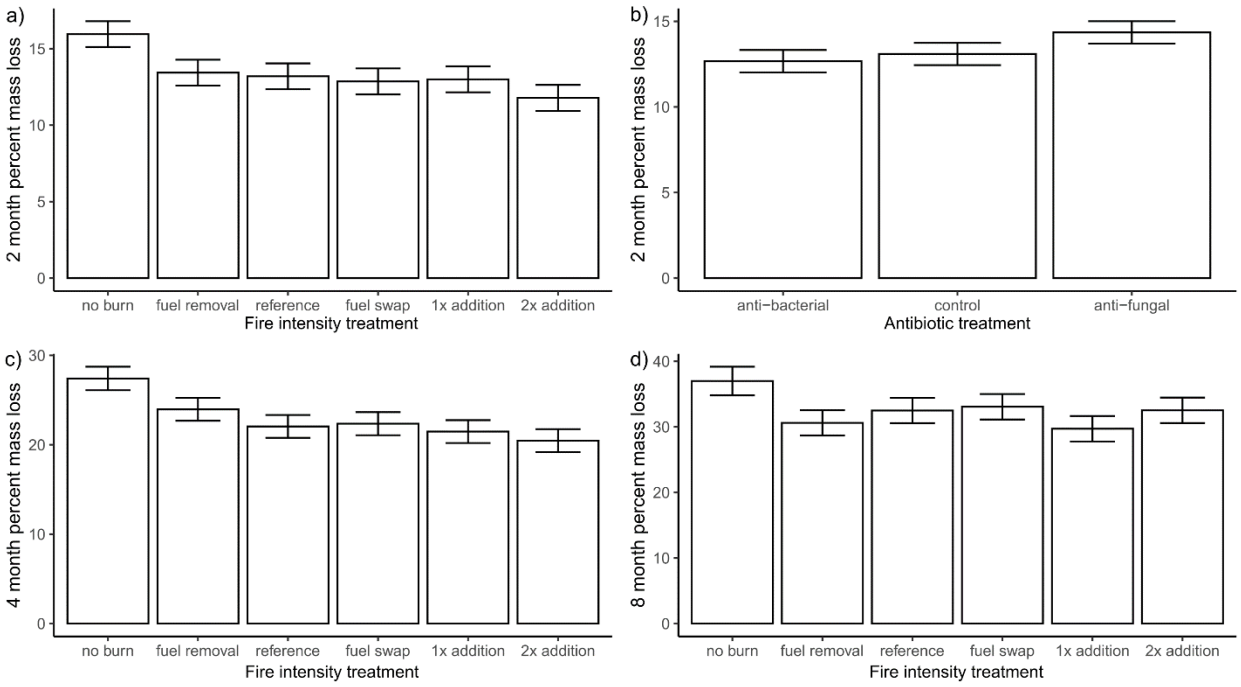

Figure 10: Effect of fire intensity and antibiotic treatments on the microbial decomposition of litter bag contents. Plots denote the mean, ± standard error. At the two month collection point, microbial decomposition a) decreased as fire intensity increased, and b) antifungal solution was added. c) At the four month collection point, microbial decomposition was lowest in the fuel addition treatments. d) At eight months, microbial decomposition was lower in burned versus unburned sites.

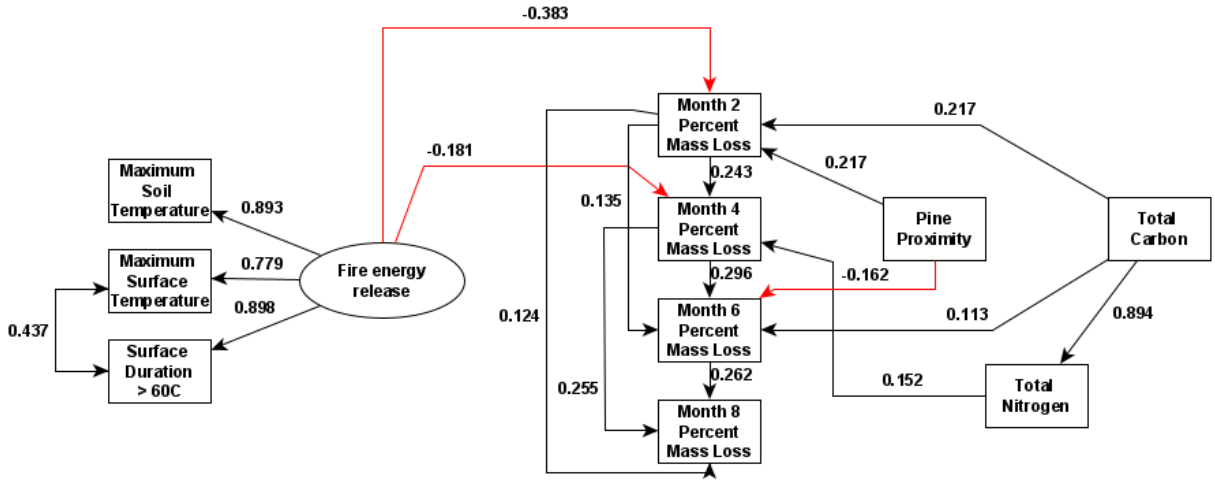

Figure 11: Final SEM and model components for fire severity's effect on microbial decomposition of fine fuels. Numerical coefficients on model paths are standardized regression coefficients. Red paths denote negative associations between linked variables, while black paths denote positive associations. Fire severity is linked to microbial decomposition at two and four months, and modifies decomposition at six and eight months through changes at prior time points. Nutrients and proximity to pines are also strong drivers of decomposition across the study despite not being linked to fire severity.

Chapter 4 - Interactions between fire and pyrophilic plants: a case study in a frequently burned Longleaf pine savanna ecosystem

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Abstract

Feedbacks between fire and pyrophilic plants engineer the recurrent, low severity fires that maintain pyrophilic ecosystems like savannas. The mechanisms that sustain fire-plant feedbacks are less clear however, but may be related to plant growth responses (i.e. fuel production) to fire associated changes in soil conditions and variations in savanna soil type (e.g. trees vs. understory). We hypothesized that pyrophilic plants would have higher germination rates than less pyrophilic species, and display rapid growth responses related to fire associated changes to abiotic (nutrient availability) and biotic (microbes) soil conditions. We further hypothesized that soil type would modify plant growth responses to fire treatments. To test these hypotheses, we conducted a greenhouse experiment using surface soil from an old-growth pine savanna exposed to low, medium, and high fire severity treatments. We compared the germination of savanna plant species from patches that burned annually (more pyrophilic plants) to species from patches that burned every two to three years (less pyrophilic plants), and then explored germination and growth responses of the more pyrophilic species to soil type and fire intensity treatments. The more pyrophilic species had higher germination rates than their less pyrophilic counterparts, and displayed varying germination and rapid growth responses to soil type and fire severity effects on biotic and abiotic soil factors. This suggests that pyrophilic plants can take advantage of post-fire conditions, and rapidly produce the fuel loads necessary for the fire regimes of pyrophilic

ecosystems. Furthermore, the varying soil type and fire severity preferences amongst plant species imply a mechanism for maintaining plant diversity in Longleaf pine savannas.

Introduction

Feedbacks between fire and pyrophilic plants produce the recurrent, low severity fires that maintain pyrophilic grasslands and savannas. Such feedbacks, prominent in warm, high-light-severity and mesic environments, are driven by plant traits that result in the rapid, post-fire production of flammable fine fuels (Singh 1993; Brewer & Platt 1994; Platt *et al.* 2016b; Beckage *et al.* 2019; Simpson *et al.* 2020). These pyrophilic traits may include enhanced germination and growth in post-fire environments, which can provide a competitive advantage over less fire tolerant species (Platt 1999; Keeley & Fotheringham 2000; Gagnon *et al.* 2010; Harms *et al.* 2017; Resco de Dios 2020) and replenish fuel loads quickly. Consequently, post-fire community-level fuel loads within 1-2 growing seasons can help fires spread, and sustain the recurrent, low severity fire regimes that favor pyrophilic taxa (Birk & Bridges 1989; Beckage & Ellingwood 2008; Beckage *et al.* 2011; Gagnon *et al.* 2015; Archibald *et al.* 2018; Schertzer & Staver 2018). How the traits of pyrophilic plants associated with growth (i.e. fuel production) are influenced by fire's effect on soil conditions and the environment needs to be examined, however.

Plant fuel production is closely linked to soil abiotic conditions that are altered by fire. Fire produces short-term flushes of mineral nutrients and pH increases that vary depending on fire severity (Certini 2005b). While C, N, and P levels generally increase after low to mid severity fires (Neary *et al.* 1999a; Butler *et al.* 2018), higher severity fires can drive net losses of these nutrients, particularly N (Raison 1979; Pellegrini *et al.* 2018). Fire also transiently

increases soil pH due to the combustion of plant fuels and denaturation of organic acids (Certini 2005). Therefore, fires in pyrophilic ecosystems may drive frequent nutrient flushes and increases in soil pH capable of stimulating post-fire germination and fuel production (Daubenmire 1968; Robson 1989; Beckage et al. 2011). The ability of pyrophilic plants to become established and produce new fuels after fires should sustain the recurrent, low severity fires that favor the persistence of pyrophilic grassland and savanna plants. Pyrophilic plant responses to fire driven soil abiotic changes may not be independent of fire-microbial interactions however, as microbes are associated with abiotic soil conditions and can alter plant germination and fuel production.

Fire effects soil microbes in ways that may modify post-fire plant-microbial interactions and fuel production. Since increases in fire severity at ground level directly increase microbial mortality (Hamman *et al.* 2007; Bárcenas-Moreno & Bååth 2009; Pressler *et al.* 2019), fuel production may be altered if microbial groups are differentially affected. For example, lower severity fires have harmful effects on pathogenic microbes in upper soil horizons (Hardison 1976; Katan 2000; Hopkins *et al.* 2021), while potentially leaving sub-surface mutualists like mycorrhizal fungi unharmed (Klopatek *et al.* 1988; Hamman *et al.* 2007). Such differential effects of fires could promote germination and fuel production, as pathogens are removed relative to mutualistic mycorrhizae. On the other hand, more intense fires may reach deeper soil horizons, killing hosts and microbes insulated in plant roots (Hamman *et al.* 2007; Dooley & Treseder 2012; Glassman *et al.* 2016). If fire's effects on microbes influence pyrophilic plant traits associated with fuel production, this could generate feedbacks on fire regimes. Interactions between fuel production and soil conditions are almost certainly spatially variable however,

particularly in savannas (e.g. trees vs. understory), such that locational effects may directly influence abiotic and biotic soil components of soil and modify fire severity.

Natural heterogeneity in soil conditions and plant fuels may play an important role in plant responses to fire. Since soil and fire characteristics are partially determined by available plant fuels (Fill *et al.* 2015; Platt *et al.* 2016; Mugnani *et al.* 2019), heterogenous plant communities such as those in savannas (e.g. trees vs. understory; Peet *et al.* 2018) could ultimately influence plant fuel production. For example, in the grass-pine matrix of Longleaf pine savannas, higher levels of soil organic carbon and lower pH are expected near pines where needlecast is higher relative to understory soils. Furthermore, differences in microbial communities are expected (e.g. arbuscular mycorrhizal understory plants vs. ectomycorrhizal pines) depending on location. These factors alone can modify plant fuel production, and may be further modified by spatial differences in fire severity (Certini 2005; Hamman *et al.* 2007; Taudière *et al.* 2017). Fires tend to be more severe near pines due to larger amounts of pine needle fuels (Platt *et al.* 2016), which could increase microbial mortality and drive nutrient flushes that effect plant responses to fire. If plant responses to different fire severities vary in ways that alter fuel production, then this could influence the fire-fuel feedbacks that maintain pyrophilic ecosystems.

Here we tested how location and fire severity impacts on soil abiotic and biotic properties impact the germination and early growth of plants from an old-growth pine savanna. Longleaf pine savannas contain grass-pine matrices, which make them broadly representative of other mesic pyrophilic grasslands and savannas, and useful for testing plant responses to fire's effect on soil conditions. In 2017, we experimentally manipulated pyrogenic fuels (shed needles) of the dominant tree species, *Pinus palustris*, in plots near and away from overstory pines, to create

three fire severity treatments (low, medium, and high). Following prescribed fires, we collected soils from the fire severity treatments, as well as seeds from pairs of pine, clonal shrubs, C4 warm season grasses, and composite forbs that occurred in annually (more pyrophilic taxa) and periodically burned (less pyrophilic taxa) sections of the savanna site. Using the collected soils and seeds, we designed a fully factorial greenhouse pot experiment that tested how locational (near vs. away from pines) and fire effects on abiotic and biotic soil components modified plant life cycle stages important in post-fire environments (germination and growth). We hypothesized that germination rates would be greater for seeds from the more pyrophilic plant taxa relative to their less pyrophilic counterparts, and that location and fire severity effects on abiotic (nutrients and pH) and biotic (microbes) soil conditions would modify plant germination rates and fuel production. Our findings suggest that pyrophilic plants can take advantage of fire in ways that promote their germination and growth in frequently burned Longleaf pine savannas.

Methods

Study System and Field Sampling: We conducted field components of our study on the Arcadia Plantation in Thomas County, GA, USA. This reserve, ~80 km north of the Gulf of Mexico, is characterized by moderately dissected terrain 25–50 m above sea level on Pliocene sediments of the Miccosukee Formation (Lawton and Friddell 1976, Sanders 1981). The soils are Ultisols (Typic and Arenic Kandiudults) characterized by sand or sandy loam A and E horizons and sandy clay loam Bt subhorizons (Robertson et al. 2019).

We used the two prescribed fire units on Arcadia Plantation that contain an exemplary old-growth conservation easement, the Wade Tract Preserve (30° 45’ N; 84° 00’ W). This easement contains a discontinuous overstory dominated by variable-aged patches of *Pinus palustris*, longleaf

pine (Platt et al. 1988. Peet et al. 2018) and a ground layer with diverse grasses, forbs, and shrubs (Mugnani et al. 2019). The fire units containing the old-growth easement are characterized by century-old stands of longleaf pine from which large trees have been periodically harvested using a single-tree selection method and an intact ground layer that has never been plowed or grazed. These fire units, historically burned every 1-3 years by lightning-ignited fires and more recently by open-woods burning, have been managed for the past several decades using annual/biennial prescribed ground layer fires (Robertson et al. 2019). Each unit has been burned in 11 prescribed fires during the 12-year period from 2009–2020 (average return interval 1.1 years). These fires have been applied from March-May with mean dates for both units in mid-April with standard deviations of about one-two weeks (Robertson et al., 2019). This prescribed fire program has resulted in shifting mosaics of unburned patches located within a more extensive background matrix of almost annually burned grassland (Robertson et al.2019, Semenova-Nelsen et al. 2019).

We established replicated plots and applied experimental treatments prior to the 2017 prescribed fires. In the fall of 2016, we selected eight locations in the Wade Tract conservation easement. Half of the locations contained an overstory of longleaf pine and the other half were open areas away from overstory pines. Within each of the four pine overstory locations, we established six 2 x 2 m ($4m^2$) plots within 10 meters of multiple overstory pines (hereafter, near pines). Within each of the four no overstory pine locations, we established six 2 x 2 m ($4m^2$) plots at least 10 meters away from overstory pines (hereafter, away from pines). Each of the 24 plots was marked and randomly assigned to a fuel treatment.

We manipulated fuel loads in plots to create three fire severity treatments on the Wade Tract. We manipulated the density of longleaf pine needles, known to increase maximum temperatures and durations of surface temperatures >60°C at the ground surface (Platt et al. 2016). We applied

one of three treatments to each plot: no manipulation of pine needles, removal of pine needles, and addition of pine needles. No-manipulation plots represented the natural accumulation of pine needles in addition to other fine fuels from plants in the ground layer vegetation. Removal of pine needles, which was accomplished by lightly raking and hand-removing pine needles within a few days of planned prescribed fires, produced fine fuels of only ground layer vegetation. Removed needles were weighed to measure the fuel reductions involved. To generate pine needle addition treatments, we collected and stored fallen pine needles from along the sides of a dirt road through the conservation easement in the fall and winter of 2016-2017. A week prior to prescribed fires in March and April 2017, we manually added 2000 g/m^2 as uniformly as we could to simulate natural needlefall in marked $4m^2$ plots.

We sampled fuel loads before and after 2017 prescribed fires. Within the week prior to 2017 prescribed fires, we randomly established paired subplots of 30 x 30 cm within each $4m^2$ plot. We collected the above-ground fuels (live vegetation and litter) within one randomly selected subplot within each plot. Fuels were sorted into five categories (pine needles, grass, forb, shrub, litter), dried, and weighed. After prescribed fires, residual fuels from the other paired plot were collected, dried, and weighed. The proportion of pre-fire fine fuel biomass combusted during the prescribed fire was then estimated.

We measured characteristics of fires in the 2017 prescribed fires. We used thermocouples and data loggers to record fire temperatures in ways described in Platt et al. (2016).

We collected and processed soil following the prescribed fires. Soil from the upper 5 cm of each plot was extracted and combined with soil collected from similar fuel/fire treatments in separate buckets. Buckets were then kept cool and shipped to the University of Kansas (Lawrence, USA), where they were stored at 4˚Celsius. Prior to sterilization, portions of the collected soil

from each severity treatment were set aside for use as live innocula treatments (low, medium, and high severities). The remainder of the soil was sterilized using ultraviolet radiation. Soil from each of the three treatments was spread ~2.54 cm deep in sterile planters and irradiated for 10 minutes with 18 Watt UV-C bulbs (Rexim LLC, Watertown, USA) placed 20 cm above the soil surface. Then soil was sterilely mixed in each planter and received two more 10 minute treatments, with mixing between each irradiation. The sterilization process was conducted separately for each soil severity treatment. Sterilization exposed the soil to a dose of ~2081 mJ/cm^2, which should be fatal for most bacterial and fungal species (ClorDiSys 2014). Following sterilization, the soil was stored in sterile, 19 L bags until use. Additionally, sterile control treatments were created using sterilized abiotic soil matching the abiotic severity treatment of the respective pot.

Seeds of eight plant species were collected for this study from the annually burned fire blocks containing the Wade Tract easement. We selected two species from each of the four major life forms in the pine savanna: tree, grass, forb, and shrub in the fall of 2017, at the times seeds were naturally being dispersed from plants. One of each pair of different life forms (hereafter, more-pyrophilic plants) occurred abundantly in the annually burned ground layer throughout the fire blocks; the other was more typical of less-frequently burned patches scattered throughout and along the periphery of the fire blocks (hereafter, less-pyrophilic plants). The four more-pyrophilic species were *Pinus palustris* (tree), *Sorghastrum secundum* (grass), *Pityopsis graminifolia* (forb), and *Callicarpa americana* (shrub). The four complimentary less-pyrophilic species were: *Pinus taeda* (tree), *Sorghastrum nutans* (grass), *Bidens bipinata* (forb), and *Rhus copallinum* (shrub).

<u>*Soil Analyses*</u>: Samples of all collected soils were analyzed for total phosphorus, ammonium, nitrate, total carbon, total nitrogen, average pH, and C:N ratios. Soil phosphorus 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). NH_4^+ and NO_3^- were extracted using 2 M KCl on 2 g of soil, and then, cadmium reduction for nitrate and colorimetric procedures were used, followed by flow analysis for ion quantification (Brown 1998). Average pH was determined by combining three separate soil sub-samples 1:1 with H_2O (pH 7) and averaging the pH output. C:N ratios were determined by dividing total carbon by total nitrogen measurements.

Greenhouse Study Procedures: Seeds were planted in a temperature regulated greenhouse at the University of Kansas Between October and November 2017. We used 1-Liter "Deep" pots (Steuwe & Sons Inc., Tangent, USA) that were first stuffed with two sheets of sterile paper towel to prevent soil loss from the pot, then filled with a 500 mL of sterilized sand, followed by 167 mL of sterilized soil (abiotic treatment), 167 mL of live soil (biotic treatment) and seeds (20 seeds for all species but pines, which received 5 seeds). Seeds were then covered with 167 mL of sterilized soil (abiotic treatment; Fig.12a). Savanna soil types were kept uniform in each pot (i.e. sterile abiotic and biotic treatments added to each pot were each from pine or understory soils). Using a fully factorial set-up (Fig. 12b), we had 8 plant species, 3 abiotic soil severity treatments, 4 biotic soil severity treatments, and 2 savanna soil types, all at 5 replications each. This gave 960 total pots, which were then randomized within fire adapted/non-fire adapted species pairings. Pots were watered every other day for two minutes using drip irrigation with 8 L/hr emitter fittings to mimic natural conditions. Above each experimental block, Sun System® Sun Blaze® light supplement lamps (Sunlight Supply® Inc., Vancouver, USA) were hung 1.22 meters from the tops of pots, and provided supplemental light from 7am to 7pm each day.

Pots were monitored monthly for germination and encroachment by non-desired species. Germination rate was quantified by dividing the total number of plants that germinated in each pot by the total number seeds added to the pot. Pots were harvested between March 26th and April 16th, 2018, which allowed for 3 months of growth time. Important to note, all non-pyrophilic species, with the exception of *P. taeda*, had germination rates too low for downstream analyses of growth. This trend was exemplified by *S. nutans*, which germinated in only 10 of 120 pots. Due to the general lack of growth, all non-pyrophilic adapted species were not considered in downstream analyses aside from analysis of germination rates.

Soil was gently washed from roots, and the above and below ground biomass were weighed separately. After drying for at least 1 month, the above and below ground biomass from each pot was weighed dry and the total combined biomass was determined.

Statistical Analysis: All statistical analyses were completed in R 3.5.1 (R Core Team 2020) using the Emmeans package (Lenth 2018). Differences in germination rates between plant species were tested using type three analyses of variance (ANOVAs) with a custom apriori contrast that compared fire tolerant and less fire tolerant plant species with the contrast function. Then, ANOVAs were used to test for treatment effects (e.g. abiotic severity, biotic severity, and savanna soil type) on pine needle fuels, soil factors, fire characteristics, fine fuel combustion, and total plant biomass for each fire tolerant plant species. When ANOVAs denoted significant main effects, specific apriori contrasts comparing fire treatment and pine proximity effects were applied using the contrast function.

RESULTS

Fuel manipulation treatment effects on fine fuel loads: The fine fuel manipulation treatments, as well as natural variation in needle fall based on proximity to overstory pines generated differences in the amount of pine needle fuels. The amount of pine needle fuels differed based on fuel manipulation treatment ($F_{2,38}$=491.8, p<0.001; Figure 13a; Table 13; Ch.4 App. Table 1), with the lowest amounts of pine fuels in the low severity treatment (0.1 g), intermediate amounts in the medium severity treatment (8.11 g), and the most pine fuels in the high severity treatments (126.5 g; Ch.4 App. Table 1). Additionally, proximity to overstory Longleaf pine trees also influenced the amount of pine needle fuels ($F_{1,38}$=3.97, p=0.054), as there were slightly higher amounts of pine fuels in near relative to away from pines plots (14 g). In summary, the amount of pine needle fuels increased with the projected severity of the fuel manipulation treatment, and due to higher needle fall in near pines plots.

Fuel manipulation treatment effects on fire severity and fine fuel combustion: The fuel manipulation treatments generated differences in fire characteristics associated with fire severity. As the projected severity of fuel manipulation treatments increased, maximum surface ($F_{2,33}$=64.5, p<0.001; Figure 13b; Table 13; Ch.4 App. Table 2) and soil temperature increases ($F_{2,33}$=21.3, p<0.001; Figure 13c; Ch.4 App. Table 3) became larger, and surface temperature durations >60 °C lengthened ($F_{2,32}$=37.2, p<0.001; Figure 13d; Ch.4 App. Table 4). Additionally, maximum soil temperature increases also differed due to an interaction between fuel manipulation treatment and proximity to overstory pines ($F_{2,33}$=3.61, p=0.04). Specifically, the maximum soil temperature increases were higher in near pines, high severity treatment plots, relative to away from pines, high severity treatment plots.

The fuel manipulation treatments also drove differences in fine fuel combustion. As the projected severity of fuel manipulation treatments increased, fine fuel combustion increased ($F_{2,33}$=26.2, P<0.001; Figure 14a; Table 14; Ch.4 App. Table 5), with the lowest combustion in the low severity plots, intermediate combustion in the medium severity plots, and the highest combustion in the high severity plots. Overall, the fuel manipulation treatments successfully generated differences in fire severity and fine fuel combustion.

Fuel manipulation treatment effects on abiotic soil characteristics: Soil factors differed between fire severity treatments. Fire manipulation treatments altered total phosphorus levels ($F_{2,38}$=3.038, p=0.06; Figure 14b; Table 14; Ch.4 App. Table 6), with phosphorus levels highest in the high severity treatment as compared to medium severity treatments (p=0.022). There were no statistical differences between the low and medium severity or low and high treatments. Ammonium levels also varied between fire severity treatments ($F_{2,38}$=3.408, p=0.044; Figure 14c; Table 14; Ch.4 App. Table 7), with ammonium availability higher in the high severity versus low severity treatment (p=0.014). Ammonium levels did not differ statistically between medium and low or medium and high treatments, however. Soil pH was affected by fire severity ($F_{2,38}$=16.38, p < 0.001; Figure 14d; Table 14; Ch.4 App. Table 8), with pH levels higher in high (p < 0.001) and medium (p < 0.001) versus low severity treatments. Soil pH in high and medium severity treatments did not differ statistically. Total nitrogen ($F_{2,38}$=2.01, p=0.15; Figure 14e; Table 14), nitrate ($F_{2,38}$=0.26, p=0.77; Figure 14f; Table 14), total carbon ($F_{2,38}$=0.3, p=0.74; Figure 14g; Table 14), and C:N ratios ($F_{2,38}$=1.27, p=0.29; figure 14h; Table 14), did not differ between fire severity treatments.

Soil factors also varied between pine proximity treatments. Total carbon ($F_{1,38}$=4.39, p = 0.043; Table 14) and C:N ratios ($F_{1,38}$=10.9, p=0.002; Table 14) differed between pine proximity

treatments. Specifically, C:N ratios were higher near pines versus away from pines (p=0.002), and these differences were due to increases in total carbon (p=0 .043), and not changes in total nitrogen ($F_{1,38}$=1.25, p=0.27). Soil pH was marginally affected by pine proximity treatments ($F_{1,38}$=3.23, p=0.081; Table 3), with pH values lower in near pines treatments (p=0.081). Total phosphorus ($F_{1,38}$=0.05, p=0.82), ammonium ($F_{1,38}$=0.13, p=0.72), and nitrate ($F_{1,38}$=0.196, p=0.66) levels did not differ between pine proximity treatments. To summarize, higher severity fires generally increased nutrient availability (total P, ammonium) and pH levels, while near pines soil had higher levels of carbon, C:N ratios, and decreased pH levels.

Germination responses to treatments: Germination rates varied between plant species and fire adaptation groups. There were overall differences between the eight plant species in terms of germination rates ($F_{7,952}$=352.73, p<0.001; Figure 15; Table 15), with pines having the highest rates (~58%) and the forbs with the lowest rates (1-10%). In addition to species differences, pyrophilic plants generally had higher germination rates than their non-pyrophilic counterparts (Appendix table 9). The two pine species (*P. palustris* & *P. taeda*) were the only exceptions to this trend, as they had similar germination rates of 57.5% and 57.8% respectively. Despite inherent differences in germination rate between plant species, pyrophilic plants germinated more reliably than their less pyrophilic counterparts.

Due to the poor germination of the less pyrophilic plant species, we were only able to assess fuel manipulation treatment effects on the more pyrophilic plant species. *Callicarpa americana* (shrub) germination rates differed between biotic severity treatments ($F_{2,96}$=2.71, p=0.05; Figure 16a; Table 16; Ch.4 App. Table 10), with higher germination rates in the sterile control and medium severity treatments relative to the low severity treatments.

Pinus palustris (pine) germination rates were influenced by biotic severity treatments, however this effect varied between abiotic severity treatments ($F_{3,96}$=2.51, p=0.03; Figure 16a,b; Table 16; Ch.4 App. Table 11). In low severity abiotic treatments, *P. palustris* germination rates were highest in sterile and low severity biotic treatments. The direction of this effect shifted in the medium severity abiotic treatments however, where *P. palustris* germination rates were higher in non-sterile treatments. In high severity abiotic treatments, *P. palustris* germination was not influenced by biotic severity treatments.

Pityopsis gramminifolia (forb) germination rates responded to abiotic ($F_{2,96}$=9.7, p<0.001; Figure 16b; Table 16; Ch.4 App. Table 12) and biotic ($F_{3,96}$=3.7, p=0.01; Figure 16a; Table 16; Ch.4 App. Table 13) severity treatments, however these responses were modified by soil type. Abiotic severity treatments favored *P. gramminifolia* germination in low and medium severity treatments when in pine soil, and in high severity treatments when in understory soil. Biotic severity treatments favored germination in sterile control and low severity treatments in pine soil, and in medium severity treatments in understory soil.

Sorghastrum secundum (grass) germination rates varied between abiotic severity treatments, and this effect was modified by soil type ($F_{2,96}$=3.9, p=0.02; Figure 16b; Table 16; Ch.4 App. Table 13). When grown in pine soil, abiotic severity treatment did not influence *S. secundum* germination, however, in understory soil germination rates were highest in medium and high severity treatments relative to low severity treatments. To summarize, pyrophilic plant germination rates varied based on biotic and abiotic severity treatments, but the direction of this effect was often dependent on soil type.

<u>*Shrub growth response to treatments*</u>: *Callicarpa americana* growth varied between abiotic soil severity treatments and savanna soil types. There was a clear trend in growth amongst abiotic

severity treatments ($F_{2,96}$=11.907, P<0.001; Figure 17a; Table 17; Ch.4 App. Table 14), with medium severity treatments having the most biomass, followed by low, and then high severity treatments. Additionally, savanna soil type had an important effect on total biomass ($F_{1,96}$=7.881, P=0.006), with plants growing larger in pine soil.

The effect of savanna soil type also interacted with abiotic severity treatments ($F_{2,96}$=8.107, P<0.001; Fig.17a; Table 17; Ch.4 App. Table 14), and showed that abiotic soil severity effects were strongest when plants grew in pine soil. Growth of *C. americana* in pine soil was largest in medium severity treatments, followed by low, and then high severities. In conclusion, savanna soil type and abiotic treatment effects were the primary drivers of *C. americana* growth, and suggested that differences between pine and understory soils can moderate the importance of abiotic severity treatment effects.

Pine growth response to treatments: *Pinus palustris* plant growth was not responsive to fire severity related effects or savanna soil types. Specifically, *P. palustris* plant biomass did not vary between abiotic soil severities ($F_{2,96}$=0.126, P=0.88; Figure 17a; Table 17), biotic soil severities ($F_{3,96}$=0.66, P=0.58; Fig.18b), savanna soil types ($F_{1,96}$=0.19, P=0.66), or any interactions of these treatments. To summarize, *P. palustris* growth was not responsive to any combination of experimental treatments.

Forb growth response to treatments: *Pityopsis graminifolia* growth was responsive to interactions between experimental treatments and soil type. In terms of biotic soil severity treatments ($F_{3,96}$=2.95, P=0.04; Fig.17b; Table 17; Ch.4 App. Table 15), *P. graminifolia* generally preferred low severity treatments (P<0.05,) and did noticeably poor in the high severity treatments (P<0.05). Response to soil biotas varied when considering savanna soil type however ($F_{3,96}$=4.116, P=0.009). When grown in pine soil, biomass was highest in the low severity treatment and lowest in the high

severity treatment (P<0.05). When grown in understory soil however, the largest differences were between live and control treatments, with more biomass when soil biota were present (P=0.044).

In addition to biotic treatment effects, *P. graminifolia* growth also varied between abiotic soil severity treatments based on savanna soil type ($F_{2,96}$=11.605, P<0.000; Fig. 17a; Table 17; Ch.4 App. Table 15). In pine soil, biomass was greater in the low (P=0.006) and medium (P=0.005) severity treatments than in the high severity treatment. In understory soil, the trend flipped however, and biomass was greater in low (P=0.025) and high treatments (P=0.002) than in medium treatments. To summarize, abiotic and biotic effects on *P. graminifolia* growth were largely dependent on savanna soil type, and generally favored plants grown in lower severity abiotic treatments and with soil biota present.

<u>*Grass growth response to treatments*</u>: *Sorghastrum secundum* plant growth varied between biotic and abiotic soil severity treatments, and was modified by an interaction between abiotic soil severity and savanna soil type. When considering the biotic soil severity treatments ($F_{3,96}$=3.98, P=0.01; Figure 17b; Table 17; Ch.4 App. Table 16), the primary differences were between sterile and live treatments. Specifically, *S. secundum* plants produced more biomass when soil microbes were present (e.g. low, medium, and high versus sterile control treatment).

Sorghastrum secundum biomass also varied between abiotic soil severity treatments ($F_{2,96}$=13.77, P<0.0001; Fig.17a; Table 17; Ch.4 App. Table 16), with a clear preference for medium (best), low, and then high (worst) severity soils. Abiotic soil severity effects were modified by savanna soil type however ($F_{2,96}$=6.243, P=0.003). Grasses generally grew larger in pine soil, with low and medium severities promoting growth the most. Plant growth was generally lower in understory as compared to pine soil, but plants produced the most biomass of any treatment in understory medium severity treatments. In summary, *S. secundum* growth was highly

responsive to experimental treatments, and displayed preferences for the presence of soil biota, pine soil treatments, and low to medium severity fires.

Discussion

Pyrophilic plants utilize high germination rates and rapid growth to take advantage of post-fire soil conditions and variations in fire severity. Work in other pyrophilic ecosystems supports these findings (Birk & Bridges 1989; Singh 1993; Beckage & Ellingwood 2008; Schertzer & Staver 2018), and suggests that post-fire regrowth by pyrophilic plants can replenish the community level fuel loads necessary for maintaining recurrent, low severity fires (Mutch 1970; Platt 1999; Beckage & Ellingwood 2008; Beckage *et al.* 2009; Nerlekar & Veldman 2020). The recurrent fires in turn can promote the formation of pyrophilic plant communities whose life histories are associated with flammability and survival in post-fire conditions (Pausas & Bond 2019; Cui *et al.* 2020; Simpson *et al.* 2020). Despite the general ability to promote and take advantage of fire, we also show that pyrophilic plants display varied responses to fire severity effects on soil conditions. Differential responses to natural variations in fire severity could generate the naturally diverse understory communities in fire frequented systems (Carr *et al.* 2009; Mugnani *et al.* 2019), and select for plants with traits that function post-fire.

Pyrophilic plants are hypothesized to possess traits that enhance germination and rapid growth in post-fire environments (Daubenmire 1968; Whelan 1995; Keeley & Fotheringham 2000). Our study supports these hypotheses, and shows that pyrophilic plants have higher germination rates than their less pyrophilic counterparts, and are able to quickly take advantage of post-fire changes to soil conditions. Successful post-fire germination should allow pyrophilic plants to rapidly occupy available space and then assimilate resources. The general ability to take

advantage of post-fire conditions may then provide a competitive advantage over less fire tolerant species (Daubenmire 1968; Keeley 1991; Singh 1993), and drive feedbacks on future fires since fuel loads would replenish quickly following fire (Beckage *et al.* 2011; Platt *et al.* 2016). While community fuel loads likely regenerate quickly in pyrophilic systems, differential responses to fire severity effects on soil conditions may influence fuel production and the severity of future fires. Therefore fire effects on soil conditions may be just as important as pyrophilic plant life history traits in determining the feedbacks between fire and plant fuels in fire recurrent ecosystems.

Pyrophilic plant germination and growth varied due to fire severity associated changes in abiotic soil conditions. Fire commonly drives changes to soil conditions and nutrient availability, and as fire severity increases, factors like pH, phosphorus, and nitrogen availability also increase (Neary *et al.* 1999; Johnson & Curtis 2001; Butler *et al.* 2018). In our study, increasingly severe fires increased soil pH, and increased the availability of phosphorus and ammonium. These conditions then influenced the germination of *Pinus palustris*, *Pityopsis graminifolia*, and *Sorghastrum secundum*. While all of the pyrophilic plants included in this study displayed high germination rates, germination responses differed based on species and fire severity. The differential responses to fire severity suggest that pyrophilic taxa are adapted to take advantage of different conditions following fire, with *P. palustris* preferring medium severity treatments, and *P. graminifolia* and *S. secundum* preferring pine, or medium to high severity treatment soils. Higher germination in the medium to high severity treatments may have been due to increased soil pH levels, that were more favorable to germination (Shoemaker & Carlson 1990). Pyrophilic plant growth also responded to soil abiotic severity treatments, however, only understory species were affected. *Calicarpa americana* (shrub) and *S. secundum* growth were promoted in medium severity soil treatments when grown in pine and understory soil respectively. *P. graminifolia*

growth was generally favored in low severity treatments, and reduced when grown in medium severity understory soil and high severity pine soil. The different soil abiotic severity preferences among understory plants suggests that pyrophilic plants benefit from natural variations in fire severity due to different nutrient and soil pH requirements. This could promote the natural heterogeneity of savanna understories (Tilman *et al.* 1997; Carr *et al.* 2009; Mugnani *et al.* 2019), as fires of varying severities stimulate nutrient pulses that favor the growth of different pyrophilic plant taxa, and potentially their associated soil microbes.

Fire severity effects on soil microbes (i.e. biotic soil severity treatments) also influenced the germination rates and growth of pyrophilic plant species. The effect of biotic soil severity treatments varied between plant species, with *C. americana* and *P. palustris* germination benefiting in higher severity treatments, *P. graminifolia* germination benefiting at lower severity treatments (particularly in pine soil), and *S. secundum* germination being unaffected by biotic fire severity treatment. The differences in germination responses may have been due to the presence/absence of microbial pathogens, and the importance of soil microbes to plant species. *C. americana* and *P. palustris* seeds may be less resistant to soil pathogens, which are killed off at higher fire severities (Hardison 1976; Katan 2000; Hopkins *et al.* 2021), such as those created following locally severe fires (Platt *et al.* 2016; Mugnani *et al.* 2019). Since increasing the severity of fire also increases microbial mortality and reduces pathogen loads (Katan 2000; Hamman *et al.* 2007; Bárcenas-Moreno & Bååth 2009; Peay *et al.* 2009, but not mycorrhizal colonization Ch. 4 App. Table 17, 18), *P. palustris* seeds may rely on severe fires to remove potentially harmful microbes. *P. graminifolia* on the other hand, may be more reliant on beneficial soil microbes that are negatively impacted as fire severity increases (Hamman *et al.* 2007; Peay *et al.* 2009; Pressler *et al.* 2019). Biotic soil severity treatments also caused differences in savanna understory plant

growth. *P. graminifolia* grew larger when microbes were present, however, when fires became more severe (particularly those near pines), growth decreased. *S. secundum* also preferred the presence of soil microbes, but, the severity of soil biotic treatment did not influence growth responses. *C. americana* and *P. palustris* growth did not vary between biotic soil severity treatments. These results mirror those for abiotic soil severity treatments in that pyrophilic plants display varying preferences for fire severity effects on soils. The preference for lower biotic severity treatments in *P. graminifolia* suggests that it does best in savanna understory sections where fires are lower in severity (Platt *et al.* 2016), which may allow important microbial mutualists to survive fire and promote the growth of post-fire colonizers. *S. secundum*'s preference for microbes across severity treatments implies that it can be an effective post-fire colonizer regardless of fire severity. The general success of *S. secundum* in post-fire environments may explain its importance in stabilizing the fire regimes of savanna systems (Beckage *et al.* 2011), and general dominance in fire recurrent, pine savanna ecosystems (Platt 1999; Peet *et al.* 2018). While specific preferences for fire severity differ, the pyrophilic plants used in this study were all able to take advantage of post-fire conditions. Since fire alters both abiotic and biotic soil conditions, and this can drive differences in post-fire fuel production, interactions between fire, plants, and soil factors should be incorporated into models of the fire-fuel feedbacks that structure pyrophilic ecosystems.

Pyrophilic ecosystems comprise ~40% of Earth's terrestrial surface (Archibald *et al.* 2018), and rely on frequent, low severity fires for their maintenance. The recurrent fires required by pyrophilic ecosystems necessitate the presence of pyrophilic plant species capable of rapidly producing flammable fine fuels after fire (Mutch 1970; Platt 1999; Beckage & Ellingwood 2008; Cui *et al.* 2020; Nerlekar & Veldman 2020). As a result of this selection, species rich, pyrophilic

plant communities develop, and are capable of generating the community level fuel loads necessary to maintain fire's spread across landscapes. The cyclical relationships between fire and pyrophilic plant species suggests a feedback process that engineers the fire recurrent regimes that maintain pyrophilic ecosystems. Furthermore, the fire-plant feedbacks that maintain these systems may also explain the occurrence of pyrophilic ecosystems in regions where environmental conditions predict the presence of forests and less fire tolerant systems (Beckage & Ellingwood 2008; Nerlekar & Veldman 2020).

In conclusion, we show that pyrophilic plant species possess life history traits that allow them to take advantage of post-fire conditions. Specifically, pyrophilic plants utilize high germination rates, followed by rapid post-fire growth which allow them to take advantage of post-fire nutrient and soil pH changes, as well as changes in soil microbial communities. The ability to succeed following fire ensures the quick production of flammable plant fuels that can maintain the frequent, low severity fires in pyrophilic systems. While pyrophilic plants possess common traits that promote their success following fire, they also display varying responses to fire severity, which suggests a potential mechanism for generating the species rich, heterogenous nature of pyrophilic plant communities. Future work can explore the exact mechanisms by which fire severity driven changes to abiotic and biotic soil factors generate plant growth responses, and how variation in plant response to fire severity influences natural differences in fuel loads and fire characteristics. Feedbacks between fire and pyrophilic plant communities result in the fire regimes that maintain pyrophilic ecosystems (Mutch 1970; Beckage *et al.* 2009; Platt *et al.* 2016). Thus, understanding the mechanisms that underly pyrophilic systems can explain their persistence amongst Earth's terrestrial ecosystems.

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Chapter 4 - Tables

Table 13: ANOVA results for fuel manipulation treatment and pine proximity effects on pine fuels and prescribed fire characteristics.

Model term	F-ratio	p-value	F-ratio	p-value	F-ratio	p-value	F-ratio	p-value
	Pine fuels (g)		Max. Surf. Temp. Inc.		Surf. Dur. >60C		Max Soil Temp. Inc.	
fuel manipulation treatment	491.799	<.0001***	69.466	<.0001**	37.199	<.0001**	21.256	<.0001**
pine proximity	3.969	0.0536*	0	0.9915	0.001	0.981	0.774	0.3855
fuel x pine	1.062	0.3559	0.297	0.7454	1.581	0.2214	3.606	0.0383*

*: p < 0.1, **: p < 0.05, ***: p < 0.001

Table 14: ANOVA results for fuel manipulation treatment and pine proximity effects on fuel combustion and soil abiotic factors.

Model term	F-ratio	p-value	F-ratio	p-value	F-ratio	p-value	F-ratio	p-value
	Fuel combustion (%)		Inorganic Phosphorus		ammonium		Soil pH	
fuel manipulation treatment	26.202	<.001***	3.038	0.0597*	3.408	0.0435**	16.382	<.001***
pine proximity	1.953	0.1715	0.05	0.8239	0.13	0.7208	3.225	0.0805*
fuel x pines	0.967	0.3907	0.324	0.7255	0.23	0.7953	1.193	0.3143
	Total Nitrogen		Nitrate		Total Carbon		C:N Ratio	
fuel manipulation treatment	0.259	0.7732	2.009	0.1482	0.303	0.7403	1.27	0.2926
pine proximity	1.245	0.2716	0.196	0.6607	4.394	0.0428**	10.927	0.0021**
fuel x pines	1.713	0.1939	0.033	0.9676	1.75	0.1875	0.591	0.5589

*: p < 0.1, **: p < 0.05, ***: p < 0.001

Table 15: ANOVA results for germination rate differences between more and less pyrophilic plant pairs.

Model term	F-ratio	p-value
plant species	352.727	<.001***

Table 16: ANOVA results for soil severity and soil type effects on pyrophilic plant germination rates.

Model term	F-ratio	p-value	F-ratio	p-value	F-ratio	p-value	F-ratio	p-value
	C. americana		P. palustris		P. graminifolia		S. secundum	
abiotic treatment	1.09	0.34	3.892	0.0237**	0.289	0.7498	13.767	<.0001***
biotic treatment	2.71	0.05**	1.231	0.3029	2.207	0.0922*	3.98	0.0101**
pine proximity	2.19	0.14	0.065	0.8	2.285	0.1339	1.789	0.1842
abiotic x biotic	1.23	0.3	2.507	0.0269**	0.854	0.5315	0.189	0.9791
abiotic x pines	2.31	0.11	0.667	0.5158	9.7	0.0001***	6.243	0.0028**
biotic x pines	1.27	0.29	1.861	0.1413	3.777	0.0131**	0.647	0.5865
abiotic x biotic x pines	0.936	0.47	1.231	0.2975	1.598	0.1561	0.353	0.9065

*: p < 0.1, **: p < 0.05, ***: p < 0.001

Table 17: ANOVA results for soil severity and soil type effects on pyrophilic plant growth.

Model term	F-ratio	p-value	F-ratio	p-value	F-ratio	p-value	F-ratio	p-value
	C. americana		P. palustris		P. graminifolia		S. secundum	
abiotic treatment	11.907	<.001***	0.126	0.8815	1.019	0.3648	13.767	<.001***
biotic treatment	2.031	0.1146	0.66	0.5787	2.95	0.0366**	3.98	0.0101**
pine proximity	7.881	0.0061**	0.19	0.6638	2.602	0.11	1.789	0.1842
abiotic x biotic	0.93	0.4775	0.387	0.8855	0.977	0.445	0.189	0.9791
abiotic x pines	8.107	<0.001***	0.394	0.6756	11.605	<.001***	6.243	0.0028**
biotic x pines	1.001	0.3961	2.087	0.107	4.116	0.0086**	0.647	0.5865
abiotic x biotic x pines	1.003	0.4278	0.607	0.7242	1.099	0.3689	0.353	0.9065

*: $p < 0.1$, **: $p < 0.05$, ***: $p < 0.001$

Chapter 4 - Figures

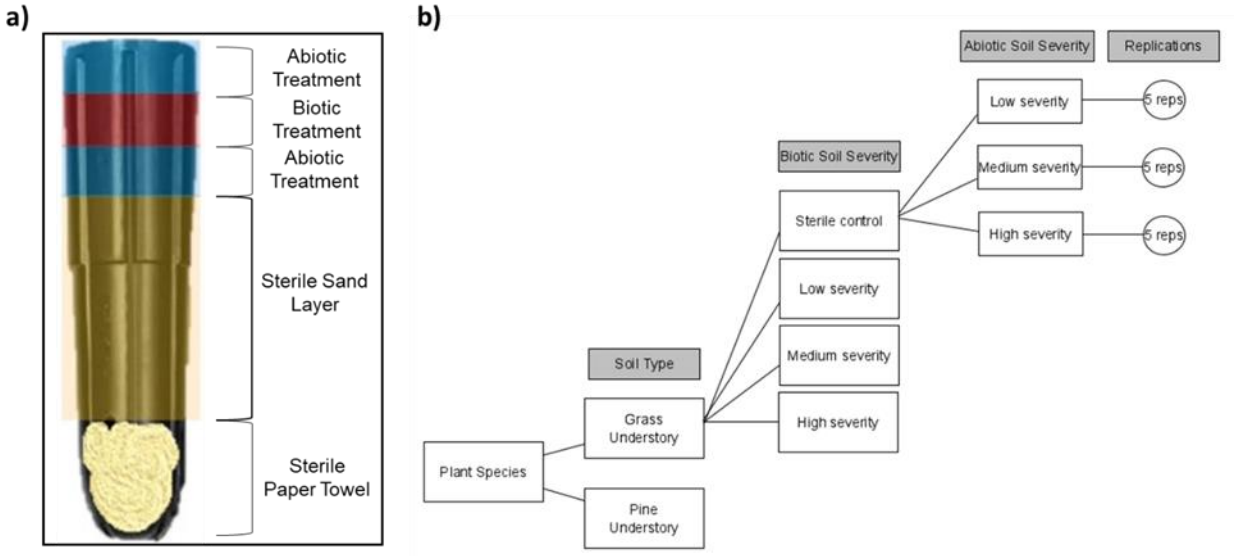

Figure 12: Pot setup and experimental design. a) Pot setup for greenhouse experiment. Two sterile paper towels were first placed in the bottom of the pot to prevent soil loss during watering. 500 mL of sterile sand was then filled in, followed by 167 mL of abiotic soil severity treatment, 167 mL of biotic soil severity treatment, and 167 mL of abiotic soil severity treatment. Plant seeds were planted in the biotic soil layer, with 20 seeds for shrubs, forbs, and grasses and 5 for tree species. Note that soil used in each pot was either all grass or pine understory soil. b) A partial diagram of the Greenhouse experimental design. Eight plant species were grown in full factorial combinations of two soil types, four biotic soil severities, and three abiotic soil severities, with five replications for each combination. These combinations combined for 960 pots total, with 120 pots per plant species.

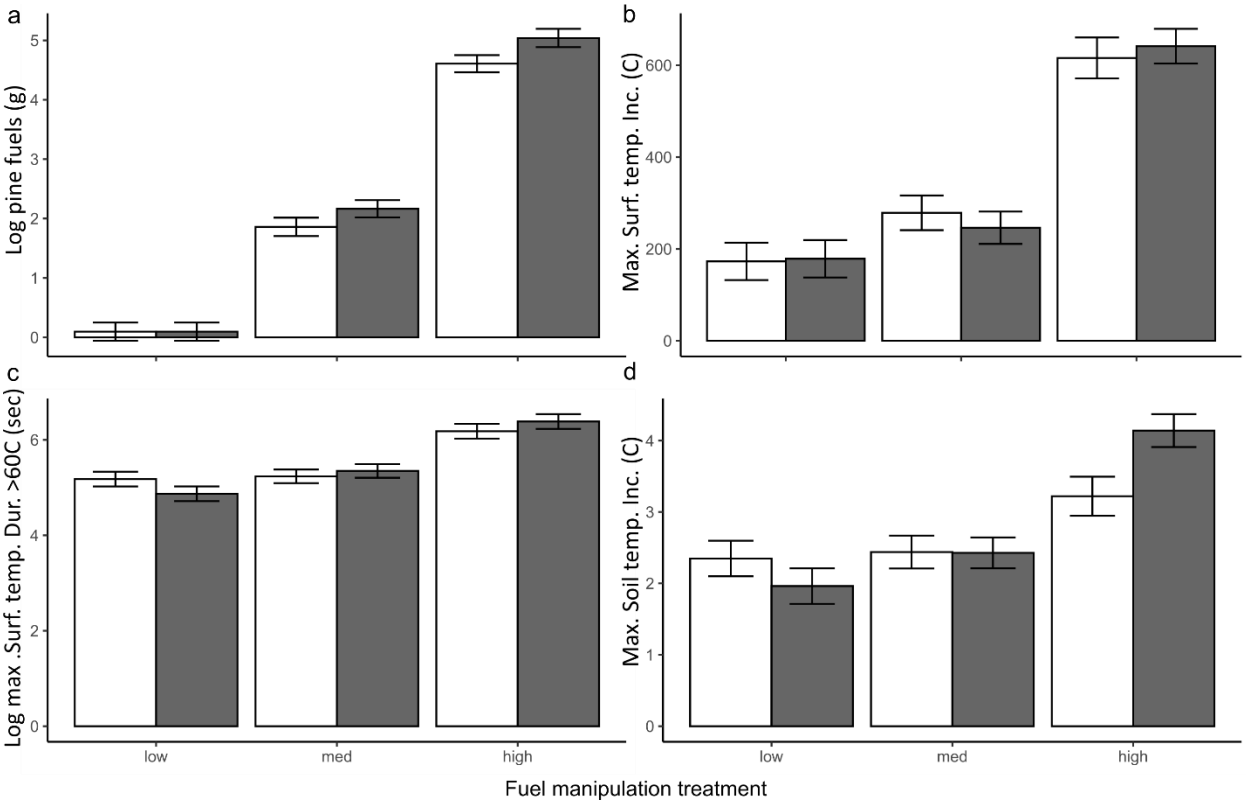

Figure 13: Pine needle fuel and fire characteristics for fuel manipulation treatments. Low indicates pine needle removal, medium indicates natural pine needle densities, and high indicates pine needle addition to natural pine needle densities. Vertical bars indicate mean ± one standard error. Dark grey bars denote plots near, and white bars denote plots away from overstory Longleaf pines. a) Pine needle fuels, b) maximum surface temperature increases, c) log surface temperature durations > 60°C, and d) maximum soil temperature increases during 2017 prescribed fires.

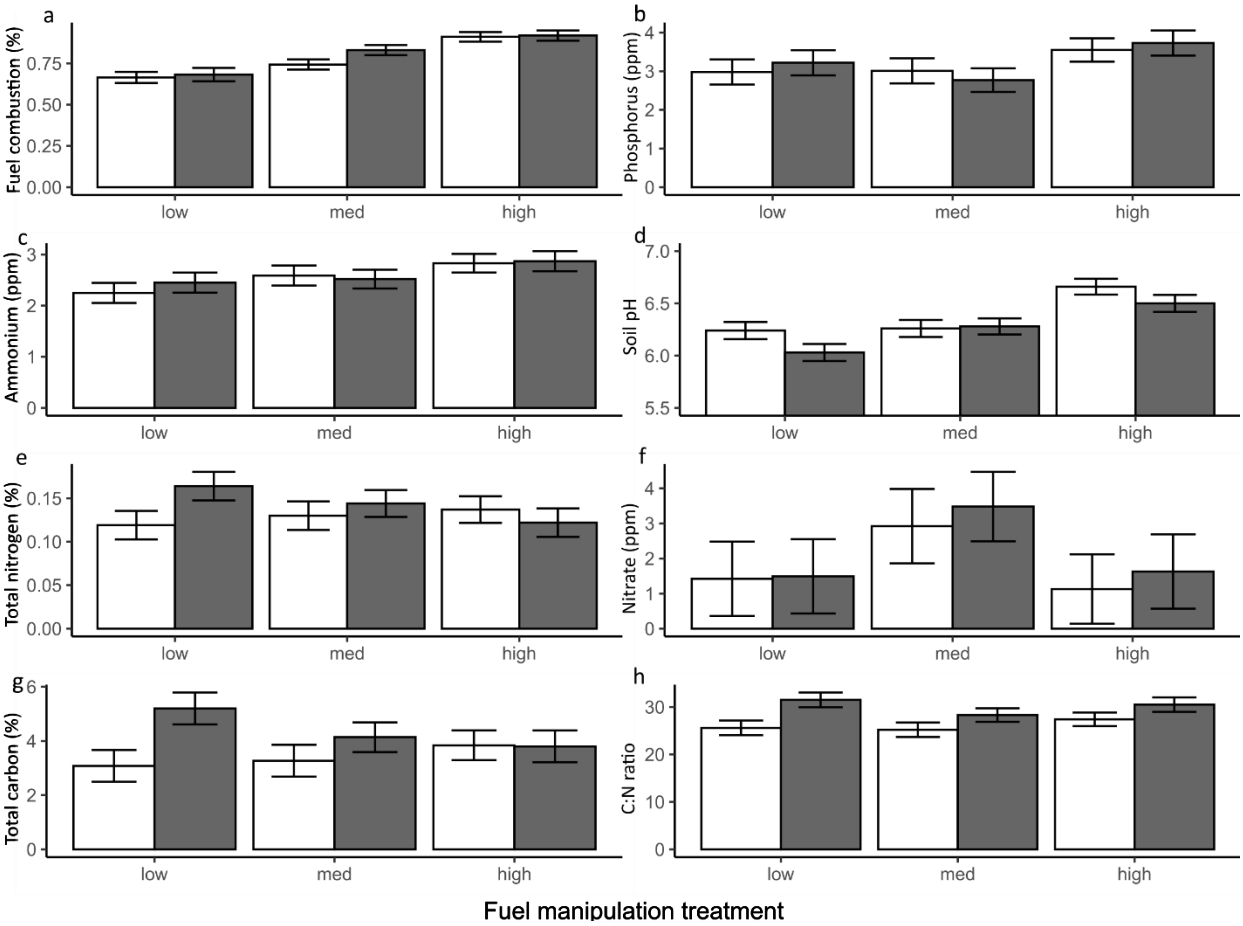

Figure 14: Fine fuel combustion and soil abiotic factor responses to fuel manipulation treatments and proximity to overstory Longleaf pine proximity. Vertical bars indicate the mean ± one standard error. Dark grey bars denote plots near overstory Longleaf pines, and white bars denote plots away from overstory Longleaf pines. a) Fuel combustion (%), b) log inorganic phosphorus (ppm), c) ammonium (ppm), d) soil pH, e) total nitrogen (%), f) nitrate (ppm), g) total carbon (%), and h) carbon to nitrogen ratios following 2017 prescribed fires.

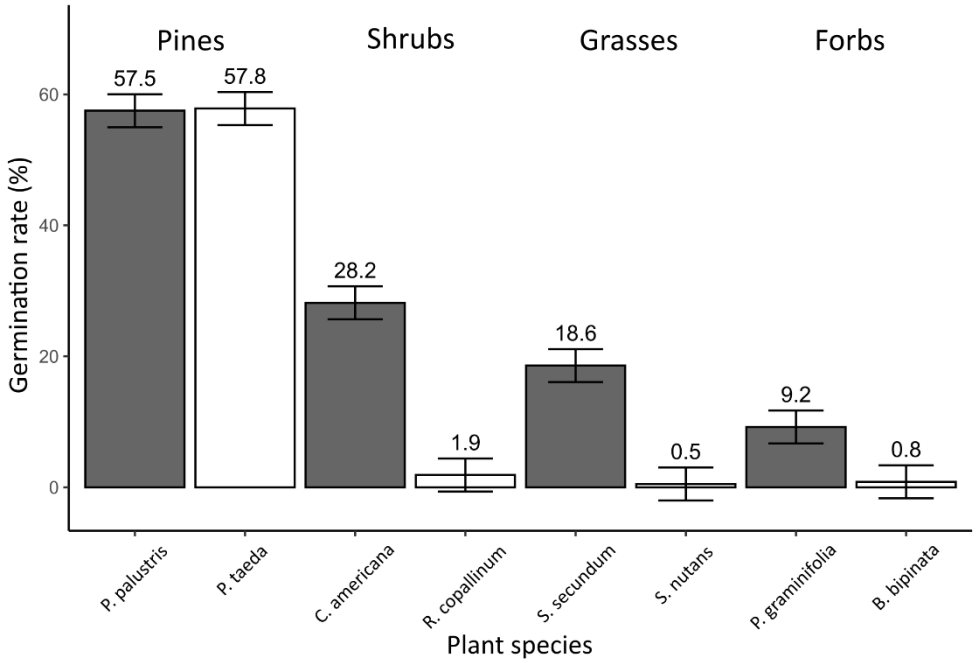

Figure 15: Germination rates of representative specie of four life forms (pines, shrubs, grasses, forbs). Vertical bars indicate means $\pm$ 95% CI. Dark grey indicates species typical of very frequently burned open pine savanna; white indicates species typical of less frequently burned transitions from pine savanna to hardwood forest. Numerical coefficients above bar are the mean germination rates (%) for each of the included species.

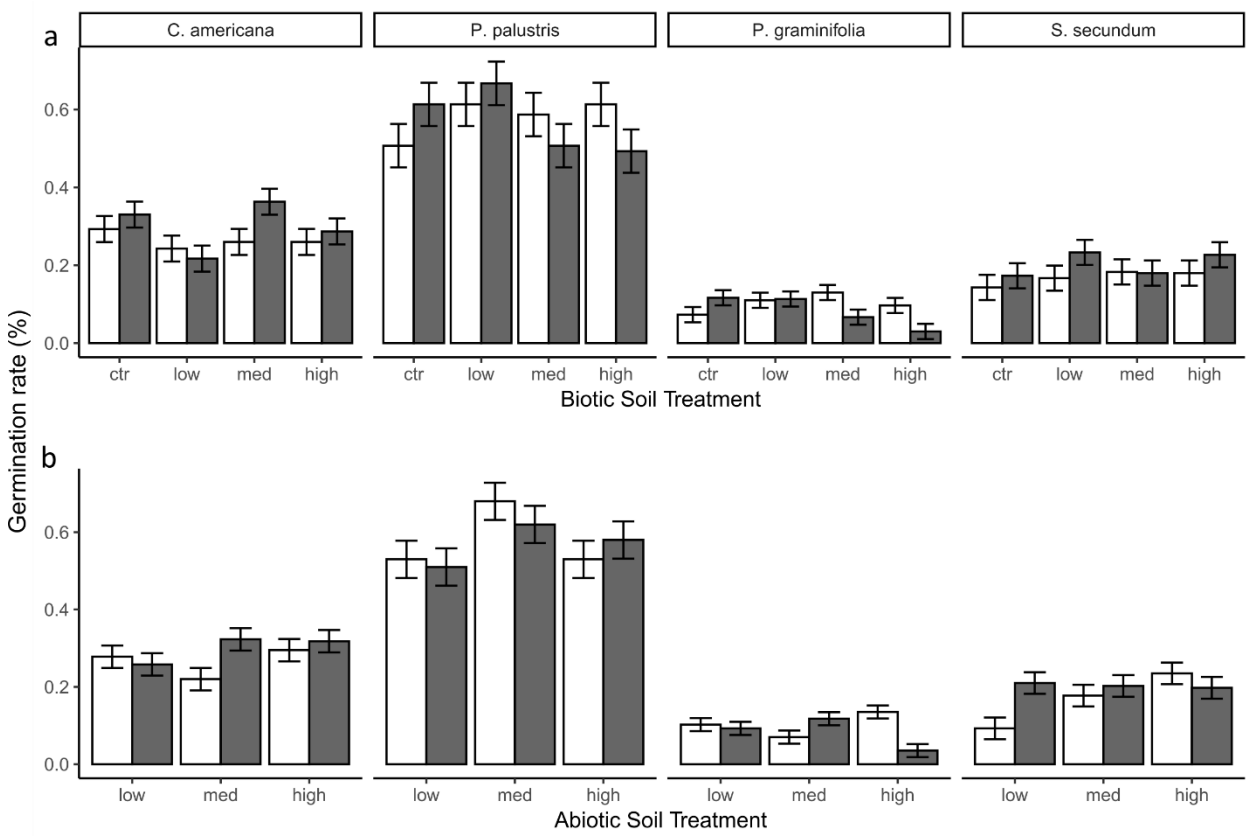

Figure 16: Pyrophilic plant germination responses to a) biotic and b) abiotic soil severity treatments and savanna soil type. Vertical bars denote the mean ± one standard error. Grey bars represent pots containing pine soil, and white bars represent pots containing understory soil.

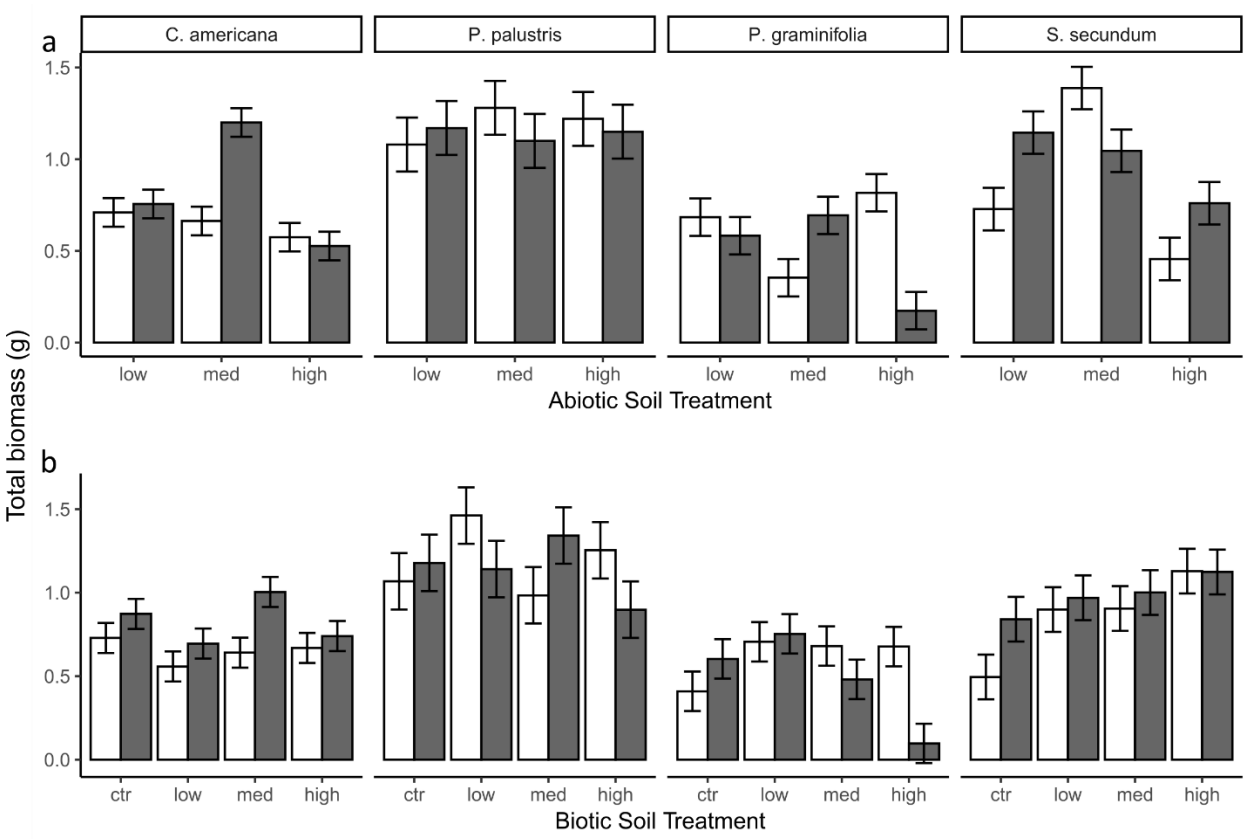

Figure 17: Pyrophilic plant growth responses to a) abiotic and b) biotic soil severity treatments and savanna soil type. Vertical bars denote the mean ± one standard error. Grey bars represent pots containing pine soil, and white bars represent pots containing understory soil.

Conclusion

Fire alters fungal community structure and function in ways that modify the plant fuel loads of pyrophilic ecosystems. Since feedbacks between plant fuel loads and fire engineer pyrophilic fire regimes (Beckage *et al.* 2009, 2011; Platt *et al.* 2016), this implies that fungi play foundational roles in pyrophilic systems. Fungal roles in pyrophilic ecosystems are dynamic across time due to the complex direct and indirect effects of fire on fungal communities. The nature of fire-fungal interactions shifts with time since fire, and is closely related to fire's well known effects on fungal community structure (Hamman *et al.* 2007; Dove & Hart 2017), environmental conditions (Raison 1979; Certini 2005; Butler *et al.* 2018), and plant-fungal interactions (Hardison 1976; Katan 2000; Hart *et al.* 2005). By putting these processes into an ecological framework, I show the relative importance of these factors across time, and produce a simplified context for understanding fire-fungal interactions in pyrophilic systems. The nature of fire-fungal interactions suggests that they are best envisioned in two parts: fire's effect on fungal communities and the local environment, and the downstream impact on fungal functions associated with plant fuel loads.

Fire altered the structure and seasonal trajectories of fungal communities in a similar manner across two pyrophilic ecosystems. Early after fire, these changes were related to the turnover of dominant taxa and altered nutrient availability, rather than heat related mortality following fire. Other studies in Longleaf pine savannas (Brown *et al.* 2013; Hansen *et al.* 2019; Semenova-Nelsen *et al.* 2019), and different pyrophilic ecosystems (e.g. Mediterranean shrublands, oak savannas, and ponderosa pine forests; Bárcenas-Moreno *et al.* 2011; Carson *et al.* 2019; Owen *et al.* 2019), mirror these changes, which suggests that fire favors specific fungal taxa across ecosystems. These taxa likely possess traits which help them survive the passage of fire (e.g. truffle forming mycorrhizae: Horton *et al.* 1998; Glassman *et al.* 2016; Owen *et al.* 2019, and

thermotolerant taxa: Sharma 1981; Peay *et al.* 2009), and persist in harsh, post-fire environments (stress tolerant taxa: McMullan-Fisher *et al.* 2011; Hansen *et al.* 2013; Persiani & Maggi 2013). Interestingly, while fire favored some mycorrhizal taxa that can promote the production of new plant fuels, it also suppressed taxa known to be plant pathogens, implying that fire may reduce pathogen loads and negative effects on plant fuel production (Hardison 1976; Katan 2000). With increasing time since fire, fire associated changes to nutrient availability were no longer strong drivers of fungal community structure, however, burned communities remained distinct from neighboring unburned communities. This suggests that initial fire related changes to fungal community structure altered the seasonal trajectory of fungi, and led to the formation of alternative communities. In summary, fire's effect on fungal communities and the local environment persisted during the year following fire, and hinted at potential shifts in post-fire fungal functions associated with plant fuel loads.

Fire driven changes to fungal communities were collocal with changes in fungal related functions like decomposition and plant-fungal interactions. While the association was not explicitly tested, burned sites exhibited both altered fungal community structure and slowed decomposition. The strength of fire's effect on decomposition was determined by fire regime components like fire history and severity, which slowed decomposition of new plant fuels as fires became more frequent and intense. This trend is supported by other studies relating frequent fires to slowed decomposition (Ficken & Wright 2017; Butler *et al.* 2019), and extends our knowledge by exploring the pathways through which fire alters decomposition across time. In our model, fire severity and history alter decomposition through direct, unmeasured pathways, as well as indirectly through changes to nutrient availability. Fire's effect on decomposition is strongest at earlier time points following fire, and gradually decreased at later time points as fire related

changes to nutrient availability become more important. The lessening of fire's direct effects may have been due to the post-fire recovery of fire effected fungal taxa (Smith *et al.* 2004; Bárcenas-Moreno *et al.* 2011), and the beginning of the growing season, at which time plant-fungal interactions may have masked fire's effect due to increased competition between plants and fungi for nutrients (Lipson *et al.* 1999; Zhu *et al.* 2016). Fire also altered plant-fungal interactions in ways that effected the production of new plant fuels. Three of the four plant taxa (*C. americana*, *P. gramminifolia*, *S. secundum*) used in these studies displayed clear preferences for low to medium severity fire treated soil. This suggests that pyrophilic plants can take advantage of fire treated microbial communities and post-fire nutrient flushes. Interestingly, these same plants showed decreases in fuel production when fires were most severe, perhaps due to the negative effect of high severity fires on beneficial members of microbial communities (e.g. mycorrhizae; Hamman *et al.* 2007; Dove & Hart 2017; Taudière *et al.* 2017, although this was not reflected in colonization data Ch. 4 appendix Table 17, 18), and/or increased volatilization and loss of nutrients like N and P (Raison 1979; Certini 2005). The complex, interacting pathways through which fire alters fungal functions like decomposition and roles in plant-fungal interactions reiterates the close associations between fungi and their local environment (Andrew *et al.* 2016; Averill *et al.* 2019). Furthermore, the dynamic nature of fire's effect on fungi and their associated functions across time illustrates the importance of considering fire-fungal interactions in a temporal context. If the sum of fire's effects across time acts to increase the production and amount of plant fuels, then this could drive feedbacks on future fire characteristics.

Feedbacks between fire and plant fuels engineer the fire regimes of pyrophilic ecosystems, and determine the characteristics of individual fires. Since interactions between fire and fungi directly modify fungal functions associated with plant fuel loads (e.g. decomposition and plant-

soil interactions), fire-fungal interactions may also contribute to fuel related feedbacks on future fires. I showed that as fires became more frequent and intense, decomposition of new plant fuels slowed, and larger portions of fuels remained at the end of the study period. Since plant fuel load accumulation and fire are not independent from one and other (Platt *et al.* 2016; Tiribelli *et al.* 2018), fire-fungal interactions may increase the frequency and severity of fire regimes. Fires may also be modified by complex interactions between pyrophilic plant taxa and fungi. Many pyrophilic plant taxa have high post-fire germination and growth rates (this work, as well as Beckage & Ellingwood 2008; Beckage *et al.* 2009; Archibald *et al.* 2018), and also possess traits which make them difficult to decompose (e.g. high lignin content, high C:N ratios, and low shoot N content; Heckathorn & Delucia 1996; Cornelissen *et al.* 2017). The rapid, post-fire production of recalcitrant plant fuels could cause fuels to accumulate quickly, and favor recurrent fires. Fire-fungal effects on fuel loads may be further modified by the timing of fire, as many fungal taxa follow seasonal trends (Santos-Gonzalez *et al.* 2007; Averill *et al.* 2019; Štursová *et al.* 2020) that could be sensitive to fire at critical points in the season. For example, fires early in the growing season can reduce fungal pathogen loads (this work, Hardison 1976; Katan 2000) and benefit plant fuel accumulation and increase the likelihood of fire. Fungi's ability to modify plant fuel loads illustrates the importance of considering both fungal and plant community roles in pyrophilic ecosystems. Since fungal functions associated with plant fuels are generally ubiquitous across ecosystems, fire-fungal interactions may also be applicable to other terrestrial ecosystems as well.

While many terrestrial systems are not adapted to recurrent fires, the processes that engineer fire regimes in pyrophilic systems likely apply. In less fire tolerant systems such as temperate and boreal forests, fungi modify plant fuels through saprotrophic, pathogenic, and mutualistic interactions, however, fire occurs far less frequently (Archibald *et al.* 2013). Longer

fire return intervals allow for greater accumulation of plant fuels and higher severity fires, with exacerbated effects on fungal communities (Kalies & Yocom Kent 2016; Kolden 2019; Roos *et al.* 2020). Manipulating the high severity fires of less fire tolerant systems is both extremely difficult and dangerous, but pyrophilic systems can act as a safe substitute for exploring fire-fungal interactions. Additionally, exploring fire-fungal interactions in pyrophilic systems is far easier, as plant fuel loads can be directly manipulated, and questions can be explored in an ecological framework. Furthermore, when comparing across ecosystems, other factors with large inter-system variation and known effects on fungal communities (e.g. pH, soil moisture, and climate; Andrew *et al.* 2016; Delgado-Baquerizo *et al.* 2018) can be readily added to this framework. Considering fire-fungal interactions in an ecological context allows for a framework that may be adaptable to other disturbance mediated systems.

Disturbance mediated ecosystems are relatively common (Lubchenco & Menge 1978; Blom 1999; Beckage & Ellingwood 2008; Archibald *et al.* 2013; Blaser 2016), and can provided useful models for understanding community assembly processes. As in pyrophilic systems, disturbance regimes influence community assembly, and may act as a recurrent, selective force if disturbances are frequent and/or intense enough. Microbiome-antibiotic interactions are one such system, where repeated doses of antibiotics can control microbiome structure and function by limiting microbial pathogens (Blaser 2016; Letten *et al.* 2021). In order to limit pathogens, antibiotic doses must be relatively frequent and intense enough to reduce the fitness of pathogens and provide a competitive advantage to other taxa. The importance of repeated doses parallels the stronger effect of frequent fires vs. fire severity on fungal communities in pyrophilic systems, and suggests that disturbance history and frequency may play larger roles in community assembly processes than single disturbance events. While single disturbance events still have important

effects on microbial community assembly, increasing the frequency of disturbance can impose a relatively constant selective filter on microbial communities that favors specific sets of traits. Similar processes may also be at work in agricultural systems (Mbuthia *et al.* 2015; Turley *et al.* 2020), where tillage and pesticide application are frequent, and known to select for specific microbial taxa and functional groups. Future work considering disturbance effects on community assembly processes can consider how disturbance regimes influence associations between taxonomic and functional diversity, modify natural seasonal variations in community structure, and interact with other assembly associated forces like dispersal, drift, and selection.

In conclusion, my thesis illustrates how fire alters fungal community assembly and function in ways that modify plant loads during the year following fire. The relative importance of different pathways linking fire and fungal communities are dynamic, and shift with time since fire. Early after fire, direct changes to fungal communities and the local environment have the strongest effect, and drive collocal shifts in decomposition and plant-fungal interactions. With increasing time since fire, indirect changes to the local environment become increasingly important as fire's direct effects weaken. By describing fire-fungal interactions in an ecological context across time, I have created a generalizable model for testing the effects of disturbance on microbial community assembly in disturbance mediated ecosystems. Since many of Earth's systems are maintained by recurrent disturbances like fire, including disturbance-microbial interactions in community assembly models can improve our understanding of the processes that structure ecosystems and biological communities. Furthermore, improving our understanding of the disturbance-microbe interactions can help us respond to climate and anthropogenic driven changes in global disturbance regimes. In summary, disturbance plays an important role in

community assembly processes and function, and has the potential to drive feedbacks on disturbance regimes.

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Chapter 1 - Appendix

Site Maps

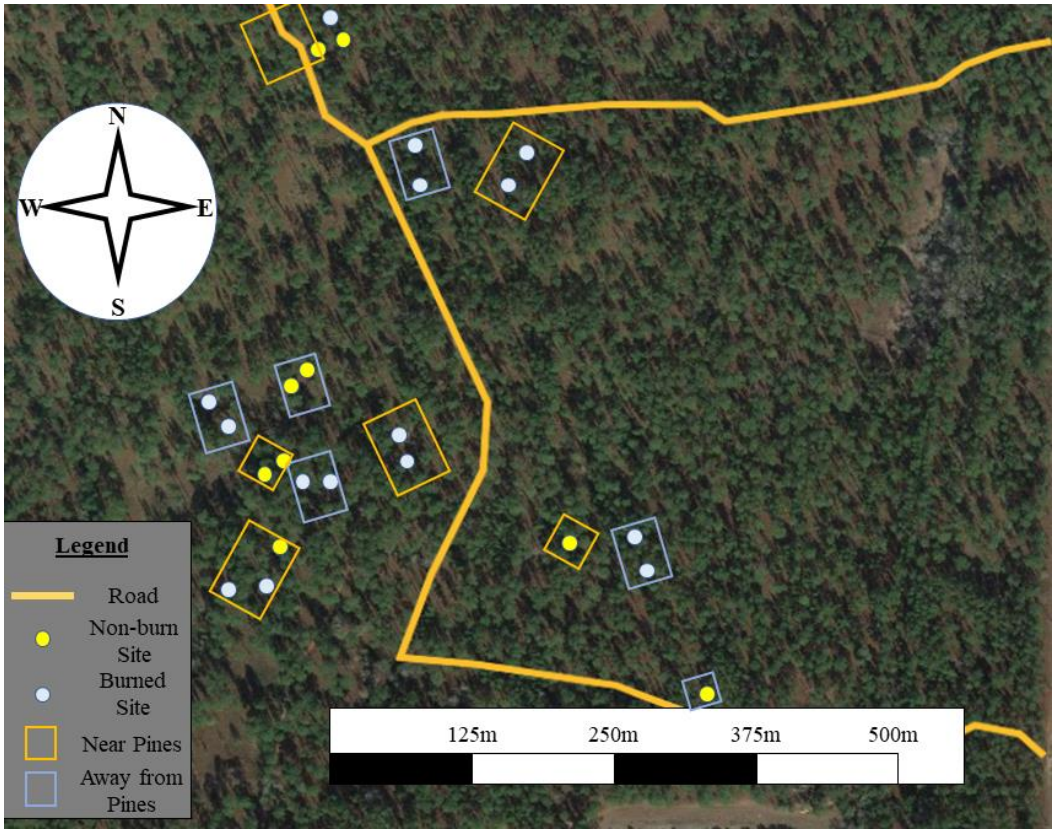

Figure S1: Longleaf pine savanna site map. Site was located at the Wade Tract (30˚ 45' N; 84˚ 00' W; Thomas County, Georgia, USA)

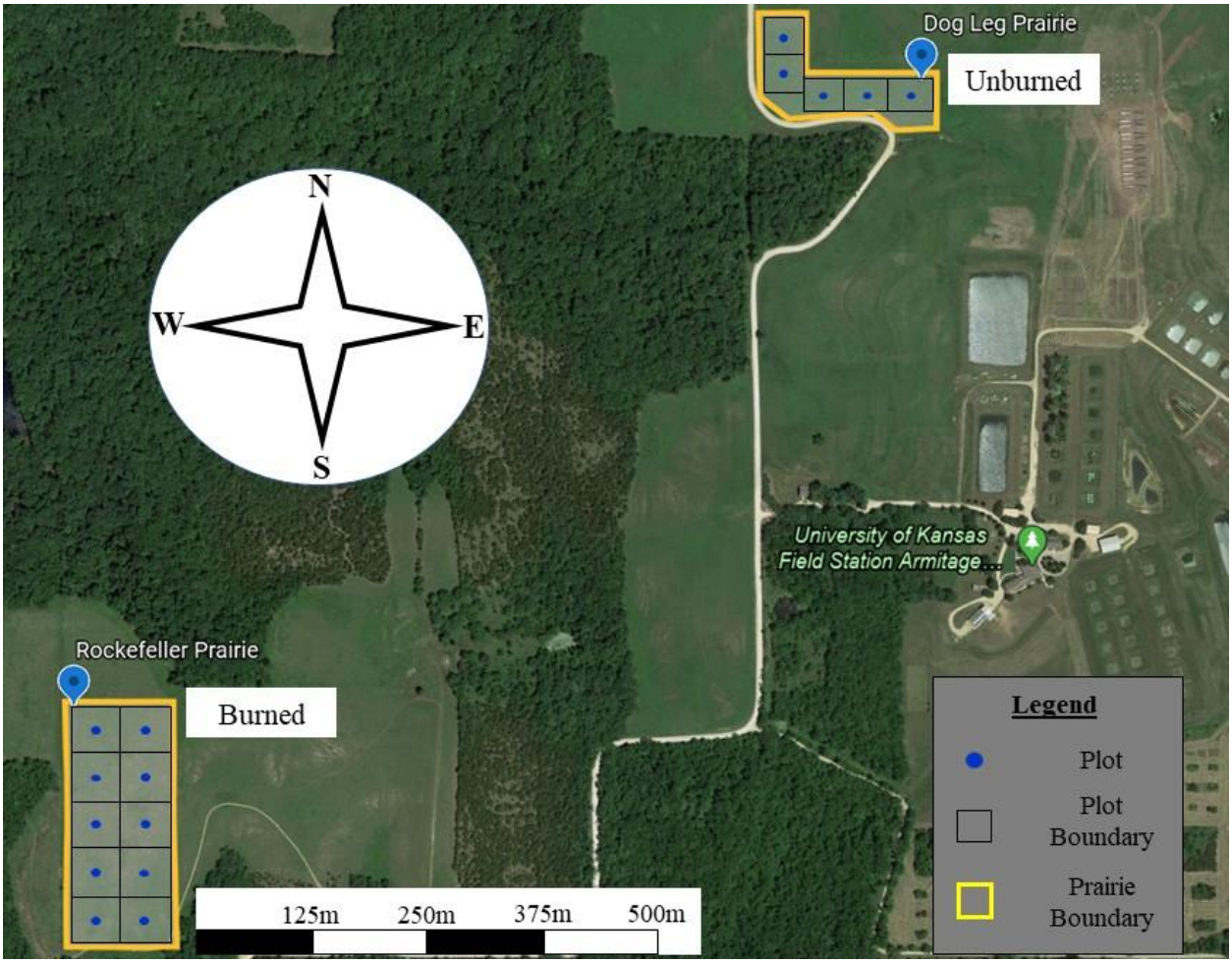

Figure S2: Tallgrass prairie site maps. Rockefeller (39° 2' N; 95° 12' W) and Dogleg prairies (39° 3' N; 95° 11' W), are located at the University of Kansas Field Station (Leavenworth County, Kansas).

Contrast Tables

Table S1: Results (t-values and significance) of PERMANOVA apriori contrasts testing differences in Longleaf pine savanna fungal community composition between pine proximity*burn treatment interaction groups. All contrasts used 999 permutations and P-values were derived from permutations.

Side	Pine Proximity	Contrast	T-value	P-value
west	near	burn vs. no burn	1.449	0.003**
west	away	burn vs. no burn	1.6251	0.001***
east	near	burn vs. no burn	1.2433	0.072*
east	away	burn vs. no burn	1.1503	0.165

*≤ 0.1, **≤0.05, ***≤0.001

Table S2: Results (t-values and significance) of PERMANOVA apriori contrasts testing changes in Longleaf pine savanna fungal community composition across sampling times. All contrasts used 999 permutations and P-values were derived from permutations.

Contrast	T-Value	P-value
pre-fire vs. 1 month	1.3671	0.021**
1 month vs 2 months	1.2578	0.018**
2 months vs. 3 months	1.3404	0.005**
2 months vs. 4 months	1.6061	0.001***
4 months vs. 6 months	1.2917	0.035**
5 months vs. 7 months	1.1638	0.168

*≤ 0.1, **≤0.05, ***≤0.001

Table S3: Results (t-values and significance) of PERMANOVA apriori contrasts testing differences in Longleaf pine savanna fungal community composition across sampling times and fire management units. All contrasts used 999 permutations and P-values were derived from permutations.

Side	Contrast	T-value	P-value
west	1 mon vs. 2 mon	1.2215	0.035**
west	1 mon vs. 4 mon	1.8469	0.001***
west	1 mon vs. 6 mon	1.6886	0.001***
west	2 mon vs. 4 mon	1.6162	0.001***
west	2 mon vs. 6 mon	1.3792	0.006**
west	4 mon vs. 6 mon	1.3184	0.038**
east	2 mon vs. 3 mon	1.2746	0.028**
east	2 mon vs. 5 mon	1.5796	0.003**
east	2 mon vs. 7 mon	1.4846	0.002**
east	3 mon vs. 5 mon	1.3211	0.024**
east	3 mon vs. 7 mon	1.0146	0.403
east	5 mon vs. 7 mon	1.1579	0.183

*≤ 0.1, **≤0.05, ***≤0.001

Table S4: Results (t-values and significance) of PERMANOVA apriori contrasts testing changes in burned Longleaf pine savanna fungal community composition across sampling times. All contrasts used 999 permutations and P-values were derived from permutations.

Contrast	T-Value	P-value
pre-fire vs. 1 month	1.3616	0.04**
1 month vs. 2 months	1.1378	0.089*
2 months vs. 3 months	1.3138	0.008**
2 months vs. 4 months	1.3977	0.005**
3 months vs. 5 months	1.3204	0.027**
4 months vs. 6 months	1.0535	0.315
5 months vs. 7 months	0.96109	0.527

*≤ 0.1, **≤0.05, ***≤0.001

Table S5: Results (t-values and significance) of PERMANOVA apriori contrasts testing changes in non-burned Longleaf pine savanna fungal community composition across sampling times. All contrasts used 999 permutations and P-values were derived from permutations.

Contrast	T-Value	P-value
1 month vs. 2 months	1.0756	0.29
2 months vs. 3 months	1.0444	0.367
2 months vs. 4 months	1.2643	0.055*
3 months vs. 5 months	0.93802	0.631
4 months vs. 6 months	1.0643	0.338
5 months vs. 7 months	1.1104	0.3371

*≤ 0.1, **≤0.05, ***≤0.001

Table S6: Results (t-values and significance) of PERMANOVA apriori contrasts testing differences in tallgrass prairie fungal community composition across sampling times. All contrasts used 999 permutations and P-values were derived from permutations.

Contrast	T-value	P-value
pre - 2 weeks	1.4493	0.013**
2 weeks - 1 month	1.1023	0.249
1 month - 2 months	1.179	0.113
2 months - 4 months	1.321	0.017**
4 months - 7 months	1.242	0.058*
7 months - 8 months	1.1136	0.234

*≤ 0.1, **≤0.05, ***≤0.001

Table S7: Results (t-values and significance) of PERMANOVA apriori contrasts testing differences in burned tallgrass prairie fungal community composition across sampling times. All contrasts used 999 permutations and P-values were derived from permutations.

Contrast	T-value	P-value
pre - 2 weeks	1.7093	0.006**
2 weeks - 1 month	1.3638	0.038**
1 month - 2 months	1.1028	0.288
2 months - 4 months	1.4171	0.019**
4 months - 7 months	1.3266	0.037**
7 months - 8 months	1.0382	0.459

*≤ 0.1, **≤0.05, ***≤0.001

Table S8: Results (t-values and significance) of PERMANOVA apriori contrasts testing differences in non-burned tallgrass prairie fungal community composition across sampling times. All contrasts used 999 permutations and P-values were derived from permutations.

Contrast	T-value	P-value
pre - 2 weeks	0.93654	0.553
2 weeks - 1 month	0.80075	0.687
1 month - 2 months	1.3778	0.082*
2 months - 4 months	1.1217	0.322
4 months - 7 months	1.1846	0.195
7 months - 8 months	1.1641	0.305

*≤ 0.1, **≤0.05, ***≤0.001

Bioinformatics
---

1) Extract Barcodes

```
extract_barcodes.py \
        -f Undetermined_S0_L001_I1_001.fastq \
        -r Undetermined_S0_L001_I2_001.fastq \
        -c barcode_paired_end --bc1_len 8 --bc2_len 8 \
        -o processed_pair_seqs12
```

2) Validate mapping file

```
validate_mapping_file.py \
        -m Map.txt \
        -o mapcheck
```

3) Split Libraries

```
split_libraries_fastq.py \
        -i Undetermined_S0_L001_R1_001.fastq \
        -b processed_pair_seqs12/barcodes.fastq \
        -m mapcheck.txt \
        -o FF17_splitF/ \
        -q 29 --max_barcode_errors 1 --barcode_type 16
```

4) Pick Open Reference OTUs

```
pick_open_refrence_otus.py \
        -i FF17_splitF/seqs.fna \
        -r "$REFERENCE_SEQ" \
        -o F17L_5OTU_sosu \
        -s 0.1 \
        -a0 16 \
        -f --suppress_taxonomy_assignment -- suppress_align_and_tree --min_otu_size 5 \
        -m sortmerna_sumaclust \
        -p "$PARAMS"*
```

* Median sequence length for quality filtering was set to 270 and 277 for pine savanna samples and tallgrass prairie samples respectively. OTUs were clustered at the 97% similarity level, and OTUS w/ fewer than 5 counts were removed.

5) Look at OTU Table

```
biom summarize-table \
        -i otu_table_mc5.biom \
        -o Fire17_sum.txt
```

6) Assign taxonomy with RDP Classifier

```
parallel_assign_taxonomy_rdp.py \
        -i rep_set.fna \
        -r sh_refs_qiime_ver7_dynamic_s_01.08.2015.txt \
        -c 0.9 \
        -O 16 --rdp_max_memory 25000 \
        -o rdp_assign_taxonomy
```

7) Add taxonomy to OTU table and transfer in excel table

```
biom add-metadata \
        -i otu_table_mc5.biom \
        -o F17try_wTax.biom --observation-metadata-fp rep_set_taxN.txt --sc-separated
        taxonomy --float-fields confidence
```

8) Normalization w/ Deseq2

```
filter_samples_from_otu_table.py \
        -i otu_table_mc5.biom \
        -o F17S_5k.biom \
        -n 5000

biom convert \
        -i F17S_5k.txt \
        -o F17S_5k.biom --to-json --table-type=”OTU table”

normalize_table.py \
        -i F17S_5k.biom -a DESeq2 --DESeq_negatives_to_zero \
        -o F17S_deseq.biom

biom add-metadata \
        -i F17S_deseq.biom \
        -o F17S_deseq.tax.biom --sample-metadata-fp map.txt --observation-metadata-fp
        rep_set_tax_N.txt --sc-separated taxonomy --float-fields confidence

biom convert \
        -i F17S_deseq.tax.biom \
        -o  F17S_deseq_tax.txt --to-tsv   --table-type=”OTU table” --header-key taxonomy
```

Table S9: Indicator species for burned Longleaf pine savanna plots. A represents the "A statistic," B the "B statistic," and Comb. Stat. is the combined A and B statistics. Taxonomy describes the lowest taxonomic level prescribed to the OTU. Putative ecology and citation are provided for each OTU when available.

A	B	Comb. Stat.	P-Value	Taxonomy	Ecology	Citation
0.78	0.67	0.73	0.001 ***	Geminibasidium	considered a pyrophilic and xerotolerant genus	Nguyen et al. 2013
0.87	0.52	0.68	0.003 **	Trichoderma	endophyte/opportunistic wood fungus and quick post-fire colonizer	Sharma 1981
0.80	0.52	0.65	0.004 **	Chaetothyriales	found in arid conditions, resistant to high temperatures	Villaseñor C. R. 2004, Sterflinger et al. 1999
0.81	0.51	0.64	0.004 **	Cordycipitaceae	insect pathogens	Kepler et al. 2017
0.92	0.44	0.64	0.002 **	Hydnangiaceae	family of ectomycorrhizal taxa	Cannon and Kirk 2007
0.86	0.48	0.64	0.011 **	Penicillium	known to be quick post-fire colonizers	McMullan-Fisher et al. 2011, Sharma 1981
0.89	0.44	0.63	0.002 **	Fungi		
0.89	0.43	0.62	0.004 **	Rhizopogon	ectomycorrhizal, false truffles, commonly found post-fire	Owen et al 2019, Glassman et al. 2016
0.80	0.48	0.62	0.007 **	Myrothecium	commonly considered plant pathogenic taxa	Chen 2016
0.79	0.44	0.59	0.011 **	Trichocomaceae	Saprobes, aggressive colonizers, adaptable to extreme conditions	McGee et al. 2016
0.82	0.41	0.58	0.014 **	Talaromyces	*Talaromyces* range from mesophilic to strongly thermophilic	Stolk and Samson 1972

Table S10: Indicator species for non-burned Longleaf pine savanna plots. A represents the "A statistic," B represents the "B statistic," and Comb. Stat. is the combined A and B statistics. Taxonomy describes the lowest taxonomic level prescribed to the OTU. Putative ecology and citation are provided for each OTU when available.

A	B	Comb. Stat.	P-Value	Taxonomy	Ecology	Citation
0.80	0.76	0.78	0.001 ***	P. livistonae	originally described in assocation with palm species	Crous et al. 2012
0.81	0.67	0.74	0.001 ***	Ascomycota		
0.81	0.67	0.74	0.001 ***	Ascomycota		
0.78	0.67	0.72	0.001 ***	Preussia	genus consists of dung and soil fungi	Kirk et al. 2008
0.76	0.58	0.66	0.004 **	Trimmatostroma	common leaf pathogens	Dick and Gadgil 2009
0.88	0.48	0.65	0.001 ***	Capnodiales	order of sooty molds and plant pathogens	Crous et al. 2009
0.88	0.48	0.65	0.001 ***	Cortinarius	common mycorrhizal species	Kirk et al . 2008
0.77	0.55	0.65	0.001 ***	Ascomycota		
0.92	0.45	0.65	0.001 ***	Ascomycota		
0.71	0.58	0.64	0.010 **	fungi		
0.84	0.48	0.64	0.001 ***	M. carmichaelii	member of plant pathogenic genus	Chen et al. 2016
0.74	0.55	0.63	0.004 **	Ascomycota		
0.72	0.52	0.61	0.004 **	Dothideomycetes	class contains endophytes, pathogens, and saprobes	Kirk et al. 2008
0.75	0.48	0.61	0.001 ***	Ascomycota		
0.84	0.42	0.60	0.003 **	Ascomycota		
0.78	0.45	0.60	0.010 **	Ascomycota		
0.83	0.42	0.59	0.002 **	Inocybe nodulosa	mycorrhizal, indicator of non-burned plots in comparitive study	Owen et al. 2019
0.72	0.48	0.59	0.008 **	Mycosphaerellaceae	family of sac fungi well known to be plant pathogens	Videira et al. 2017
0.71	0.42	0.55	0.014 *	Ascomycota		
0.62	0.42	0.51	0.011 *	Basidiomycota		

Table S11: Tallgrass prairie indicator species: 2 weeks post-fire. A represents the "A statistic," B represents the "B statistic," and Comb. Stat. is the combined A and B statistics. Taxonomy describes the lowest taxonomic level prescribed to the OTU. Putative ecology and citation are provided for each OTU when available.

A	B	Comb. Stat.	P-Value	Taxonomy	Ecology	Citation
0.82	1.00	0.91	0.002 **	Fungi		
0.82	1.00	0.91	0.002 **	Spizellomyces plurigibbosus	commercially available	
0.82	1.00	0.91	0.004 **	Fungi		
0.88	0.89	0.88	0.005 **	Fusarium tricinctum	plant pathogen isolated from surface sterilize	DOE Joint Genome Institute - JFI MycoCosm: Fusarium tricinctum
0.87	0.89	0.88	0.004 **	Fungi		
0.95	0.78	0.86	0.006 **	Fungi		
0.83	0.89	0.86	0.010 **	Cortinarius diasemospermus	some Cortinarius taxa are indicative of freque	McMullan-Fisher et al. 2011
0.94	0.78	0.86	0.003 **	Fungi		
0.91	0.78	0.84	0.012 *	Myrothecium cinctum	member of plant pathogenic genus	Chen et al. 2016
0.89	0.78	0.83	0.010 **	Lophiostoma sp.	wood saprotroph	Holm L. 1988
0.86	0.78	0.82	0.011 *	Powellomyces sp.	chytrid	Simmons D.R. 2011
1.00	0.67	0.82	0.011 *	Fungi		
0.83	0.78	0.81	0.022 *	Coprinellus disseminatus	wood saprotroph	Kuo M. 2008
0.97	0.67	0.81	0.013 *	Fungi		
0.83	0.78	0.80	0.012 *	Fungi		
0.94	0.67	0.79	0.011 *	Sporobolomyces beijingensis	likely saprotrophic	Wang and Bai 2004
0.80	0.78	0.79	0.036 *	Fungi		
0.93	0.67	0.79	0.024 *	Talaromyces purpureus	Talaromyces is a sexual state of Penicillium,	McMullan-Fisher et al. 2011, Sharma 1981
0.92	0.67	0.78	0.026 *	Agaricales	large order containing saprobes, parasites, an	Kirk et al. 2008
0.68	0.89	0.78	0.049 *	Basidiomycota		
0.78	0.78	0.78	0.041 *	Ascomycota		
0.88	0.67	0.77	0.029 *	Entoloma	most members are saprotrophic, with some n	Kirk et al. 2008
0.86	0.67	0.76	0.044 *	Capnodium sp.	sooty molds, plant pathogens	Lumbsch and Huhndorf 2007
0.85	0.67	0.75	0.035 *	Dothideomycetes	diverse class of fungi containing endophytes,	Kirk et al. 2008
0.84	0.67	0.75	0.046 *	Ascomycota		
1.00	0.56	0.75	0.027 *	Agaricomycetes	large class containing saprobes, parasites, and	Kirk et al. 2008
1.00	0.56	0.75	0.033 *	Basidiomycota		
1.00	0.56	0.75	0.034 *	Geastraceae	earth stars, wood saprotrophs	Kirk et al. 2008
1.00	0.56	0.75	0.027 *	Lasiosphaeriaceae	wood and dung associated	Cannon and Kirk 2007
1.00	0.56	0.75	0.037 *	Pleosporales	order of saprotrophic, pathogenic, and endopl	Zhang et al. 2009
1.00	0.56	0.75	0.033 *	Coprinellus sp.	many member taxa are associated with wood	Peiris et al. 2007
1.00	0.56	0.75	0.032 *	Fungi		
1.00	0.56	0.75	0.033 *	Fungi		
1.00	0.56	0.75	0.032 *	Fungi		
0.97	0.56	0.73	0.032 *	Pyronemataceae	specialization to burnt ground, saprobe/ecton	Hansen et al. 2013
0.94	0.56	0.72	0.034 *	Dothideomycetes	diverse class of fungi containing endophytes,	Kirk et al. 2008
0.93	0.56	0.72	0.034 *	Fungi		
0.91	0.56	0.71	0.035 *	Minimedusa sp.	likely a cellulytic saprotroph	Pinzari et al. 2014
0.91	0.56	0.71	0.028 *	Entolomataceae	saprotrophic	Noordeloos and Gates 2012
0.89	0.56	0.70	0.029 *	Fungi		
0.89	0.56	0.70	0.034 *	Scolecobasidium constrictum	dark walled mold	
0.87	0.56	0.70	0.036 *	Urnula craterium	wood saprotroph and Oak parasite	Huffman et al. 2008

Table S12: Tallgrass prairie indicator species: pre-fire. A represents the "A statistic," B represents the "B statistic," and Comb. Stat. is the combined A and B statistics. Taxonomy describes the lowest taxonomic level prescribed to the OTU. Putative ecology and citation are provided for each OTU when available.

A	B	Comb. Stat.	P-Value	Taxonomy	Ecology	Citation
0.98	1.00	0.99	0.002 **	Acremonium implicatum	endophytic fungus of Brachiaria grasses	Dongyi H. and Kelemu S. 2004
0.90	1.00	0.95	0.002 **	Pleosporales	order of saprotrophic, pathogenic, and endophytic fung	Zhang et al. 2009
1.00	0.89	0.94	0.002 **	Ascomycota		
1.00	0.89	0.94	0.002 **	Ascomycota		
1.00	0.89	0.94	0.002 **	Phaeosphaeriaceae	family of plant associated nectrotrophs and saprobes	Cannon and Kirk 2007
1.00	0.89	0.94	0.002 **	Stictis	genera of wood saprobes and lichenized fungi	Wedin et al. 2006
0.88	1.00	0.94	0.002 **	Tubeufiaceae	family of plant pathogens and saprobes	Rossman 1987
0.98	0.89	0.93	0.003 **	Glomerobolus gelineus	halotolerant species	Schoch et al. 2006
0.87	1.00	0.93	0.002 **	Capnodiales	order of sooty molds and plant pathogens	Crous et al. 2009
0.87	1.00	0.93	0.003 **	Cryptococcus	common genus of soil saprobes and opportunistic huma	May et al. 2016
0.96	0.89	0.93	0.002 **	trimmatostroma	common leaf pathogens	Dick and Gadgil 2009
0.85	1.00	0.92	0.002 **	Ascomycota		
0.94	0.89	0.92	0.002 **	Phoma	genus containing many plant pathogenic taxa	Kirk et al. 2008, Harshberger 1917
0.83	1.00	0.91	0.002 **	Ascomycota		
0.83	1.00	0.91	0.005 **	Ascomycota		
0.81	1.00	0.90	0.003 **	bulleribasidium oberjochense	anamorphic form of Bullera, likely myco-parasite	Kirk et al. 2008, Golubev et al. 1997
0.91	0.89	0.90	0.006 **	Mrakiella aquatica	isolated from freshwater samples	Jones and Slooff 1966
0.80	1.00	0.89	0.003 **	Ascomycota		
0.79	1.00	0.89	0.002 **	Phaeosphaeriaceae	family of plant associated nectrotrophs and saprobes	Cannon and Kirk 2007
0.79	1.00	0.89	0.002 **	Periconia	saprotroph	Markovskaja and Kačergius 2014
0.89	0.89	0.89	0.003 **	Lachnum	genus of wood and leaf saprotrophs	
0.79	1.00	0.89	0.004 **	Phialophora livistonae	originally described in assocation with palm species	Crous et al. 2012
1.00	0.78	0.88	0.004 **	Ascomycota		
0.87	0.89	0.88	0.004 **	Capnodiales	order of sooty molds and plant pathogens	Crous et al. 2009
1.00	0.78	0.88	0.008 **	Tremellales	order of mycoparsitic taxa	Kirk et al. 2008
1.00	0.78	0.88	0.004 **	Cryptococcus dimennae	genus of saprotrophic and opportunistic human pathoge	May et al. 2016
0.83	0.89	0.86	0.007 **	articulospora	aquatic associated	Seena et al. 2018
0.93	0.78	0.85	0.002 **	Ascomycota		
0.82	0.89	0.85	0.009 **	Tubeufiaceae	family of plant pathogens and saprobes	Rossman 1987
0.81	0.89	0.85	0.014 *	Helotiales	order predominantly composed of wood saprobes	Kirk et al. 2008
0.92	0.78	0.85	0.007 **	Capnodiales	order of sooty molds and plant pathogens	Crous et al. 2009
0.90	0.78	0.84	0.017 *	Ascomycota		
0.90	0.78	0.84	0.013 *	Zymoseptoria	genus of grass pathogens	Quaedvlieg et al. 2011
0.79	0.89	0.84	0.020 *	Pleosporales	order of saprotrophic, pathogenic, and endophytic fung	Zhang et al. 2009
0.88	0.78	0.83	0.016 *	Leotiomycetes	class of plant pathogens and saprobes	Wang Z. 2007
0.76	0.89	0.82	0.018 *	Dothideomycetes	diverse class of fungi containing endophytes, pathogens	Kirk et al. 2008
0.86	0.78	0.82	0.010 **	Ascomycota		
0.75	0.89	0.82	0.032 *	Fungi		
1.00	0.67	0.82	0.015 *	Ascomycota		
1.00	0.67	0.82	0.009 **	Basidiomycota		
0.86	0.78	0.82	0.023 *	Basidiomycota		
1.00	0.67	0.82	0.012 *	Capnodiales	order of sooty molds and plant pathogens	Crous et al. 2009
1.00	0.67	0.82	0.015 *	Rachicladosporium	genus containing several plant pathogens	Crous et al. 2014, Crous et al. 2018
1.00	0.67	0.82	0.010 **	Tubeufiaceae	family of plant pathogens and saprobes	Rossman 1987
1.00	0.67	0.82	0.013 *	Tubeufiaceae	family of plant pathogens and saprobes	Rossman 1987
0.85	0.78	0.82	0.021 *	Herpotrichiellaceae	family of wood saprobes and animal pathogens	Gueidan C. 2008
0.74	0.89	0.81	0.010 **	Dothioraceae	family of nectrotroph and wood associated taxa	Cannon and Kirk 2007
0.83	0.78	0.81	0.015 *	Mycosphaerellaceae	plant pathogen	Pérez et al. 2013, Taylor et al. 2003
0.96	0.67	0.80	0.010 **	Orbiliomycetes	class of nematode and invertebrate trapping taxa	Baral et al. 2018
0.95	0.67	0.80	0.006 **	Chaetomiaceae	family of human and animal pathogens	Plumlee et al. 2017
0.95	0.67	0.80	0.022 *	Chaetothyriales	often found in arid conditions and known to be resistan	aseñor C. R. 2004, Sterflinger et al. 19
0.81	0.78	0.80	0.020 *	Dioszegia	genus of plant associated taxa	Renker et al. 2004
0.95	0.67	0.80	0.011 *	Eurotiomycetes		
0.81	0.78	0.79	0.029 *	Ascochyta	genus of grass pathogens	Sprague and Johnson 2018
0.81	0.78	0.79	0.040 *	Fungi		
0.71	0.89	0.79	0.037 *	Phaeosphaeria	genus of plant pathogens	El-Demerdash 2018
0.94	0.67	0.79	0.018 *	Hannaella luteola	isolated from plant leaves	atalogue of Microorganisms - Hannael
0.94	0.67	0.79	0.023 *	Periconia	saprotroph	Markovskaja and Kačergius 2014

0.93	0.67	0.79	0.018 *	Ramichloridium	likely plant pathogen	Arzanlou et al. 2007
0.91	0.67	0.78	0.018 *	Periconia	saprotroph	Markovskaja and Kačergius 2014
0.78	0.78	0.78	0.033 *	Savoryella aquatica	water associated	Hyde K.D. 1993
0.89	0.67	0.77	0.027 *	Helotiales	order predominantly composed of wood saprobes	Kirk et al. 2008
0.88	0.67	0.77	0.036 *	Geoglossum	likely saprotroph	Kuo M. 2019
0.85	0.67	0.75	0.047 *	Ascomycota		
0.85	0.67	0.75	0.042 *	Metacordyceps chlamydosporia	arthropod parasite	Kepler et al. 2012
0.84	0.67	0.75	0.048 *	Cyphellophora	associatd with some plant diseases	Gao et al. 2015
0.84	0.67	0.75	0.033 *	Montagnulaceae	family containing saprotrophic, endophytic, and pathog	Ariyawansa et al. 2014
1.00	0.56	0.75	0.037 *	Ascomycota		
1.00	0.56	0.75	0.038 *	Ascomycota		
1.00	0.56	0.75	0.032 *	Ascomycota		
1.00	0.56	0.75	0.032 *	Ascomycota		
1.00	0.56	0.75	0.034 *	Ascomycota		
1.00	0.56	0.75	0.021 *	Ascomycota		
1.00	0.56	0.75	0.049 *	Basidiomycota		
1.00	0.56	0.75	0.038 *	Capnodiales	order of sooty molds and plant pathogens	Crous et al. 2009
1.00	0.56	0.75	0.043 *	Chaetothyriales	often found in arid conditions and known to be resistanaseñor C. R. 2004, Sterflinger et al. 1
1.00	0.56	0.75	0.027 *	Eurotiomycetes		
1.00	0.56	0.75	0.028 *	Ophiocordycipitaceae	insect parasite	Sung et al. 2007
1.00	0.56	0.75	0.022 *	Periconia	saprotroph	Markovskaja and Kačergius 2014
1.00	0.56	0.75	0.027 *	Bullera pseudoalba	saprotroph	Nakase and Suzuki 1986
1.00	0.56	0.75	0.037 *	Eurotiomycetes		
1.00	0.56	0.75	0.030 *	Eurotiomycetes		
1.00	0.56	0.75	0.038 *	Helicoma	many member taxa are found on dead wood and leave	Goos R.D. 1986
1.00	0.56	0.75	0.021 *	Pleomassariaceae	wood saprotrophs	Cannon and Kirk 2007
0.82	0.67	0.74	0.037 *	Sarcinomyces		
0.98	0.56	0.74	0.037 *	Stictis	genera of wood saprobes and lichenized fungi	Weden et al. 2006
0.96	0.56	0.73	0.034 *	Adisciso	contains leaf associated taxa	Tanaka et al. 2011
0.96	0.56	0.73	0.029 *	Ascomycota		
0.94	0.56	0.72	0.028 *	Ascomycota		
0.78	0.67	0.72	0.043 *	Stachybotrys	mold	
0.93	0.56	0.72	0.047 *	Capnodiales	order of sooty molds and plant pathogens	Crous et al. 2009
0.91	0.56	0.71	0.039 *	Ascomycota		
0.91	0.56	0.71	0.047 *	Fungi		

Nutrients

Table S13: Longleaf pine savanna fungal community variance explained by nutrient availability table. Numerical values in the table represent R^2 values from the envfit() function.

	Non-Burned				Burned		
Sampling Time	**Total Carbon**	**Total Nitrogen**	**Total Phosphorus**	**Sampling Time**	**Total Carbon**	**Total Nitrogen**	**Total Phosphorus**
month 1	0.7	0.94	0.04	*month 1*	0.14	0.16	0.29
month 2	0.06	0.17	0.5	*month 2*	0.17	0.13	0.04
month 3	na	na	na	*month 3*	0.56	0.62	0.8**
month 4	na	na	na	*month 4*	0.09	0.09	0.4
month 5	na	na	na	*month 5*	0.31	0.39	0.28
month 6	0.51	0.99	0.14	*month 6*	0.15	0.25	0.2
month 7	na	na	na	*month 7*	0.2	0.14	0.18

*≤0.1, **≤0.05, ***≤0.001

Table S14: Treatment effects on Longleaf pine savannas total soil carbon (%). Type III ANOVAs were used.

Pine Savanna - Total Carbon (%)				
model term	**d.f.1**	**d.f.2**	**F ratio**	**P-value**
fire treatment	1	63.02	6.683	0.0121**
pine proximity	1	63.01	1.776	0.1874
time	6	56.06	0.003	1
fire x pine	1	63.01	3.958	0.051*
fire x time	6	63.05	0.303	0.933

*≤0.1, **≤0.05, ***≤0.001

Table S15: Treatment effects on Longleaf pine savannas total soil nitrogen (%). Type III ANOVAs were used.

Pine Savanna - Total Nitrogen (%)				
model term	**d.f.1**	**d.f.2**	**F ratio**	**P-value**
fire treatment	1	63.01	5.338	0.0242**
pine proximity	1	63.01	2.02	0.1602
time	6	62.92	0.082	0.9978
fire x pine	1	63.01	0.194	0.6613
fire x time	6	63.02	0.474	0.8249

*≤0.1, **≤0.05, ***≤0.001

Table S16: Treatment effects on Longleaf pine savannas total inorganic soil phosphorus (ppm). Type III ANOVAs were used.

Pine Savanna - Log Total Inorganic Phosphorus (ppm)				
model term	**d.f.1**	**d.f.2**	**F ratio**	**P-value**
fire treatment	1	63.01	0.578	0.4501
pine proximity	1	63	2.431	0.124
time	6	63.08	0.074	0.9983
fire x pine	1	63	8.184	0.0057***
fire x time	6	63.02	0.548	0.7694

*≤0.1, **≤0.05, ***≤0.001

Table S17: Tallgrass prairie fungal community variance explained by nutrient availability table. Numerical values in the table represent R^2 values from the envfit() function.

	Non-Burned				**Burned**		
Sampling Time	**Total Carbon**	**Total Nitrogen**	**Total Phosph.**	**Sampling Time**	**Total Carbon**	**Total Nitrogen**	**Total Phosph.**
pre-treatment	0.6	0.2	0.75	*pre-treatment*	0.2	0.14	0.39

week 2	0.5	0.63	0.34	*week 2*	0.59**	0.54*	0.59**
month 1	0.53	0.86	0.34	*month 1*	0.74**	0.81**	0.61*
month 2	0.71	0.29	0.67	*month 2*	0.74**	0.6**	0.01
month 4	0.24	0.08	0.49	*month 4*	0.22	0.24	0.29
month 7	0.67	0.77	0.74	*month 7*	0.04	0.03	0.09
month 8	0.79	0.78	0.9	*month 8*	0.22	0.35	0.15

*≤0.1, **≤0.05, ***≤0.001

Table S18: Treatment effects on tallgrass prairie total soil carbon (%). Type III ANOVAs were used.

model term	**d.f.1**	**d.f.2**	**F ratio**	**p-value**
fire treatment	1	89	55.251	<.0001***
time	6	89	8.321	<.0001***
fire x time	6	89	0.401	0.8767

*≤0.1, **≤0.05, ***≤0.001

Table S19: Treatment effects on tallgrass prairie total soil nitrogen (%). Type III ANOVAs were used.

model term	**d.f.1**	**d.f.2**	**F ratio**	**p-value**
fire treatment	1	89	61.222	<.0001***
time	6	89	7.122	<.0001***
fire x time	6	89	0.474	0.8263

*≤0.1, **≤0.05, ***≤0.001

Table S20: Treatment effects on tallgrass prairie total inorganic soil phosphorus (ppm). Type III ANOVAs were used.

model term	**d.f.1**	**d.f.2**	**F ratio**	**p-value**
fire treatment	1	89	18.387	<.0001***
time	6	89	3.209	0.0067**
fire x time	6	89	0.945	0.467

*≤0.1, **≤0.05, ***≤0.001

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Chapter 2 - Appendix

Table S1: Description of variables included in structural equation model fitting process. Table contains the name, type, coding, applied transformations, and a brief description of each variable.

Variable name	SEM code	Transform	Mean	sd	Description
Number of Fires	0-0-0 = 0 0-0-1 = 1 1-0-1 = 2 0-1-1 = 2 1-1-1 = 3	none	0-0-0 is reference	na	Describes the number of fires during the prior three years as a summation.
Pine Proximity	near, away	none	away is reference	na	Type of litter contained by litter bags at each site. Near = litter within 10m of nearest pine, Away = litter from 10 m away of nearest pine.
Surface Fire Duration above 60˚C		square root	193.02	144.79	Amount of time (seconds) 2016 fires remained over 60˚C.
Maximum Instant Surface Temperature Increase (°C)		square root	177.72	113.08	Maximum temperature (˚C) recorded in the plant litter layer.
Maximum Instant Soil Temperature Increase (°C)		square root	12.26	12.26	Maximum temperature (˚C) recorded in the upper soil layer.
Pine Needle Fuels (g)		square root	11.72	10.35	Weight of longleaf pine needles in each plot (grams).
Total Fine Fuels (g)		none	85.08	36.53	Weight of all fine fuels in each plot (grams).
NH4+ (ppm)		square root	16.14	12.08	Concentration of ammonium in plot (parts per million).
NO3- (ppm)		square root	1.66	1.48	Concentration of nitrate in plot (ppm).
Inorganic Soil Phosphorus (ppm)		log10	44.74	42.85	Determined by Mehlich III method (ppm).
Total Soil Carbon (%)		none	4.28	1.71	Total organic and inorganic carbon.
Total Soil Nitrogen (%)		natural log	0.23	0.64	Total organic and inorganic nitrogen.
2 Month Decomposition Rate (%/day)		none	0.15	0.07	Decomposition rate through 2 month post-fire time point.
4 Month Decomposition Rate (%/day)		none	0.12	0.05	Decomposition rate through 4 month post-fire time point.

6 Month Decomposition Rate (%/day)	none	0.08	0.04	Decomposition rate through 6 month post-fire time point.
8 Month Decomposition Rate (%/day)	none	0.07	0.03	Decomposition rate through 8 month post-fire time point.

Table S1.5: Initial SEM model.

Response Variable	Explanatory Variable(s)	Justification	Citation
NH_4^+ (ppm)	Number of Fires	NH_4^+ decreases w/ recurrent fires	Christensen 1977
	Max Ins. Surface Temp. Increase (˚C)	Nitrogen volatilizes at 200°C	Raison 1979
	Max Ins. Soil Temp. Increase (˚C)	Nitrogen volatilizes at 200°C	Raison 1979
	Surface Fire Duration > 60˚C	longer duration leads to greater combustion and loss	
2 Month Decomposition Rate	Number of Fires	High fire frequencies are associated with slow decomposition	Ficken and Wright 2017
	NH4+	NH_4^+ is readily utilized by microbes for enzyme production	Taylor et al. 1989
	Pine Proximity	Pine needles are harder to decompose and produce more intense fires	Hobbie 2000
	Phosphorus	N:P ratios control decomposition	
	Soil Carbon	High C:N ratios slow decomposition	Taylor et al. 1989
	Soil Nitrogen	Low C:N ratios increase decomposition	Taylor et al. 1989
	NO_3^-	NO_3^- is readily utilized by microbes for enzyme production	Taylor et al. 1989
	Max Ins. Surface Temp. Increase (˚C)	High temperature jumps can increase microbial mortality	Bárcenas-Moreno and Bååth 2009
	Max Ins. Soil Temp. Increase (˚C)	High temperature jumps can increase microbial mortality	Bárcenas-Moreno and Bååth 2010
	Surface Fire Duration > 60˚C	Longer heating durations can increase microbial mortality	Peay et al. 2009
4 Month Decomposition Rate	Number of Fires	High fire frequencies are associated with slow decomposition	Ficken and Wright 2017
	Soil Carbon	High C:N ratios slow decomposition	Taylor et al. 1989
	Soil Nitrogen	Low C:N ratios increase decomposition	Taylor et al. 1989
	2 Month Decomposition Rate	Decomposition is successional process	Voříšková and Baldrian 2013
	Phosphorus	N:P ratios control decomposition	
	NH4+	NH_4^+ is readily utilized by microbes for enzyme production	Taylor et al. 1989
	NO3-	NO_3^- is readily utilized by microbes for enzyme production	Taylor et al. 1989
	Pine Proximity	Pine needles are harder to decompose and produce more intense fires	
	Max Ins. Surface Temp. Increase (˚C)	High temperature jumps can increase microbial mortality	Bárcenas-Moreno and Bååth 2009
	Surface Fire Duration > 60˚C	High temperature jumps can increase microbial mortality	Bárcenas-Moreno and Bååth 2009
	Max Ins. Soil Temp. Increase (˚C)	Longer heating durations can increase microbial mortality	Peay et al. 2009
6 Month Decomposition Rate	Soil Carbon	High C:N ratios slow decomposition	Taylor et al. 1989
	Soil Nitrogen	Low C:N ratios increase decomposition	Taylor et al. 1989
	NO3-	NO_3^- is readily utilized by microbes for enzyme production	Taylor et al. 1989
	NH_4+	NH_4^+ is readily utilized by microbes for enzyme production	Taylor et al. 1989
	Pine Proximity	Pine needles are harder to decompose and produce more intense fires	Hobbie 2000
	Number of Fires	High fire frequencies are associated with slow decomposition	Ficken and Wright 2017
	Phosphorus	N:P ratios control decomposition	
	Max Ins. Surface Temp. Increase (˚C)	High temperature jumps can increase microbial mortality	Bárcenas-Moreno and Bååth 2009
	Max Ins. Soil Temp. Increase (˚C)	High temperature jumps can increase microbial mortality	Bárcenas-Moreno and Bååth 2009
	Surface Fire Duration > 60˚C	Longer heating durations can increase microbial mortality	Peay et al. 2009
	2 Month Decomposition Rate	Decomposition is successional process	Voříšková and Baldrian 2013
	4 Month Decomposition Rate	Decomposition is successional process	Voříšková and Baldrian 2013
8 Month Decomposition Rate	Number of Fires	High fire frequencies are associated with slow decomposition	Ficken and Wright 2017
	2 Month Decomposition Rate	Decomposition is successional process	Voříšková and Baldrian 2013
	4 Month Decomposition Rate	Decomposition is successional process	Voříšková and Baldrian 2014
	6 Month Decomposition Rate	Decomposition is successional process	Voříšková and Baldrian 2013
	Soil Carbon	High C:N ratios slow decomposition	Taylor et al. 1989
	Soil Nitrogen	Low C:N ratios increase decomposition	Taylor et al. 1989
	Phosphorus	N:P ratios control decomposition	
	NO3-	NO_3^- is readily utilized by microbes for enzyme production	Taylor et al. 1989
	NH4+	NH_4^+ is readily utilized by microbes for enzyme production	Taylor et al. 1989
	Pine Proximity	plant fuel traits differ near/away from pines	
	Max Ins. Surface Temp. Increase (˚C)	High temperature jumps can increase microbial mortality	Bárcenas-Moreno and Bååth 2009
	Max Ins. Soil Temp. Increase (˚C)	High temperature jumps can increase microbial mortality	Bárcenas-Moreno and Bååth 2009
	Surface Fire Duration > 60˚C	Longer heating durations can increase microbial mortality	Peay et al. 2009
Maximum Instantaneous Surface Temperature Rise (˚C)	Pine Needles	Pine needles are highly flammable fuels that control fire characteristics	Ellair and Platt 2013
	Total Fine Fuels	more fuels should intensify fire characteristics	
	Pine Proximity	plant fuel traits differ near/away from pines	Platt et al. 2016
	Number of Fires	frequent fires may reduce plant fuel loads	
Maxiumum Instantaneous Soil Temperature Rise (˚C)	Pine Needles	Pine needles are highly flammable fuels that control fire characteristics	Ellair and Platt 2013
	Pine Proximity	plant fuel traits differ near/away from pines	
	Total Fine Fuels	more fuels should intensify fire characteristics	
	Number of Fires	frequent fires should reduce fuel load	
	Litter Fire Duration > 60˚C	longer heating duration at surface should increase soil temperatures	Peay et al. 2009
Surface Fire Duration > 60˚C	Max Ins. Surface Temp. Increase (˚C)	higher temperatures are associated with longer burn durations	Bárcenas-Moreno and Bååth 2009
	Number of Fires	frequent fires should reduce fuel load	
	Pine Needles	Pine needles are highly flammable fuels that control fire characteristics	Ellair and Platt 2013
	Total Fine Fuels	more fuels should intensify fire characteristics	
	Pine Proximity	plant fuel traits differ near/away from pines	
Soil Carbon (%)	Number of Fires	frequent low severity fires are associated with increased C storage	Czimczik et al. 2005
	Soil Nitrogen	High amounts of available N can control C released through decomposition	linked through C:N ratio
	Max Ins. Surface Temp. Increase (˚C)	higher temps. Increase nutrient availability	
	Max Ins. Soil Temp. Increase (˚C)	higher temps. Increase nutrient availability	
	Surface Fire Duration > 60˚C	longer duration leads to greater combustion and loss	
Soil Nitrogen (%)	Number of Fires	frequent fires reduce nitrogen availability	Bell and Binkley 1989
	NO3-	more nitrate = more soil nitrogen	
	Max Ins. Surface Temp. Increase (˚C)	Nitrogen volatilizes at 200°C	Raison 1979
	Max Ins. Soil Temp. Increase (˚C)	Nitrogen volatilizes at 200°C	Raison 1979
	Surface Fire Duration > 60˚C	longer duration leads to greater combustion and loss	
	NH_4+	more nitrate = more soil nitrogen	
Soil Phosphorus (ppm)	Number of Fires	fire increases phosphorus availability	Butler et al. 2018
	Soil Carbon	linked through C:P ratio	
	Soil Nitrogen	linked through C:N ratio	
	Max Ins. Surface Temp. Increase (˚C)	P availability increases with fire temperature	Butler et al. 2018
	Max Ins. Soil Temp. Increase (˚C)	P availability increases with fire temperature	Butler et al. 2018
	Surface Fire Duration > 60˚C	longer duration leads to more P released from organic matter	longer duration leads to greater combustion and loss
NO_3^- (ppm)	Number of Fires	frequent fires reduce nitrogen availability	Christensen 1977
	Max Ins. Surface Temp. Increase (˚C)	Nitrogen volatilizes at 200°C	Raison 1979
	Max Ins. Soil Temp. Increase (˚C)	Nitrogen volatilizes at 200°C	Raison 1979
	Surface Fire Duration > 60˚C	longer duration leads to greater combustion and loss	
Pine Needles (g)	Pine Proximity	more pine needles near longleaf pines	

Fine Fuels Section

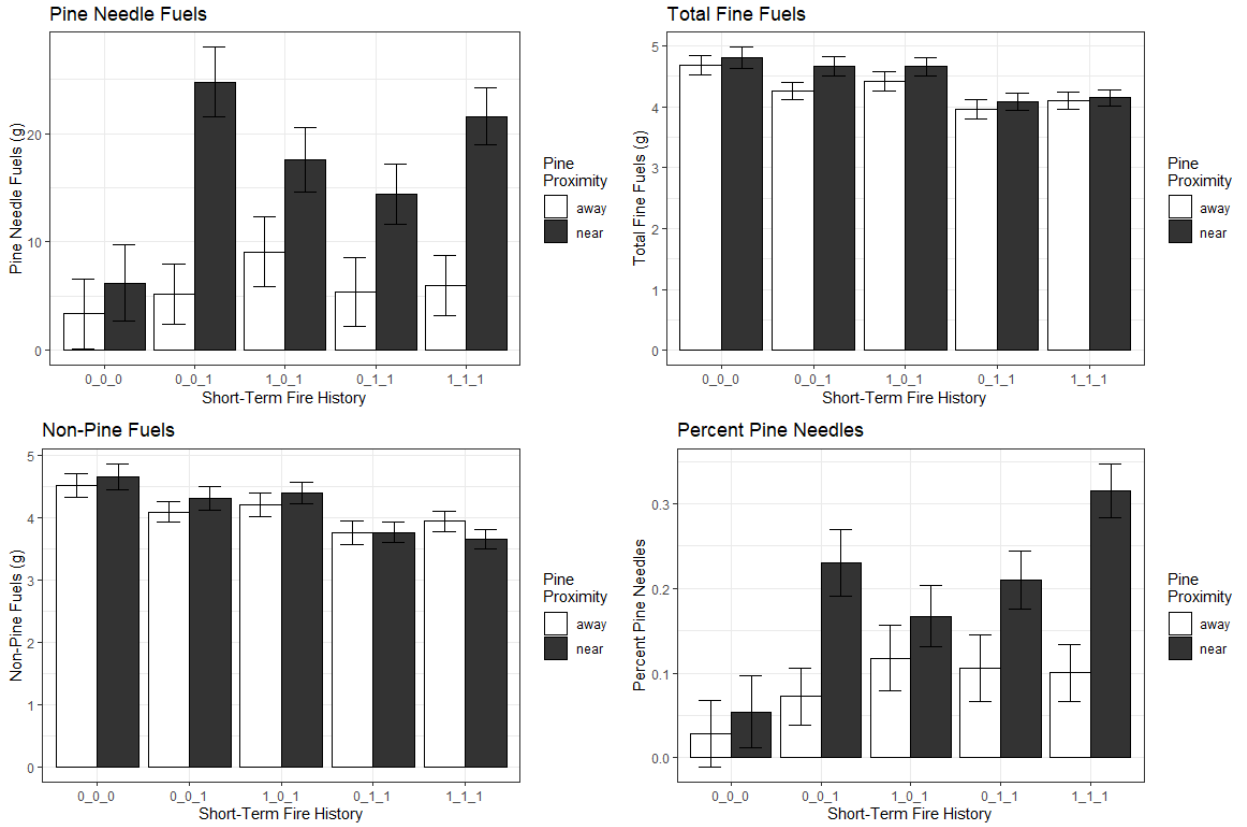

Figure S1: Pre-fire fine fuel characteristics for experimental plots. When comparing near and away from pines plots, the only differences in fine fuels were amounts and proportions of pine needles. In near plots, there were larger amounts of pine needles and pine needles made up larger proportions of fuel loads. The other differences in plant fuel loads were with total fine fuel loads and non-pine fuels. In plots that burned twice in the final two years of the experiment, plant fuel loads were smaller and contained fewer non-pine fuels.

Table S2: Fine Fuel ANOVA tables.

Fine Fuel ANOVA Tables				
Pine Needle Fuels				
model term	df1	df2	F.ratio	p.value
fire	4	59	3.314	0.0162**
litter	1	59	33.363	<.0001***
fire:litter	4	59	2.222	0.0774*
Total Final Fuels				
model term	df1	df2	F.ratio	p.value
fire	4	59	7.338	0.0001***
litter	1	59	3.66	0.0606*
fire:litter	4	59	0.434	0.7837
Non-Pine Fuels				
model term	df1	df2	F.ratio	p.value
fire	4	59	7.372	0.0001***
litter	1	59	0.217	0.6429
fire:litter	4	59	0.791	0.536
Percent Pine Needles				
model term	df1	df2	F.ratio	p.value
fire	4	59	5.089	0.0014**
litter	1	59	22.276	<.0001***
fire:litter	4	59	2.296	0.0697*

* = $p \leq 0.1$, ** = $p \leq 0.05$, *** = $p \leq 0.001$.

Post-Fire Nutrients Section

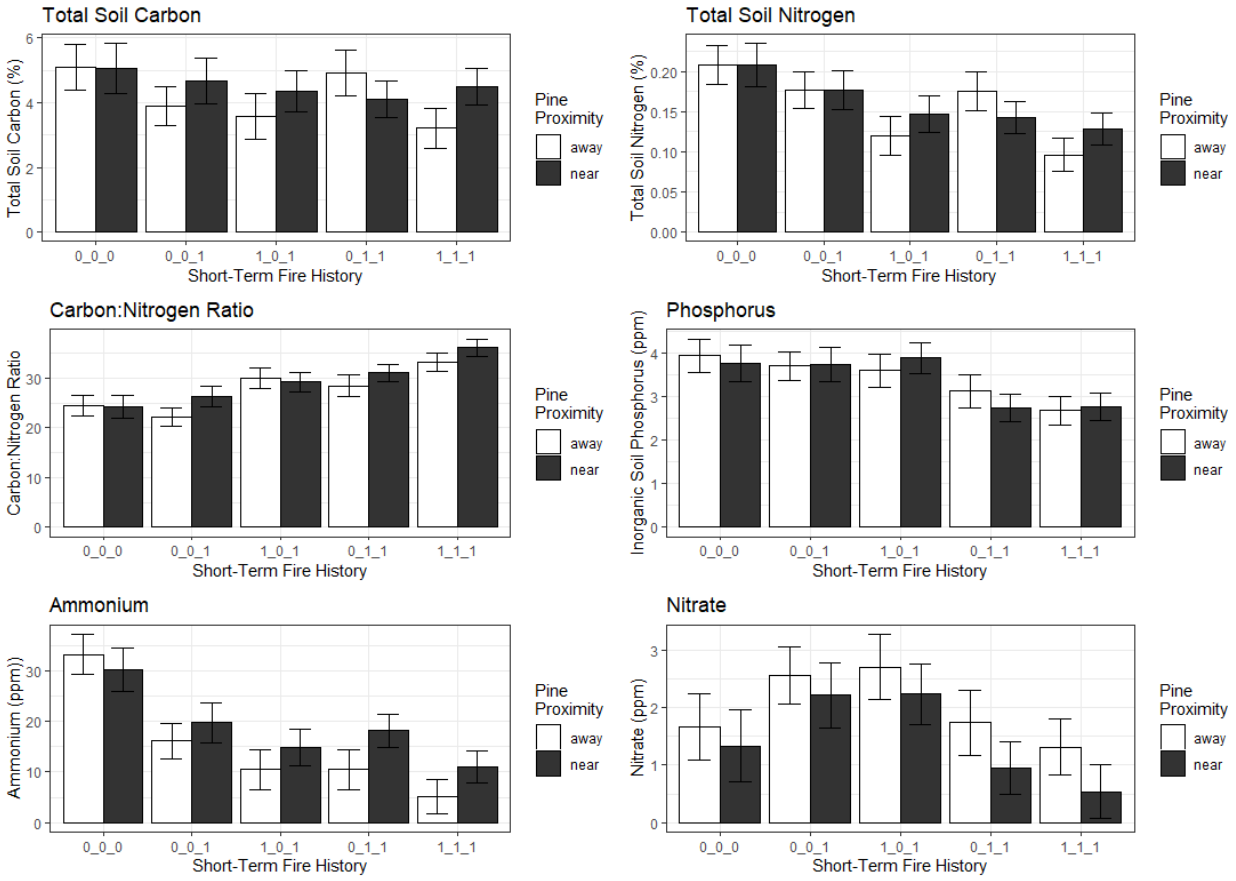

Figure S2: Post-fire nutrient levels. As fire frequency increased, total soil nitrogen, phosphorus, and ammonium levels decreased, while C:N ratios and Nitrate levels increased. Note that nitrate actually decreased when sites were burned twice in the final two years of the study.

Table S3: Nutrient ANOVA tables.

Nutrient ANOVA Tables

Total Soil Carbon

model term	df1	df2	F.ratio	p.value
fire	4	60	1.023	0.4028
litter	1	60	0.923	0.3405
fire:litter	4	60	0.846	0.5016

Total Soil Nitrogen

model term	df1	df2	F.ratio	p.value
fire	4	59	5.172	0.0012**
litter	1	59	0.117	0.7338
fire:litter	4	59	0.686	0.6044

C:N

model term	df1	df2	F.ratio	p.value
fire	4	60	10.391	<.0001***
litter	1	60	1.821	0.1822
fire:litter	4	60	0.552	0.6985

Ammonium

model term	df1	df2	F.ratio	p.value
fire	4	60	10.636	<.0001***
litter	1	60	2.452	0.1226
fire:litter	4	60	0.535	0.7106

Nitrate

model term	df1	df2	F.ratio	p.value
fire	4	60	3.385	0.0146**
litter	1	60	2.556	0.1151
fire:litter	4	60	0.09	0.9852

Phosphorus

model term	df1	df2	F.ratio	p.value
fire	4	60	4.492	0.0031**
litter	1	60	0.016	0.9001
fire:litter	4	60	0.261	0.902

* = $p \leq 0.1$, ** = $p \leq 0.05$, *** = $p \leq 0.001$.

2016 Prescribed Burn Characteristics Section

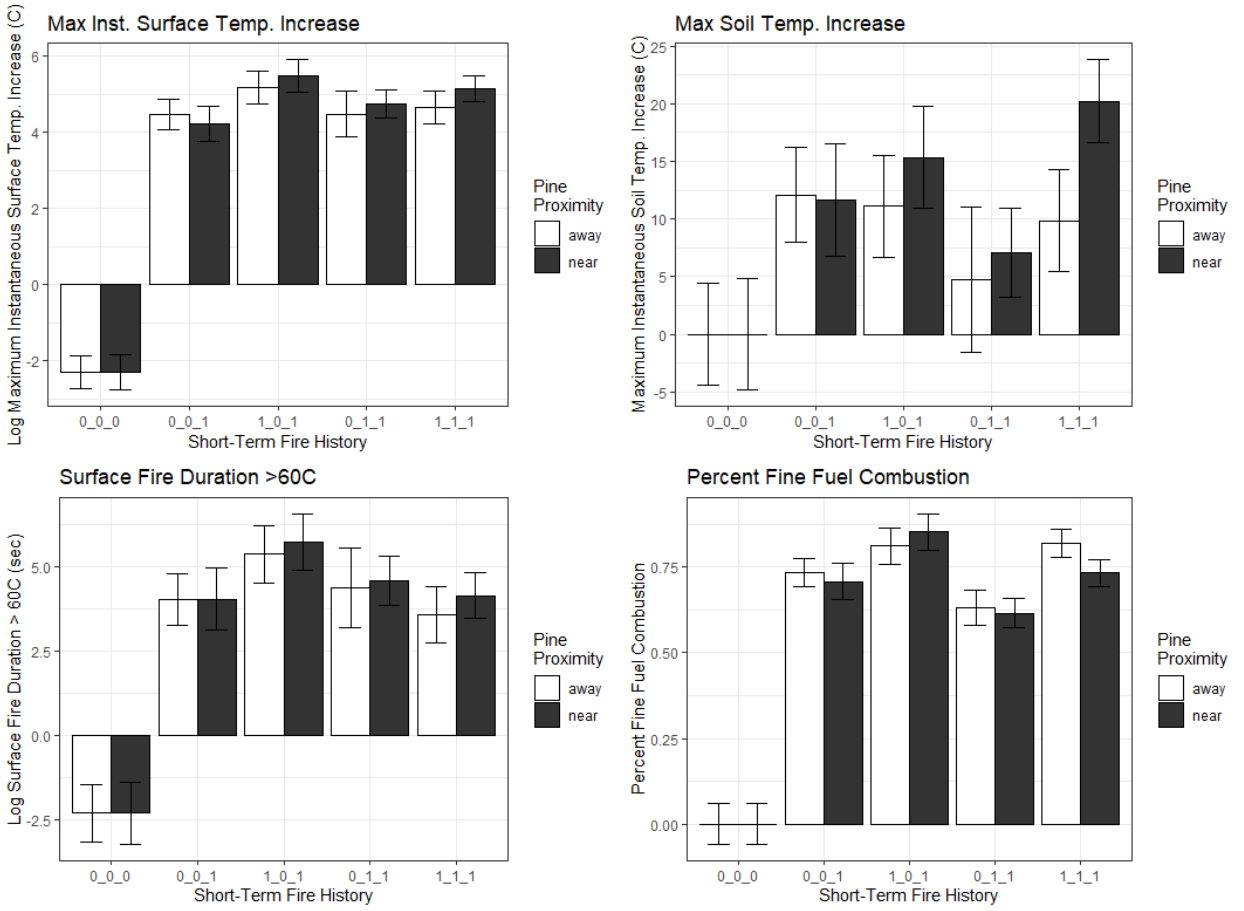

Figure S3: 2016 fire characteristics. During the 2016 prescribed burns, burned treatments did not vary significantly from one and other in terms of fire characteristics. The primary differences were between the unburned and burned plots. Note that no fire characteristics were recorded for unburned (000) sites. Any negative values reflect the addition of 0.1 prior to natural log transformation for surface temperature increase and surface fire duration.

Table S4: Fire characteristic ANOVA tables.

Fire Characteristic ANOVA Tables

Max. Inst. Surface Temp. Increase

model term	df1	df2	F.ratio	p.value
fire	4	51	105.116	<.0001***
litter	1	51	0.355	0.5538
fire:litter	4	51	0.24	0.9144

Surface Fire Duration >60C

model term	df1	df2	F.ratio	p.value
fire	4	51	25.3	<.0001***
litter	1	51	0.175	0.6779
fire:litter	4	51	0.043	0.9963

Max Inst. Soil Temp. Increase

model term	df1	df2	F.ratio	p.value
fire	4	51	3.753	0.0094**
litter	1	51	1.303	0.259
fire:litter	4	51	0.537	0.709

Percent Fine Fuel Combustion

model term	df1	df2	F.ratio	p.value
fire	4	51	72.553	<.0001***
litter	1	51	0.311	0.5793
fire:litter	4	51	0.526	0.7171

* = $p \leq 0.1$, ** = $p \leq 0.05$, *** = $p \leq 0.001$.

Decomposition Rate ANOVA Tables:

Table S5: Analysis of Variance and apriori contrast table for 2 month decomposition rates. * = p ≤ 0.1, ** = p ≤ 0.05, *** = p ≤ 0.001.

Month 2 Decomp Rate ANOVA Table				
model term	**df1**	**df2**	**F.ratio**	**p.value**
fire	4	46	26.752	<.0001***
litter	1	46	0.013	0.9099
fire:litter	4	46	3.83	0.0091**

Month 2 Decomp Rate Contrasts					
contrast	**estimate**	**SE**	**df**	**t.ratio**	**p.value**
near vs. away	0.00586	0.0515	46	-0.114	0.9099
no fire vs. fire	0.91862	0.1031	46	8.914	<.0001***
1 vs 2 fires	0.20648	0.0564	46	3.662	0.0006***
1 vs 3 fires	0.15769	0.0326	46	4.836	<.0001***
2 vs 3 fires	0.10889	0.0564	46	1.931	0.0596*

Table S6: Analysis of Variance and apriori contrasts table for 4 month decomposition rates

Month 4 Decomp Rate ANOVA Table				
model term	**df1**	**df2**	**F.ratio**	**p.value**
fire	4	47	22.088	<.0001***
litter	1	47	0.451	0.5054
fire:litter	4	47	0.558	0.6941

Month 4 Decomp Rate Contrasts					
contrast	**estimate**	**SE**	**df**	**t.ratio**	**p.value**
near vs. away	-0.0289	0.043	47	-0.671	0.5054
no fire vs. fire	0.77	0.0871	47	8.839	<.0001***
1 vs 2 fires	0.1246	0.0484	47	2.577	0.0132**
1 vs 3 fires	0.0726	0.0283	47	2.567	0.0135**
2 vs 3 fires	0.0205	0.0469	47	0.438	0.6637

Table S7: Analysis of Variance and apriori contrasts table for 6 month decomposition rates

Month 6 Decomp Rate ANOVA Table				
model term	**df1**	**df2**	**F.ratio**	**p.value**
fire	4	46	16.1	<.0001***
litter	1	46	8.441	0.0056**
fire:litter	4	46	1.441	0.2356

Month 6 Decomp Rate Contrasts					
contrast	**estimate**	**SE**	**df**	**t.ratio**	**p.value**
near vs. away	-0.0953	0.0328	46	-2.905	0.0056**
no fire vs. fire	0.4463	0.0661	46	6.754	<.0001***
1 vs 2 fires	0.1358	0.036	46	3.771	0.0005***
1 vs 3 fires	0.0739	0.0209	46	3.528	0.001***
2 vs 3 fires	0.012	0.036	46	0.334	0.7398

Table S8: Analysis of Variance and apriori contrasts table for 8 month decomposition rates

Month 8 Decomp Rate ANOVA Table				
model term	**df1**	**df2**	**F.ratio**	**p.value**
fire	4	44	19.327	<.0001***
litter	1	44	2.081	0.1562
fire:litter	4	44	2.235	0.0805*

Month 8 Decomp Rate Contrasts					
contrast	**estimate**	**SE**	**df t**	**.ratio**	**p.value**
near vs. away	-0.042	0.0291	44	-1.443	0.1562
no fire vs. fire	0.4297	0.0598	44	7.187	<.0001***
1 vs 2 fires	0.1285	0.0316	44	4.063	0.0002***
1 vs 3 fires	0.0832	0.0183	44	4.559	<.0001***
2 vs 3 fires	0.038	0.0316	44	1.201	0.2362

Structural Equation Model Fitting Steps:

Data was first checked for normality and homoscedasticity of variance between variables. Due to some deviations from normality and heteroscedastic variance, the data transformed appropriately and then scaled before further analysis.

After scaling the data, a highly saturated model containing decomposition rates, nutrient variables, short-term fire history treatments, fire characteristics, and fuel traits was specified. See lavaan code below:

Step 1:

```
Model 1 <- '
decomprate2 ~ burns + C + N + P + no3 + nh4 + prox + litt.inc + soil.inc + dur60
decomprate4 ~ burns + C + N + P + no3 + nh4 + prox + decrate2 + litt.inc + soil.inc + dur60
decomprate6 ~ burns + C + N + P + no3 + nh4 + prox + decrate2 + decrate4 + litt.inc + soil.inc +
                dur60
decomprate8 ~ burns + C + N + P + no3 + nh4 + prox + decrate2 + decrate4 + decrate6 + litt.inc
               + soil.inc + dur60
litt.inc ~ needle + tot.fuel + burns + prox
soil.inc ~ litt.inc + needle + tot.fuel + burns + dur60 + prox
dur60 ~ litt.inc + needle + tot.fuel + burns + prox
C ~ burns + N + litt.inc + soil.inc + dur60
N ~ burns + nh4 + no3 + litt.inc + soil.inc + dur60
P ~ burns + C + N + litt.inc + soil.inc + dur60
no3 ~ burns + litt.inc + soil.inc + dur60
nh4 ~ burns + litt.inc + soil.inc + dur60
needle ~ prox
'
```

```
lavaan 0.6-4 ended normally after 89 iterations

  Optimization method                           NLMINB
  Number of free parameters                        113

                                                  Used       Total
  Number of observations                            68          69
  Number of missing patterns                         9

  Estimator                                         ML
  Model Fit Test Statistic                      83.625
  Degrees of freedom                                30
  P-value (Chi-square)                           0.000

Model test baseline model:

  Minimum Function Test Statistic              899.498
  Degrees of freedom                               117
  P-value                                        0.000

User model versus baseline model:

  Comparative Fit Index (CFI)                    0.931
```

```
  Tucker-Lewis Index (TLI)                       0.733

Loglikelihood and Information Criteria:

  Loglikelihood user model (H0)               -727.653
  Loglikelihood unrestricted model (H1)       -685.840

  Number of free parameters                        113
  Akaike (AIC)                                1681.306
  Bayesian (BIC)                              1932.110
  Sample-size adjusted Bayesian (BIC)         1576.266

Root Mean Square Error of Approximation:

  RMSEA                                          0.162
  90 Percent Confidence Interval          0.121  0.204
  P-value RMSEA <= 0.05                          0.000

Standardized Root Mean Square Residual:

  SRMR                                           0.076
```

Despite convergence, the model was not a good fit for the data (X^2=83.625, p = 0). Note that in structural equation modeling, Chi-Squared tests compare the covariance matrix of the model to the covariance matrix of the included data. A significant difference between the two covariance matrices is considered a poor fitting model, signified by $p < 0.05$. To improve model fit, relationships in the model with a p value of 0.75 or greater were removed.

Step 2:

```
Model 2 <- '
decomprate2 ~ burns + C + N + P + no3 + nh4 + prox + litt.inc + dur60
decomprate4 ~ burns + P + no3 + nh4 + prox + decrate2 + litt.inc + soil.inc + dur60
decomprate6 ~ burns + C + N + P + no3 + nh4 + prox + decrate2 + decrate4 + litt.inc
decomprate8 ~ burns + decrate2 + decrate4 + decrate6 + litt.inc + soil.inc + dur60
litt.inc ~ needle + tot.fuel + burns + prox
soil.inc ~ litt.inc + needle + tot.fuel + burns + dur60 + prox
dur60 ~ litt.inc + needle + burns + prox
C ~ burns + N + litt.inc + soil.inc + dur60
N ~ burns + nh4 + no3 + litt.inc + soil.inc + dur60
P ~ burns + C + N + litt.inc + soil.inc + dur60
no3 ~ burns + litt.inc + soil.inc + dur60
nh4 ~ burns + litt.inc + soil.inc + dur60
needle ~ prox
'
```

```
lavaan 0.6-4 ended normally after 78 iterations

  Optimization method                           NLMINB
  Number of free parameters                        101

                                                  Used       Total
  Number of observations                            68          69
  Number of missing patterns                         9
```

```
  Estimator                                      ML
  Model Fit Test Statistic                   85.112
  Degrees of freedom                             42
  P-value (Chi-square)                        0.000

Model test baseline model:

  Minimum Function Test Statistic           899.498
  Degrees of freedom                            117
  P-value                                     0.000

User model versus baseline model:

  Comparative Fit Index (CFI)                 0.945
  Tucker-Lewis Index (TLI)                    0.847

Loglikelihood and Information Criteria:

  Loglikelihood user model (H0)            -728.396
  Loglikelihood unrestricted model (H1)    -685.840

  Number of free parameters                     101
  Akaike (AIC)                             1658.792
  Bayesian (BIC)                           1882.963
  Sample-size adjusted Bayesian (BIC)      1564.907

Root Mean Square Error of Approximation:

  RMSEA                                       0.123
  90 Percent Confidence Interval          0.085  0.160
  P-value RMSEA <= 0.05                       0.002

Standardized Root Mean Square Residual:

  SRMR                                        0.077
```

This model was an improvement compared to step 1, however its Chi-Square value suggested that it was still a poor fit to the data (X^2=85.112, p = 0). To improve model fit, relationships in the model with a p value of 0.5 or greater were removed.

Step 3:

```
Model 3 <- '
decomprate2 ~ burns + N + P + no3 + nh4 + prox + litt.inc + dur60
decomprate4 ~ burns + P + nh4 + prox + decrate2 + soil.inc + dur60
decomprate6 ~ C + N + P + no3 + nh4 + prox + decrate4 + litt.inc
decomprate8 ~ burns + decrate2 + decrate4 + decrate6 + litt.inc + dur60
litt.inc ~ needle + burns + prox
soil.inc ~ litt.inc + needle + tot.fuel + burns + prox
dur60 ~ litt.inc + burns + prox
C ~ burns + N + litt.inc + dur60
N ~ burns + nh4 + litt.inc + soil.inc + dur60
P ~ burns + C + N + litt.inc + soil.inc + dur60
no3 ~ burns + litt.inc + dur60
nh4 ~ burns + soil.inc
needle ~ prox
```

'

```
lavaan 0.6-4 ended normally after 79 iterations

  Optimization method                           NLMINB
  Number of free parameters                         87

                                                  Used       Total
  Number of observations                            68          69
  Number of missing patterns                         9

  Estimator                                         ML
  Model Fit Test Statistic                      91.963
  Degrees of freedom                                56
  P-value (Chi-square)                           0.002

Model test baseline model:

  Minimum Function Test Statistic              899.498
  Degrees of freedom                               117
  P-value                                        0.000

User model versus baseline model:

  Comparative Fit Index (CFI)                    0.954
  Tucker-Lewis Index (TLI)                       0.904

Loglikelihood and Information Criteria:

  Loglikelihood user model (H0)                -731.822
  Loglikelihood unrestricted model (H1)        -685.840

  Number of free parameters                         87
  Akaike (AIC)                                1637.644
  Bayesian (BIC)                              1830.741
  Sample-size adjusted Bayesian (BIC)         1556.772

Root Mean Square Error of Approximation:

  RMSEA                                          0.097
  90 Percent Confidence Interval          0.060  0.132
  P-value RMSEA <= 0.05                          0.024

Standardized Root Mean Square Residual:

  SRMR                                           0.077
```

Model 3 converged, and fit statistics suggested that it was a better fit to the data than previous models (X^2=91.963, p = 0.002, RMSEA = 0.097, SRMR = 0.077). The model covariance matrix was still significantly different than the data covariance matrix however, so poorly supported relationships were again parsed out of the model. In this case, poorly suppored relationships were defined as p > 0.5 and/or estimate effects < 0.1.

Step 4:

Model 4 <- '

```
decomprate2 ~ burns + P + no3 + nh4 + prox + litt.inc + dur60
decomprate4 ~ burns + litter + decrate2 + soil.inc + dur60
decomprate6 ~ C + N + P + no3 + nh4 + prox + decrate4 + litt.inc
decomprate8 ~ decrate2 + decrate4 + decrate6 + litt.inc + dur60
litt.inc ~ needle + burns + prox
soil.inc ~ litt.inc + needle + tot.fuel + burns + prox
dur60 ~ litt.inc + burns + prox
C ~ burns + N + litt.inc + dur60
N ~ burns + nh4 + litt.inc + soil.inc + dur60
P ~ burns + C + N + litt.inc + soil.inc + dur60
no3 ~ burns + litt.inc + dur60
nh4 ~ burns + soil.inc
needle ~ prox
'
```

```
lavaan 0.6-4 ended normally after 77 iterations

  Optimization method                           NLMINB
  Number of free parameters                         83

                                                  Used       Total
  Number of observations                            68          69
  Number of missing patterns                         9

  Estimator                                         ML
  Model Fit Test Statistic                      94.598
  Degrees of freedom                                60
  P-value (Chi-square)                           0.003

Model test baseline model:

  Minimum Function Test Statistic              899.498
  Degrees of freedom                               117
  P-value                                        0.000

User model versus baseline model:

  Comparative Fit Index (CFI)                    0.956
  Tucker-Lewis Index (TLI)                       0.914

Loglikelihood and Information Criteria:

  Loglikelihood user model (H0)                -733.139
  Loglikelihood unrestricted model (H1)        -685.840

  Number of free parameters                         83
  Akaike (AIC)                                1632.279
  Bayesian (BIC)                              1816.498
  Sample-size adjusted Bayesian (BIC)         1555.125

Root Mean Square Error of Approximation:

  RMSEA                                          0.092
  90 Percent Confidence Interval          0.054  0.126
  P-value RMSEA <= 0.05                          0.036

Standardized Root Mean Square Residual:

  SRMR                                           0.077
```

Model 4 converged and was a marginally good fit to the data (X^2=94.598, p = 0.003, RMSEA = 0.092, SRMR = 0.077). The chi-squared test and non-significant relationships in the model still suggested that the model could be improved, however. To improve model fit, relationships with p > 0.25 were removed.

Step 5:

```
Model 5 <- '
decomprate2 ~ burns + P + no3 + nh4 + dur60
decomprate4 ~ burns + decrate2 + soil.inc
decomprate6 ~ C + N + no3 + nh4 + prox + decrate4 + litt.inc
decomprate8 ~ decrate2 + decrate4 + decrate6 + litt.inc + dur60
litt.inc ~ needle + burns + prox
soil.inc ~ litt.inc + needle + burns
dur60 ~ litt.inc + burns + prox
C ~ burns + N + litt.inc + dur60
N ~ burns + nh4 + soil.inc + dur60
P ~ burns + C + N + litt.inc + soil.inc + dur60
no3 ~ burns + litt.inc + dur60
nh4 ~ burns + soil.inc
needle ~ prox
'
```

```
lavaan 0.6-4 ended normally after 74 iterations

  Optimization method                           NLMINB
  Number of free parameters                         75

  Number of observations                            69
  Number of missing patterns                        10

  Estimator                                         ML
  Model Fit Test Statistic                      65.801
  Degrees of freedom                                55
  P-value (Chi-square)                           0.151

Model test baseline model:

  Minimum Function Test Statistic              874.536
  Degrees of freedom                               104
  P-value                                        0.000

User model versus baseline model:

  Comparative Fit Index (CFI)                    0.986
  Tucker-Lewis Index (TLI)                       0.973

Loglikelihood and Information Criteria:

  Loglikelihood user model (H0)                -746.934
  Loglikelihood unrestricted model (H1)        -714.033
```

```
  Number of free parameters                         75
  Akaike (AIC)                                1643.868
  Bayesian (BIC)                              1811.426
  Sample-size adjusted Bayesian (BIC)         1575.215

Root Mean Square Error of Approximation:

  RMSEA                                          0.053
  90 Percent Confidence Interval          0.000  0.097
  P-value RMSEA <= 0.05                          0.433

Standardized Root Mean Square Residual:

  SRMR                                           0.057
```

Model 5 converged and was an excellent fit to the data (X^2=65.801, p = 0.151*, RMSEA = 0.053*, SRMR = 0.057*). At this point, further model fitting stopped, as fit statistics determined that the model covariance matrix and residuals did not differ significantly from the full covariance matrix and residual errors did not differ significantly from 0.

Table S10: Expanded Fit Statistics for SEM Fitting. Table contains all fit statistics used for model fitting. Note that Chi-Square and RMSEA P-Values are significant when greater than 0.05. RMSEA statistics are considered optimal when less than 0.05. RMR and SRMR suggest good fit when less than 0.1. CFI and NNFI suggest good fit when greater than 0.95. Finally, all model R squared coefficients should be greater than 0.2.

model name	Converged	DF	X^2 value	X^2 p-value	RMSEA	RMSEA 90% CI	RMSEA CI p-value	CFI	RMR	NNFI	SRMR	R2 < 0.2
model 1	TRUE	30	83.625	0	0.162	0.121-0.204	0	0.931	0.072	0.733	0.076	none
model 2	TRUE	42	85.112	0	0.123	0.085-0.16	0.002	0.945	0.072	0.847	0.077	none
model 3	TRUE	56	91.963	0.002	0.097	0.06-0.132	0.024	0.954	0.073	0.904	0.077	none
model 4	TRUE	60	94.598	0.003	0.092	0.054-0.126	0.036	0.956	0.073	0.914	0.077	none
model 5	TRUE	55	65.801	0.151*	0.053	0-0.097	0.433*	0.986*	0.051*	0.973*	0.057*	none

Chapter 3 - Appendix

Table S1: Initial SEM model table.

Response Variable	Explanatory Variable(s)	Justification
	Latent variables	
Fire energy release	maximum surface temperature increase	Ellair & Platt (2013) & Platt et al. (2016)
	maximum soil temperature increase	Ellair & Platt (2013) & Platt et al. (2016)
	surface duration > 60˚C	Ellair & Platt (2013) & Platt et al. (2016)
	Regression paths	
fire energy release	fire intensity treatment	intensity treatments increase fuel loads
total nitrogen	total carbon	linked through C:N ratio
	fire energy release	Raison 1979
	fire intensity treatment	Raison 1979
total carbon	fire energy release	Johnson and Curtis 2001
	fire intensity treatment	Johnson and Curtis 2001
month 2 percent mass loss	fire energy release	Bárcenas-Moreno and Bååth 2009
	fire intensity treatment	Bárcenas-Moreno and Bååth 2009
	total carbon	Taylor et al. 1989
	total nitrogen	Taylor et al. 1989
	pine proximity	Hobbie 2000
month 4 percent mass loss	fire energy release	Bárcenas-Moreno and Bååth 2009
	fire intensity treatment	Bárcenas-Moreno and Bååth 2009
	total carbon	Taylor et al. 1989
	total nitrogen	Taylor et al. 1989
	pine proximity	Hobbie 2000
	mon. 2 mass loss	Voříšková and Baldrian 2013
month 6 percent mass loss	fire energy release	Bárcenas-Moreno and Bååth 2009
	fire intensity treatment	Bárcenas-Moreno and Bååth 2009
	total carbon	Taylor et al. 1989
	total nitrogen	Taylor et al. 1989
	pine proximity	Hobbie 2000
	mon. 2 mass loss	Voříšková and Baldrian 2013
	mon. 4 mass loss	Voříšková and Baldrian 2013
month 8 percent mass loss	fire energy release	Bárcenas-Moreno and Bååth 2009
	fire intensity treatment	Bárcenas-Moreno and Bååth 2009
	total carbon	Taylor et al. 1989
	total nitrogen	Taylor et al. 1989
	pine proximity	Hobbie 2000
	mon. 2 mass loss	Voříšková and Baldrian 2013
	mon. 4 mass loss	Voříšková and Baldrian 2013
	mon. 8 mass loss	Voříšková and Baldrian 2013
	Covariance structures	
maximum surface temperature increase ~ surface duration >60°C		

SEM model specification:

Data was first checked for normality and homoscedasticity of variance between variables. Due to some deviations from normality and heteroscedastic variance, the data was transformed appropriately before further analysis (see table 2).

After transforming the data, a latent variable construct was created to model fire severity using maximum litter and soil temperatures and litter duration > 60˚C (see lavaan code below).

```
latent <- '
# latent variable
severity =~ surfpeak + surfdur + soilpeak
# regressions
mon2loss ~ severity
# variance structures
surfpeak ~~ surfdur
'
```

```
lavaan 0.6-3 ended normally after 30 iterations

  Optimization method                           NLMINB
  Number of free parameters                          9

  Number of observations                           285

  Estimator                                         ML
  Model Fit Test Statistic                       0.013
  Degrees of freedom                                 1
  P-value (Chi-square)                           0.911
```

The latent variable model converged, and implied excellent fit (see model fit table at end of appendix). Since the latent variable model described fire severity well, we moved forward in the modeling process by including other measured variables. The first model tested was a highly saturated one, which contained microbial decomposition, soil nutrient, pine proximity, and fire severity related variables. Antibiotics were not included in the SEM due to their weak effect in the LMER models and because of the difficulty in using non-ordinal factor variables in SEM.

```
sem1 <- '
# latent variable
severity =~ surfpeak + surfdur + soilpeak

# regressions
sev.code ~ severity
totn ~ totc + severity + sev.code
totc ~ severity + sev.code
mon2per ~ severity + sev.code + totc + totn + pines
mon4per ~ severity + sev.code + totc + totn + pines + mon2per
mon6per ~ severity + sev.code + totc + totn + pines + mon2per + mon4per
mon8per ~ severity + sev.code + totc + totn + pines + mon2per + mon4per + mon6per

# variance structures
```

surfpeak ~~ surfdur
'

```
lavaan 0.6-3 ended normally after 194 iterations

  Optimization method                           NLMINB
  Number of free parameters                         85

                                                  Used       Total
  Number of observations                           278         285

  Estimator                                         ML
  Model Fit Test Statistic                     344.974
  Degrees of freedom                                34
  P-value (Chi-square)                           0.000
```

This model converged, however it was not well supported by the data (MFTS = 344.974, P-value = 0, for more fit measures see model fit table at end of appendix). To improve model fit, all model pathways with an r-squared coefficient < 0.1 were removed. This led to the creation of a second model:

```
sem2 <- '
# latent variable
severity =~ surfpeak + surfdur + soilpeak

# regressions
sev.code ~ severity
totn ~ totc + severity + sev.code
mon2per ~ severity + sev.code + totc + totn + pines
mon4per ~ severity + sev.code + totc + totn + pines + mon2per
mon6per ~ severity + sev.code + totc + totn + pines + mon2per + mon4per
mon8per ~ severity + sev.code + totc + totn + pines + mon2per + mon4per + mon6per

# variance structures
surfpeak ~~ surfdur
'
```

```
lavaan 0.6-2 ended normally after 110 iterations

  Optimization method                           NLMINB
  Number of free parameters                         43

                                                  Used       Total
  Number of observations                           278         285

  Estimator                                         ML
  Model Fit Test Statistic                     140.452
  Degrees of freedom                                20
  P-value (Chi-square)                           0.000
```

The second model converged, and while it had a lower MFTS than the prior model, it was still poorly supported by the data (MFTS = 140.452, P-value = 0). To further improve model fit, all relationships in the model with p-values > 0.5 were removed.

```
sem3 <- '
# latent variable
severity =~ surfpeak + surfdur + soilpeak

# regressions
sev.code ~ severity
totn ~ totc
mon2per ~ severity + sev.code + totc + pines
mon4per ~ severity + totn + pines + mon2per
mon6per ~ severity + totc + pines + mon2per + mon4per
mon8per ~ severity + sev.code + totc + totn + mon2per + mon4per + mon6per

# variance structures
surfpeak ~~ surfdur
'
```

```
lavaan 0.6-2 ended normally after 98 iterations

  Optimization method                           NLMINB
  Number of free parameters                         35

                                                Used       Total
  Number of observations                         278         285

  Estimator                                       ML
  Model Fit Test Statistic                   145.841
  Degrees of freedom                              28
  P-value (Chi-square)                         0.000
```

The third model also, converged but again was poorly supported (MFTS = 145.841, P-value = 0). Due to the continued presence of non-significant relationships in the model, all relationships with P-values > 0.2 were parsed to create a fourth model.

```
sem4 <- '
# latent variable
severity =~ surfpeak + surfdur + soilpeak

# regressions
sev.code ~ severity
totn ~ totc
mon2per ~ severity + totc + pines
mon4per ~ severity + totn + mon2per
mon6per ~ totc + pines + mon2per + mon4per
mon8per ~ severity + sev.code + mon2per + mon4per + mon6per

# variance structures
surfpeak ~~ surfdur
'
```

```
lavaan 0.6-2 ended normally after 83 iterations
```

```
  Optimization method                           NLMINB
  Number of free parameters                         30

                                                  Used       Total
  Number of observations                           278         285

  Estimator                                         ML
  Model Fit Test Statistic                     157.008
  Degrees of freedom                                33
  P-value (Chi-square)                           0.000
```

The fourth model was poorly supported (MFTS = 157.008, P-value = 0), so in a final attempt to improve model fit all non-significant relationships (P > 0.05) were removed.

```
sem5 <- '
# latent variable
severity =~ surfpeak + surfdur + soilpeak

# regressions
sev.code ~ severity
totn ~ totc
mon2per ~ severity + totc + pines
mon4per ~ severity + totn + mon2per
mon6per ~ totc + pines + mon2per + mon4per
mon8per ~ mon2per + mon4per + mon6per

# variance structures
surfpeak ~~ surfdur
'
```

```
lavaan 0.6-2 ended normally after 78 iterations

  Optimization method                           NLMINB
  Number of free parameters                         29

                                                  Used       Total
  Number of observations                           278         285

  Estimator                                         ML
  Model Fit Test Statistic                     150.913
  Degrees of freedom                                34
  P-value (Chi-square)                           0.000
```

This model converged, and was a better fit than the prior model, however it was still not well supported by the data (MFTS = 150.913, P-value = 0). After running the fifth model, it was noticed that the severity code ("sev.code") was not a good predictor of microbial decomposition, so the pathway linking the severity latent variable to sev.code was removed, and the model rerun.

```
sem6 <- '
# latent variable
severity =~ surfpeak + surfdur + soilpeak

# regressions
```

```
totn ~ totc
mon2per ~ severity + totc + pines
mon4per ~ severity + totn + mon2per
mon6per ~ totc + pines + mon2per + mon4per
mon8per ~ mon2per + mon4per + mon6per

# variance structures
surfpeak ~~ surfdur
'
```

```
lavaan 0.6-2 ended normally after 73 iterations

  Optimization method                           NLMINB
  Number of free parameters                         26

                                                  Used       Total
  Number of observations                           278         285

  Estimator                                         ML
  Model Fit Test Statistic                      35.382
  Degrees of freedom                                26
  P-value (Chi-square)                           0.104
```

This final model converged, and was an excellent fit to the data (MFTS 35.382, P-value = 0.104). SEM fit statistics for this model were then determined (see table at end of appendix) according to Hooper et al. 2008. The fit statistics described the model as an excellent fit to the data, so model fitting was stopped, and interpretation of the model began.

Table S1:

Model	MFTS	P-value	DF	RMSEA 90% CI	RMSEA CI p-value	CFI	RMR	NNFI	SRMR	$R^2 < 0.2$
fire severity latent variable	0.013	0.911	1	0 - 0.064**	0.937	1**	0**	1.009**	0.001**	yes
model 1	344.974	0	34	0.164-0.199	0	0.908*	0.025**	0.715	0.067	yes
model 2	140.452	0	20	0.125-0.171	0	0.926*	0.025**	0.8	0.054*	yes
model 3	145.81	0	28	0.104-0.143	0	0.928*	0.025**	0.86	0.052*	yes
model 4	157.008	0	33	0.098-0.135	0	0.924*	0.025**	0.876	0.053*	yes
model 5	150.913	0	34	0.093-0.130	0	0.928*	0.025**	0.886	0.053*	yes
final model	35.382	0.104	26	0 - 0.063**	0.774	0.993**	0.017**	0.988**	0.054*	yes

** = good fit in Hooper et al. 2008

* = acceptable fit in Hooper et al. 2008

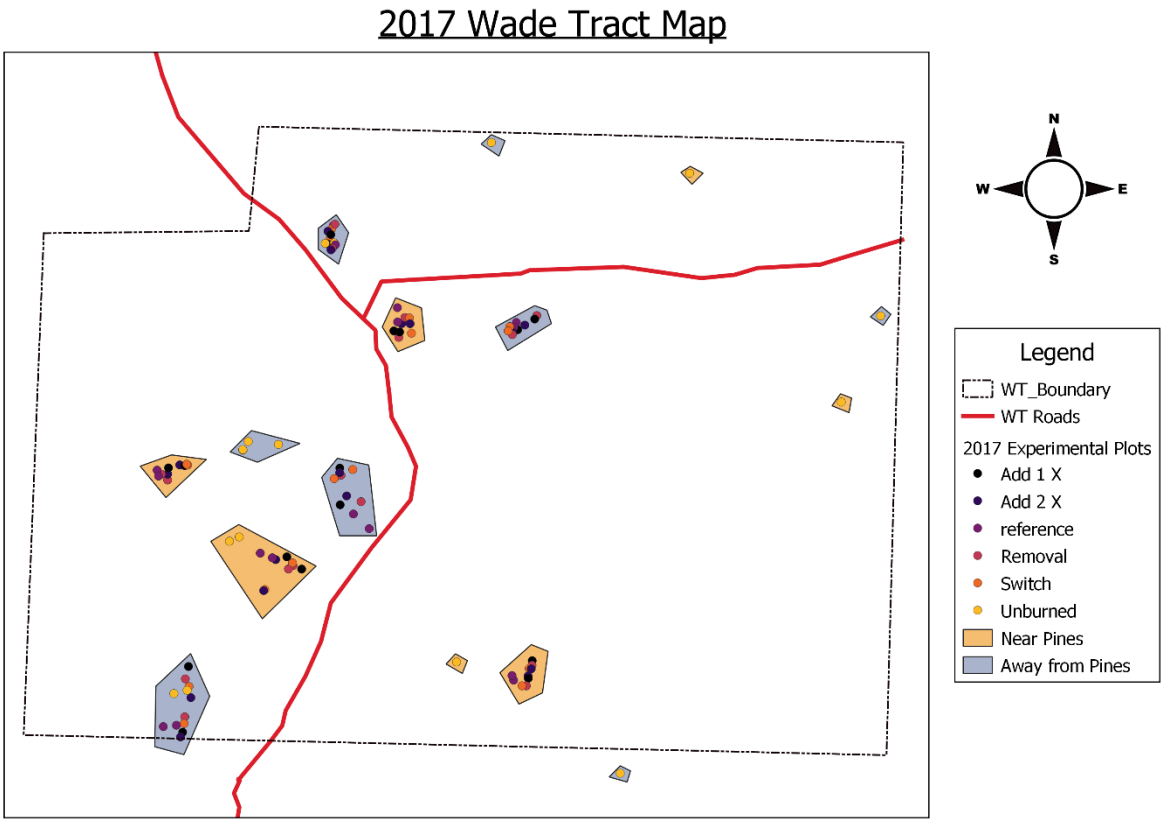

Figure S1: Map of Wade Tract and experimental plots. The above map shows the extent of the Wade Tract (black dashed line), fire severity treatment plots (see legend for treatment type), access roads (solid red line), and near/away from pines classifications (orange polygon = near pines, blue polygon = away from pines). Note that unburned plots are not always grouped with other experimental units, however they are still classified near/away.

Chapter 4 - Appendix

Appendix Tables

Table 1: Apriori contrasts for fuel manipulation treatment x pine proximity effects on pine fuels.

Contrast	Estimate	SE	d.f.	T-ratio	P-value
low vs. med	-3.834	0.304	38	-12.609	<.0001***
low vs. high	-9.457	0.304	38	-31.103	<.0001***
med vs. high	-5.623	0.299	38	-18.8	<.0001***
away vs. near	-0.738	0.37	38	-1.992	0.0536*
low:away vs. low:near	0	0.218	38	0	1
med:away vs. med:near	-0.305	0.211	38	-1.443	0.1571
high:away vs. high:near	-0.433	0.211	38	-2.045	0.0478**

*: $p < 0.1$, **: $p < 0.05$, ***: $p < 0.001$

Table 2: Apriori contrasts for fuel manipulation treatment effects on maximum surface temperature increase.

Contrast	Estimate	SE	d.f.	T-ratio	P-value
low vs. med	-173	77.4	33	-2.239	0.032**
low vs. high	-906	82.1	33	-11.035	<.0001***
med vs. high	-732	78	33	-9.39	<.0001***

*: $p < 0.1$, **: $p < 0.05$, ***: $p < 0.001$

Table 3: Apriori contrasts for fuel manipulation treatment effects on log surface temperature duration >60°C.

Contrast	Estimate	SE	d.f.	T-ratio	P-value
low vs. med	-0.538	0.298	32	-1.806	0.0803*
low vs. high	-2.518	0.309	32	-8.147	<.0001***
med vs. high	-1.98	0.298	32	-6.648	<.0001***

*: $p < 0.1$, **: $p < 0.05$, ***: $p < 0.001$

Table 4: Apriori contrasts for fuel manipulation treatment x pine proximity effects on log maximum soil temperature increase.

Contrast	Estimate	SE	d.f.	T-ratio	P-value
low vs. med	-0.5563	0.473	33	-1.176	0.2479
low vs. high	-3.0503	0.502	33	-6.082	<.0001***
med vs. high	-2.494	0.477	33	-5.232	<.0001***
away vs. near	-0.5212	0.593	33	-0.88	0.3855
low:away vs. low:near	0.386	0.352	33	1.096	0.2809
med:away vs. med:near	0.0115	0.316	33	0.037	0.9711
high:away vs. high:near	-0.9188	0.357	33	-2.573	0.0148**

*: $p < 0.1$, **: $p < 0.05$, ***: $p < 0.001$

Table 5: Apriori contrasts for fuel manipulation treatment effects on fuel combustion.

Contrast	Estimate	SE	d.f.	T-ratio	P-value
low vs. med	-0.226	0.0685	33	-3.307	0.0023**
low vs. high	-0.482	0.0676	33	-7.134	<.0001***
med vs. high	-0.256	0.0608	33	-4.208	0.0002**

*: p < 0.1, **: p < 0.05, ***: p < 0.001

Table 6: Apriori contrasts for fuel manipulation treatment effects on log inorganic phosphorus.

Contrast	Estimate	SE	d.f.	T-ratio	P-value
medium vs. low	-0.427	0.64	38	-0.667	0.5088
high vs. medium	1.506	0.629	38	2.394	0.0217**
high vs. low	1.08	0.64	38	1.688	0.0995*

Table 7: Apriori contrasts for fuel manipulation treatment effects on ammonium.

Contrast	Estimate	SE	d.f.	T-ratio	P-value
medium vs. low	0.411	0.385	38	1.066	0.2931
high vs. medium	0.588	0.379	38	1.55	0.1293
high vs. low	0.999	0.385	38	2.591	0.0135**

*: p < 0.1, **: p < 0.05, ***: p < 0.001

Table 8: Apriori contrasts for fuel manipulation treatment effects on soil pH.

Contrast	Estimate	SE	d.f.	T-ratio	P-value
medium vs. low	0.266	0.161	38	1.655	0.1061
high vs. medium	0.626	0.158	38	3.956	0.0003***
high vs. low	0.893	0.161	38	5.548	<.0001***

*: p < 0.1, **: p < 0.05, ***: p < 0.001

Table 9: Apriori contrasts for differences in germination rates between more and less pyrophilic plant species.

Contrast	Estimate	SE	d.f.	T-ratio	P-value
pyrophilic vs. non-pyrophilic	0.524	0.0363	952	14.43	<.001***

*: p < 0.1, **: p < 0.05, ***: p < 0.001

Table 10: Apriori contrasts for biotic soil severity effects on *C. americana* germination rate.

Contrast	Estimate	SE	d.f.	T-ratio	P-value
live vs. sterile	0.12	0.0819	96	1.465	0.1463
ctr vs. low	0.0817	0.0334	96	2.442	0.0165**
ctr vs. med	0	0.0334	96	0	1
ctr vs. high	0.0383	0.0334	96	1.146	0.2546
low vs. med	-0.0817	0.0334	96	-2.442	0.0165**
low vs. high	-0.0433	0.0334	96	-1.296	0.1982
med vs. high	0.0383	0.0334	96	1.146	0.2546

*: p < 0.1, **: p < 0.05, ***: p < 0.001

Table 11: Apriori contrasts for abiotic x biotic soil severity interaction effects on *P. palustris* germination rate.

Contrast	Estimate	SE	d.f.	T-ratio	P-value
abiotic effects					
low v med	-0.52	0.1929	96	-2.696	0.0083**
low v high	-0.14	0.1929	96	-0.726	0.4697
med v high	0.38	0.1929	96	1.97	0.0517*
low					
sterile vs. live	0.48	0.2362	96	2.032	0.0449**
ctr v low	0.06	0.0964	96	0.622	0.5353
ctr v med	0.26	0.0964	96	2.696	0.0083**
ctr v high	0.16	0.0964	96	1.659	0.1004
low v med	0.2	0.0964	96	2.074	0.0408**
low v high	0.1	0.0964	96	1.037	0.3024
med v high	-0.1	0.0964	96	-1.037	0.3024
reference					
sterile vs. live	-0.6	0.2362	96	-2.54	0.0127**
ctr v low	-0.26	0.0964	96	-2.696	0.0083**
ctr v med	-0.14	0.0964	96	-1.452	0.1498
ctr v high	-0.2	0.0964	96	-2.074	0.0408**
low v med	0.12	0.0964	96	1.244	0.2164
low v high	0.06	0.0964	96	0.622	0.5353
med v high	-0.06	0.0964	96	-0.622	0.5353
high					
sterile vs. live	-0.06	0.2362	96	-0.254	0.8
ctr v low	-0.04	0.0964	96	-0.415	0.6792
ctr v med	-0.08	0.0964	96	-0.83	0.4088
ctr v high	0.06	0.0964	96	0.622	0.5353
low v med	-0.04	0.0964	96	-0.415	0.6792
low v high	0.1	0.0964	96	1.037	0.3024
med v high	0.14	0.0964	96	1.452	0.1498

Table 12: Apriori contrasts for abiotic severity treatment x soil type and biotic severity treatment x soil type interaction effects on *P. graminifolia* germination rate.

Contrast	Estimate	SE	d.f.	T-ratio	P-value
abiotic x pines					
near: l vs m	-0.025	0.0239	96	-1.047	0.2976
near: l vs h	0.0575	0.0239	96	2.409	0.0179**
near: m vs h	0.0825	0.0239	96	3.456	0.0008***
away: l vs m	0.0325	0.0239	96	1.362	0.1765
away: l vs h	-0.0325	0.0239	96	-1.362	0.1765
away: m vs h	-0.065	0.0239	96	-2.723	0.0077**
biotic x pines					
near: ctr vs l	0.00333	0.0276	96	0.121	0.904
near: ctr vs m	0.05	0.0276	96	1.814	0.0728
near: ctr vs h	0.08667	0.0276	96	3.144	0.0022**
near: l vs m	0.04667	0.0276	96	1.693	0.0937
near: l vs h	0.08333	0.0276	96	3.023	0.0032**
near: m vs h	0.03667	0.0276	96	1.33	0.1866
away: ctr vs l	-0.03667	0.0276	96	-1.33	0.1866
away: ctr vs m	-0.05667	0.0276	96	-2.056	0.0425**
away: ctr vs h	-0.02333	0.0276	96	-0.847	0.3994
away: l vs m	-0.02	0.0276	96	-0.726	0.4698
away: l vs h	0.01333	0.0276	96	0.484	0.6297
away: m vs h	0.03333	0.0276	96	1.209	0.2295

Table 13: Apriori contrasts for abiotic soil severity treatment x soil type interaction effects on *S. secundum* germination rate.

Contrast	Estimate	SE	d.f.	T-ratio	P-value
near: low vs. med	0.0075	0.0396	96	0.189	0.8504
near: low vs. high	0.0125	0.0396	96	0.315	0.7532
near: med vs. high	0.005	0.0396	96	0.126	0.8999
away: low vs. med	-0.085	0.0396	96	-2.144	0.0346*
away: low vs high	-0.1425	0.0396	96	-3.594	0.0005***
away: med vs high	-0.0575	0.0396	96	-1.45	0.1502

Table 14: Apriori contrasts for abiotic soil severity treatment and abiotic soil severity treatment x soil type interaction effects on *C. americana* plant biomass.

Contrast	Estimate	SE	d.f.	T-ratio	P-value
abiotic treatment					
low vs med	-1.59	0.624	96	-2.55	0.0124**
low vs high	1.452	0.624	96	2.328	0.022**
med vs high	3.042	0.624	96	4.878	<.0001***
abiotic x pines					
near: low vs med	-1.776	0.441	96	-4.028	0.0001***
near: low vs high	0.914	0.441	96	2.073	0.0409**
near: med vs high	2.69	0.441	96	6.101	<.0001***
away: low vs med	0.186	0.441	96	0.422	0.6741
away: low vs high	0.538	0.441	96	1.22	0.2254
away: med vs high	0.352	0.441	96	0.798	0.4267

Table 15: Apriori contrasts for biotic soil severity treatment, biotic soil severity x soil type, and abiotic soil severity x soil type interaction effects on *P. graminifolia* biomass.

Contrast	Estimate	SE	d.f.	T-ratio	P-value
biotic treatment					
bio: ctr vs live	-1.0706	1.734	96	-0.618	0.5384
bio: ctr vs low	-1.34	0.708	96	-1.893	0.0613*
bio: ctr vs med	-0.4446	0.708	96	-0.628	0.5314
bio: ctr vs high	0.714	0.708	96	1.009	0.3156
bio: low vs med	0.8954	0.708	96	1.265	0.2089
bio: low vs high	2.054	0.708	96	2.902	0.0046**
bio: med vs high	1.1586	0.708	96	1.637	0.1049
biotic x pines					
near: ctr vs live	1.436	1.226	96	-1.171	0.2444
near: ctr vs low	-0.45	0.5	96	-0.899	0.3708
near: ctr vs med	0.368	0.5	96	0.735	0.464
near: ctr vs high	1.518	0.5	96	3.033	0.0031**
near: low vs med	0.818	0.5	96	1.634	0.1054
near: low vs high	1.968	0.5	96	3.932	0.0002***
near: med vs high	1.15	0.5	96	2.298	0.0237**
away: ctr vs live	-2.5066	1.226	96	2.045	0.0436**
away: ctr vs low	-0.89	0.5	96	-1.778	0.0785*
away: ctr vs med	-0.8126	0.5	96	-1.624	0.1077
away: ctr vs high	-0.804	0.5	96	-1.606	0.1115
away: low vs med	0.0774	0.5	96	0.155	0.8774
away: low vs high	0.086	0.5	96	0.172	0.8639
away: med vs high	0.0086	0.5	96	0.017	0.9863
abiotic x pines					
near: low vs med	-0.444	0.578	96	-0.768	0.4442
near: low vs high	1.636	0.578	96	2.831	0.0057**
near: med vs high	2.08	0.578	96	3.599	0.0005***
away: low vs med	1.32	0.578	96	2.284	0.0246**
away: low vs high	-0.5306	0.578	96	-0.918	0.3608
away: med vs high	-1.8506	0.578	96	-3.202	0.0019**

Table 16: Apriori contrasts for abiotic soil severity treatment, biotic soil severity treatment, and abiotic soil severity treatment x soil type interaction effects on *S. secundum* biomass.

Contrast	Estimate	SE	d.f.	T-ratio	P-value
abiotic treatment					
low vs. medium	-2.242	0.929	96	-2.413	0.0177**
low vs. high	2.628	0.929	96	2.829	0.0057**
medium vs. high	4.87	0.929	96	5.242	<.0001***
abiotic x pines					
near: low vs. med	0.4	0.657	96	0.609	0.5441
near: low vs. high	1.54	0.657	96	2.344	0.0211**
near: med vs. high	1.14	0.657	96	1.735	0.0859*
away: low vs. med	-2.642	0.657	96	-4.022	0.0001***
away: low vs high	1.088	0.657	96	1.656	0.101
away: med vs high	3.73	0.657	96	5.678	<.0001***
biotic treatment					
ctr vs live	-6.058	1.971	96	-3.074	0.0028**
ctr vs low	-1.598	0.805	96	-1.986	0.0499**
ctr vs med	-1.71	0.805	96	-2.125	0.0361**
ctr vs high	-2.75	0.805	96	-3.418	0.0009***
low vs med	-0.112	0.805	96	-0.139	0.8896
low vs high	-1.152	0.805	96	-1.432	0.1555
med vs high	-1.04	0.805	96	-1.293	0.1993

Table 17: Arbuscular mycorrhizal fungi colonization data for chapter 4.

Number	Species	Function	Miss	Hit	Arbuscule	Vesicle	Coil
178	*Rhus sp.*	shrub					
180	*Rhus sp.*	shrub					
233	*Rhus sp.*	shrub					
64	*C. americana*	shrub	0	20	3	4	0
206	*Rhus sp.*	shrub					
230	*Rhus sp.*	shrub	6	14	7	5	0
21	*C. americana*	shrub	0	20	3	4	1
214	*Rhus sp.*	shrub	3	17	12	1	0
127	*Rhus sp.*	shrub					
104	*C. americana*	shrub	0	20	16	4	0
168	*Rhus sp.*	shrub					
207	*Rhus sp.*	shrub					
62	*C. americana*	shrub	0	20	15	6	0
50	*C. americana*	shrub	0	20	9	0	1
95	*C. americana*	shrub	0	20	10	5	0
134	*Rhus sp.*	shrub					
73	*C. americana*	shrub	4	16	12	1	0

220	*Rhus sp.*	shrub					
5	*C. americana*	shrub	1	19	6	2	1
66	*C. americana*	shrub	0	20	14	2	0
57	*C. americana*	shrub	0	20	4	6	2
6	*C. americana*	shrub	0	20	3	10	0
9	*C. americana*	shrub	0	20	21	2	0
167	*Rhus sp.*	shrub					
101	*C. americana*	shrub	0	20	11	3	0
132	*Rhus sp.*	shrub	11	9	9	3	0
48	*C. americana*	shrub	0	20	11	10	0
14	*C. americana*	shrub	3	17	16	0	0
193	*Rhus sp.*	shrub					
86	*C. americana*	shrub	0	20	11	4	1
17	*C. americana*	shrub	2	18	13	3	0
75	*C. americana*	shrub	0	20	24	2	0
82	*C. americana*	shrub	0	20	18	11	0
237	*Rhus sp.*	shrub					
221	*Rhus sp.*	shrub					
154	*Rhus sp.*	shrub					
124	*Rhus sp.*	shrub	2	18	9	2	0
7	*C. americana*	shrub					
52	*C. americana*	shrub	0	20	12	10	1
199	*Rhus sp.*	shrub					
147	*Rhus sp.*	shrub	0	20	5	4	0
161	*Rhus sp.*	shrub					
27	*C. americana*	shrub	1	19	14	4	0
171	*Rhus sp.*	shrub					
201	*Rhus sp.*	shrub					
18	*C. americana*	shrub	2	18	11	2	3
139	*Rhus sp.*	shrub					
85	*C. americana*	shrub	1	19	20	0	0
158	*Rhus sp.*	shrub					
42	*C. americana*	shrub	0	20	10	8	3
43	*C. americana*	shrub	0	20	7	7	0
166	*Rhus sp.*	shrub	7	13	7	2	0
218	*Rhus sp.*	shrub					
223	*Rhus sp.*	shrub	3	17	6	9	0
126	*Rhus sp.*	shrub					
25	*C. americana*	shrub	2	18	14	2	0
80	*C. americana*	shrub	0	20	10	11	2
212	*Rhus sp.*	shrub	14	6	3	1	0
68	*C. americana*	shrub	0	20	16	0	0
58	*C. americana*	shrub	2	18	13	17	0
22	*C. americana*	shrub	0	20	11	12	0
190	*Rhus sp.*	shrub	12	8	2	1	0

92	*C. americana*	shrub					
117	*C. americana*	shrub	0	20	19	4	0
74	*C. americana*	shrub	0	20	14	4	0
164	*Rhus sp.*	shrub	4	16	6	8	0
163	*Rhus sp.*	shrub					
179	*Rhus sp.*	shrub					
234	*Rhus sp.*	shrub	4	16	15	5	0
219	*Rhus sp.*	shrub					
184	*Rhus sp.*	shrub					
70	*C. americana*	shrub	0	20	13	2	0
111	*C. americana*	shrub	0	20	14	5	0
90	*C. americana*	shrub	0	20	9	3	0
175	*Rhus sp.*	shrub					
13	*C. americana*	shrub	1	19	11	7	0
182	*Rhus sp.*	shrub					
236	*Rhus sp.*	shrub	7	13	4	2	0
112	*C. americana*	shrub	0	20	15	8	0
34	*C. americana*	shrub	0	20	13	3	0
186	*Rhus sp.*	shrub					
129	*Rhus sp.*	shrub					
118	*C. americana*	shrub	0	20	18	5	0
145	*Rhus sp.*	shrub					
183	*Rhus sp.*	shrub					
150	*Rhus sp.*	shrub					
1	*C. americana*	shrub	3	17	5	4	0
225	*Rhus sp.*	shrub					
191	*Rhus sp.*	shrub					
177	*Rhus sp.*	shrub	3	17	13	1	0
203	*Rhus sp.*	shrub	1	19	16	5	0
94	*C. americana*	shrub	2	18	19	3	0
192	*Rhus sp.*	shrub					
217	*Rhus sp.*	shrub					
71	*C. americana*	shrub	0	20	5	4	0
224	*Rhus sp.*	shrub					
93	*C. americana*	shrub	1	19	14	8	2
174	*Rhus sp.*	shrub					
131	*Rhus sp.*	shrub	4	16	8	7	2
173	*Rhus sp.*	shrub	5	15	4	1	0
98	*C. americana*	shrub	0	20	9	2	0
202	*Rhus sp.*	shrub					
87	*C. americana*	shrub	6	14	5	1	0
128	*Rhus sp.*	shrub					
138	*Rhus sp.*	shrub					
185	*Rhus sp.*	shrub					
176	*Rhus sp.*	shrub					

156	*Rhus sp.*	shrub					
222	*Rhus sp.*	shrub					
31	*C. americana*	shrub	2	18	10	1	0
10	*C. americana*	shrub	0	20	7	5	0
169	*Rhus sp.*	shrub					
210	*Rhus sp.*	shrub	5	15	14	3	0
89	*C. americana*	shrub	0	20	9	2	0
41	*C. americana*	shrub	1	19	15	1	0
148	*Rhus sp.*	shrub	2	18	16	1	0
196	*Rhus sp.*	shrub					
113	*C. americana*	shrub	0	20	16	6	1
195	*Rhus sp.*	shrub					
19	*C. americana*	shrub	2	18	14	9	0
197	*Rhus sp.*	shrub					
123	*Rhus sp.*	shrub	0	20	10	15	1
162	*Rhus sp.*	shrub	1	19	5	3	0
88	*C. americana*	shrub	0	20	12	2	0
49	*C. americana*	shrub	1	19	6	4	1
45	*C. americana*	shrub	0	20	14	8	0
38	*C. americana*	shrub	0	20	13	3	1
103	*C. americana*	shrub	0	20	16	9	6
78	*C. americana*	shrub	0	20	17	2	0
24	*C. americana*	shrub	0	20	12	11	0
211	*Rhus sp.*	shrub					
32	*C. americana*	shrub	2	18	17	2	0
151	*Rhus sp.*	shrub					
181	*Rhus sp.*	shrub					
227	*Rhus sp.*	shrub					
157	*Rhus sp.*	shrub					
76	*C. americana*	shrub	1	19	16	5	1
67	*C. americana*	shrub	2	18	5	5	0
116	*C. americana*	shrub	2	18	6	8	1
84	*C. americana*	shrub	1	19	11	6	2
142	*Rhus sp.*	shrub	2	18	12	9	0
72	*C. americana*	shrub	2	18	13	8	0
20	*C. americana*	shrub	3	17	7	3	1
33	*C. americana*	shrub	0	20	10	7	0
140	*Rhus sp.*	shrub	3	17	1	6	0
200	*Rhus sp.*	shrub					
81	*C. americana*	shrub	0	20	13	14	2
2	*C. americana*	shrub	0	20	16	5	0
53	*C. americana*	shrub	1	19	18	3	1
102	*C. americana*	shrub	1	19	13	4	0
106	*C. americana*	shrub	5	15	7	10	0
209	*Rhus sp.*	shrub					

39	*C. americana*	shrub	0	20	23	6	1
56	*C. americana*	shrub	2	18	8	2	0
4	*C. americana*	shrub	4	16	2	8	0
59	*C. americana*	shrub	1	19	9	11	0
122	*Rhus sp.*	shrub	9	11	3	2	0
159	*Rhus sp.*	shrub	8	12	2	6	0
187	*Rhus sp.*	shrub					
231	*Rhus sp.*	shrub	14	6	4	0	0
60	*C. americana*	shrub	2	18	14	5	0
240	*Rhus sp.*	shrub	1	19	7	8	0
120	*C. americana*	shrub	0	20	9	2	0
54	*C. americana*	shrub	4	16	13	3	0
23	*C. americana*	shrub	0	20	20	7	1
16	*C. americana*	shrub	0	20	12	7	0
110	*C. americana*	shrub	0	20	14	4	0
146	*Rhus sp.*	shrub					
37	*C. americana*	shrub					
30	*C. americana*	shrub	0	20	8	3	0
79	*C. americana*	shrub	0	20	11	5	0
29	*C. americana*	shrub	0	20	17	4	0
28	*C. americana*	shrub	0	20	12	4	1
119	*C. americana*	shrub	0	20	12	1	1
213	*Rhus sp.*	shrub					
194	*Rhus sp.*	shrub	1	19	4	6	0
26	*C. americana*	shrub	2	18	11	3	2
198	*Rhus sp.*	shrub	8	12	3	1	0
160	*Rhus sp.*	shrub	3	17	5	6	0
152	*Rhus sp.*	shrub					
107	*C. americana*	shrub					
130	*Rhus sp.*	shrub	2	18	12	4	0
115	*C. americana*	shrub	0	20	10	10	0
189	*Rhus sp.*	shrub					
61	*C. americana*	shrub	0	20	20	4	0
165	*Rhus sp.*	shrub	3	17	10	3	0
133	*Rhus sp.*	shrub					
144	*Rhus sp.*	shrub	0	20	12	6	0
65	*C. americana*	shrub	2	18	6	10	1
100	*C. americana*	shrub	0	20	14	5	0
155	*Rhus sp.*	shrub					
136	*Rhus sp.*	shrub					
141	*Rhus sp.*	shrub	3	17	14	4	0
108	*C. americana*	shrub	2	18	6	3	1
188	*Rhus sp.*	shrub					
96	*C. americana*	shrub	0	20	23	5	2
36	*C. americana*	shrub	0	20	18	5	1

69	*C. americana*	shrub	0	20	13	4	1
97	*C. americana*	shrub	0	20	11	10	2
215	*Rhus sp.*	shrub					
170	*Rhus sp.*	shrub					
35	*C. americana*	shrub	0	20	7	5	0
143	*Rhus sp.*	shrub					
77	*C. americana*	shrub					
205	*Rhus sp.*	shrub					
15	*C. americana*	shrub	0	20	6	1	0
114	*C. americana*	shrub	1	19	16	2	0
229	*Rhus sp.*	shrub	7	13	9	1	0
109	*C. americana*	shrub	6	14	10	1	0
55	*C. americana*	shrub	1	19	21	5	1
232	*Rhus sp.*	shrub					
83	*C. americana*	shrub	0	20	8	8	3
12	*C. americana*	shrub	1	19	12	8	1
125	*Rhus sp.*	shrub					
63	*C. americana*	shrub	1	19	18	4	1
208	*Rhus sp.*	shrub					
228	*Rhus sp.*	shrub	9	11	5	1	0
172	*Rhus sp.*	shrub					
105	*C. americana*	shrub	0	20	15	9	0
47	*C. americana*	shrub	0	20	18	4	0
238	*Rhus sp.*	shrub					
8	*C. americana*	shrub	2	18	12	7	0
153	*Rhus sp.*	shrub	0	20	10	3	1
216	*Rhus sp.*	shrub	0	20	10	4	2
235	*Rhus sp.*	shrub					
91	*C. americana*	shrub	0	20	12	13	2
239	*Rhus sp.*	shrub					
226	*Rhus sp.*	shrub					
204	*Rhus sp.*	shrub					
135	*Rhus sp.*	shrub					
40	*C. americana*	shrub	0	20	18	4	2
121	*Rhus sp.*	shrub	4	16	10	7	0
3	*C. americana*	shrub	0	20	10	5	0
149	*Rhus sp.*	shrub	2	18	8	8	0
44	*C. americana*	shrub	2	18	10	1	1
51	*C. americana*	shrub	2	18	7	10	1
99	*C. americana*	shrub	0	20	19	5	1
137	*Rhus sp.*	shrub					
46	*C. americana*	shrub	2	18	16	3	2
11	*C. americana*	shrub	1	19	11	3	1
393	*S. nutans*	grass					
266	*S. secundum*	grass	6	14	7	1	0

282	*S. secundum*	grass	14	6	0	0	0
463	*S. nutans*	grass					
265	*S. secundum*	grass	4	16	11	3	0
346	*S. secundum*	grass	12	8	4	0	0
284	*S. secundum*	grass					
434	*S. nutans*	grass					
316	*S. secundum*	grass	9	11	3	0	0
300	*S. secundum*	grass	8	12	8	1	1
375	*S. nutans*	grass					
365	*S. nutans*	grass					
326	*S. secundum*	grass	1	19	6	2	2
352	*S. secundum*	grass	7	13	10	0	2
283	*S. secundum*	grass	11	9	7	2	0
255	*S. secundum*	grass					
415	*S. nutans*	grass					
412	*S. nutans*	grass					
384	*S. nutans*	grass					
423	*S. nutans*	grass	8	12	1	0	0
438	*S. nutans*	grass					
400	*S. nutans*	grass					
405	*S. nutans*	grass					
409	*S. nutans*	grass					
345	*S. secundum*	grass	12	8	4	2	0
468	*S. nutans*	grass	3	17	17	1	0
337	*S. secundum*	grass	2	18	15	3	0
342	*S. secundum*	grass	14	6	4	0	0
444	*S. nutans*	grass					
385	*S. nutans*	grass					
359	*S. secundum*	grass	9	11	5	1	0
280	*S. secundum*	grass	5	15	4	1	2
397	*S. nutans*	grass					
272	*S. secundum*	grass	6	14	5	2	0
462	*S. nutans*	grass					
360	*S. secundum*	grass	10	10	8	1	0
248	*S. secundum*	grass	12	8	3	1	0
257	*S. secundum*	grass	16	4	0	0	0
436	*S. nutans*	grass					
407	*S. nutans*	grass					
418	*S. nutans*	grass					
376	*S. nutans*	grass					
301	*S. secundum*	grass	11	9	4	0	0
420	*S. nutans*	grass					
358	*S. secundum*	grass	15	5	3	0	0
250	*S. secundum*	grass					
432	*S. nutans*	grass					

254	*S. secundum*	grass	9	11	5	1	0
348	*S. secundum*	grass	10	10	3	1	1
275	*S. secundum*	grass	6	14	14	1	0
338	*S. secundum*	grass	7	13	2	2	0
390	*S. nutans*	grass					
267	*S. secundum*	grass	7	13	11	1	0
336	*S. secundum*	grass	6	14	7	3	1
480	*S. nutans*	grass					
363	*S. nutans*	grass					
330	*S. secundum*	grass	6	14	4	1	0
477	*S. nutans*	grass					
268	*S. secundum*	grass	14	6	1	1	0
404	*S. nutans*	grass	10	10	1	1	0
386	*S. nutans*	grass					
333	*S. secundum*	grass	14	6	1	1	0
295	*S. secundum*	grass					
335	*S. secundum*	grass	7	13	6	4	0
429	*S. nutans*	grass					
322	*S. secundum*	grass	7	13	10	1	0
399	*S. nutans*	grass					
454	*S. nutans*	grass					
364	*S. nutans*	grass					
357	*S. secundum*	grass	5	15	7	3	1
293	*S. secundum*	grass	0	20	15	0	0
356	*S. secundum*	grass	8	12	9	1	0
378	*S. nutans*	grass					
421	*S. nutans*	grass					
311	*S. secundum*	grass	4	16	5	1	1
461	*S. nutans*	grass					
410	*S. nutans*	grass					
302	*S. secundum*	grass	10	10	3	1	0
245	*S. secundum*	grass	9	11	1	1	0
403	*S. nutans*	grass					
473	*S. nutans*	grass	11	9	3	0	0
288	*S. secundum*	grass					
374	*S. nutans*	grass					
321	*S. secundum*	grass	9	11	3	0	1
472	*S. nutans*	grass					
290	*S. secundum*	grass	2	18	7	0	0
362	*S. nutans*	grass					
424	*S. nutans*	grass					
285	*S. secundum*	grass	0	20	3	1	0
247	*S. secundum*	grass					
433	*S. nutans*	grass					
391	*S. nutans*	grass					

244	*S. secundum*	grass	3	17	8	2	0
320	*S. secundum*	grass	4	16	10	3	0
243	*S. secundum*	grass					
329	*S. secundum*	grass	11	9	4	0	1
269	*S. secundum*	grass	5	15	9	4	0
367	*S. nutans*	grass					
297	*S. secundum*	grass	5	15	2	2	0
445	*S. nutans*	grass					
349	*S. secundum*	grass	5	15	10	2	0
474	*S. nutans*	grass					
453	*S. nutans*	grass					
327	*S. secundum*	grass	5	15	22	2	0
307	*S. secundum*	grass	15	5	1	1	1
402	*S. nutans*	grass					
340	*S. secundum*	grass	1	19	8	3	1
377	*S. nutans*	grass					
467	*S. nutans*	grass					
439	*S. nutans*	grass	3	17	3	2	0
289	*S. secundum*	grass	4	16	10	0	1
323	*S. secundum*	grass	3	17	15	1	1
372	*S. nutans*	grass					
478	*S. nutans*	grass					
366	*S. nutans*	grass					
331	*S. secundum*	grass	2	18	11	1	1
317	*S. secundum*	grass	11	9	3	0	0
313	*S. secundum*	grass	7	7	5	0	0
371	*S. nutans*	grass					
303	*S. secundum*	grass	9	11	8	0	0
383	*S. nutans*	grass					
296	*S. secundum*	grass	4	16	1	0	0
262	*S. secundum*	grass	7	13	6	3	0
394	*S. nutans*	grass					
455	*S. nutans*	grass					
310	*S. secundum*	grass	8	12	4	1	0
281	*S. secundum*	grass					
343	*S. secundum*	grass					
464	*S. nutans*	grass					
370	*S. nutans*	grass					
251	*S. secundum*	grass	12	8	5	4	2
419	*S. nutans*	grass					
450	*S. nutans*	grass	4	16	9	0	0
279	*S. secundum*	grass					
465	*S. nutans*	grass					
287	*S. secundum*	grass	2	18	8	3	0
430	*S. nutans*	grass					

422	*S. nutans*	grass					
315	*S. secundum*	grass	6	14	8	2	0
286	*S. secundum*	grass	0	20	8	1	0
440	*S. nutans*	grass					
264	*S. secundum*	grass	7	13	7	1	0
426	*S. nutans*	grass					
425	*S. nutans*	grass					
460	*S. nutans*	grass					
249	*S. secundum*	grass	11	9	6	0	1
273	*S. secundum*	grass	13	7	6	0	0
373	*S. nutans*	grass					
319	*S. secundum*	grass	4	16	13	1	1
475	*S. nutans*	grass					
270	*S. secundum*	grass	4	16	4	6	0
392	*S. nutans*	grass					
459	*S. nutans*	grass					
291	*S. secundum*	grass					
442	*S. nutans*	grass					
344	*S. secundum*	grass	10	10	7	0	0
354	*S. secundum*	grass	12	8	2	2	0
387	*S. nutans*	grass					
458	*S. nutans*	grass					
242	*S. secundum*	grass					
298	*S. secundum*	grass	7	13	5	1	0
369	*S. nutans*	grass					
308	*S. secundum*	grass	10	10	7	0	0
446	*S. nutans*	grass					
379	*S. nutans*	grass	9	11	0	1	0
241	*S. secundum*	grass	11	9	1	0	0
334	*S. secundum*	grass					
398	*S. nutans*	grass					
256	*S. secundum*	grass	11	9	2	0	0
325	*S. secundum*	grass	10	10	2	0	0
414	*S. nutans*	grass					
469	*S. nutans*	grass					
260	*S. secundum*	grass	5	15	7	1	0
246	*S. secundum*	grass	14	6	3	0	0
261	*S. secundum*	grass	4	16	13	1	0
259	*S. secundum*	grass	11	9	7	0	1
411	*S. nutans*	grass					
437	*S. nutans*	grass					
292	*S. secundum*	grass	3	17	10	4	0
341	*S. secundum*	grass	11	9	14	2	0
294	*S. secundum*	grass					
435	*S. nutans*	grass					

304	*S. secundum*	grass	3	17	5	3	0
448	*S. nutans*	grass					
382	*S. nutans*	grass					
328	*S. secundum*	grass	7	13	8	3	0
353	*S. secundum*	grass	6	14	8	0	0
427	*S. nutans*	grass	6	14	4	0	1
456	*S. nutans*	grass					
351	*S. secundum*	grass	7	13	7	2	0
457	*S. nutans*	grass					
355	*S. secundum*	grass	13	7	4	0	0
449	*S. nutans*	grass					
441	*S. nutans*	grass					
451	*S. nutans*	grass					
396	*S. nutans*	grass					
274	*S. secundum*	grass	9	11	1	1	0
476	*S. nutans*	grass					
452	*S. nutans*	grass					
380	*S. nutans*	grass					
350	*S. secundum*	grass	9	11	14	0	0
413	*S. nutans*	grass					
278	*S. secundum*	grass	10	10	2	4	0
389	*S. nutans*	grass					
401	*S. nutans*	grass					
431	*S. nutans*	grass					
263	*S. secundum*	grass	3	17	10	1	0
417	*S. nutans*	grass	8	12	6	0	0
332	*S. secundum*	grass	7	13	12	0	0
299	*S. secundum*	grass	5	15	9	3	2
277	*S. secundum*	grass	5	15	10	2	1
381	*S. nutans*	grass					
339	*S. secundum*	grass	3	17	17	6	0
305	*S. secundum*	grass	9	11	7	1	0
395	*S. nutans*	grass					
388	*S. nutans*	grass					
253	*S. secundum*	grass	11	9	6	1	0
318	*S. secundum*	grass	5	15	8	3	0
470	*S. nutans*	grass	3	17	14	2	0
258	*S. secundum*	grass	8	12	3	0	0
276	*S. secundum*	grass	9	11	8	4	0
479	*S. nutans*	grass					
309	*S. secundum*	grass	6	14	7	1	0
271	*S. secundum*	grass	10	10	9	0	0
312	*S. secundum*	grass	13	7	5	0	0
443	*S. nutans*	grass					
324	*S. secundum*	grass	3	17	4	0	0

306	*S. secundum*	grass	3	17	12	3	0
447	*S. nutans*	grass					
428	*S. nutans*	grass					
466	*S. nutans*	grass					
314	*S. secundum*	grass	3	17	10	0	0
471	*S. nutans*	grass					
347	*S. secundum*	grass					
406	*S. nutans*	grass					
408	*S. nutans*	grass					
252	*S. secundum*	grass					
416	*S. nutans*	grass					
368	*S. nutans*	grass					
361	*S. nutans*	grass					
942	*pityopsis*	forb					
863	*pityopsis*	forb					
1026	*Bidens bipinata*	forb	2	18	6	1	0
878	*pityopsis*	forb	0	20	6	2	0
967	*Bidens bipinata*	forb	4	16	2	4	1
963	*Bidens bipinata*	forb	11	9	4	1	1
955	*pityopsis*	forb					
1030	*Bidens bipinata*	forb	8	12	3	5	0
1034	*Bidens bipinata*	forb					
1025	*Bidens bipinata*	forb					
949	*pityopsis*	forb					
968	*Bidens bipinata*	forb	10	10	1	0	1
973	*Bidens bipinata*	forb	1	19	6	0	0
1048	*Bidens bipinata*	forb	1	19	7	1	2
982	*Bidens bipinata*	forb	9	11	5	0	0
846	*pityopsis*	forb	0	20	2	10	1
958	*pityopsis*	forb	2	18	10	8	0
1004	*Bidens bipinata*	forb	9	11	4	0	0
953	*pityopsis*	forb	9	11	7	0	0
932	*pityopsis*	forb	0	20	7	9	0
872	*pityopsis*	forb					
844	*pityopsis*	forb	0	20	5	1	0
1053	*Bidens bipinata*	forb	3	17	3	1	2
919	*pityopsis*	forb					
943	*pityopsis*	forb					
864	*pityopsis*	forb					
852	*pityopsis*	forb	2	18	8	3	1
977	*Bidens bipinata*	forb					
861	*pityopsis*	forb					
1005	*Bidens bipinata*	forb	8	12	2	0	0
1055	*Bidens bipinata*	forb	12	8	8	0	0
901	*pityopsis*	forb	0	20	7	2	0

969	*Bidens bipinata*	forb	0	20	11	3	0
841	*pityopsis*	forb	0	20	7	3	0
941	*pityopsis*	forb	8	12	6	3	3
1008	*Bidens bipinata*	forb	1	19	3	5	0
882	*pityopsis*	forb	9	11	3	2	1
929	*pityopsis*	forb	3	17	0	2	1
1022	*Bidens bipinata*	forb					
1056	*Bidens bipinata*	forb	3	17	9	1	0
885	*pityopsis*	forb	1	19	2	18	0
1040	*Bidens bipinata*	forb	5	15	8	1	0
858	*pityopsis*	forb	5	15	2	2	0
948	*pityopsis*	forb	7	13	3	0	0
961	*Bidens bipinata*	forb	7	13	3	3	6
915	*pityopsis*	forb					
1052	*Bidens bipinata*	forb					
1073	*Bidens bipinata*	forb					
847	*pityopsis*	forb	3	17	8	4	2
1023	*Bidens bipinata*	forb	5	15	3	0	0
1019	*Bidens bipinata*	forb					
918	*pityopsis*	forb	1	19	6	2	2
853	*pityopsis*	forb					
860	*pityopsis*	forb					
854	*pityopsis*	forb	1	19	4	3	2
924	*pityopsis*	forb					
867	*pityopsis*	forb	1	19	4	0	2
908	*pityopsis*	forb	1	19	5	6	1
1029	*Bidens bipinata*	forb	17	3	0	0	0
1020	*Bidens bipinata*	forb	3	17	3	1	6
894	*pityopsis*	forb	1	19	9	1	1
1079	*Bidens bipinata*	forb					
913	*pityopsis*	forb	8	12	1	1	0
1039	*Bidens bipinata*	forb	2	18	14	2	2
922	*pityopsis*	forb	0	20	8	11	0
971	*Bidens bipinata*	forb					
1064	*Bidens bipinata*	forb	0	20	6	2	0
923	*pityopsis*	forb	0	20	2	22	1
1069	*Bidens bipinata*	forb					
946	*pityopsis*	forb					
904	*pityopsis*	forb	2	18	7	1	0
933	*pityopsis*	forb	0	20	1	15	1
892	*pityopsis*	forb	1	19	5	7	0
1046	*Bidens bipinata*	forb	4	16	1	1	0
964	*Bidens bipinata*	forb	0	20	5	1	0
927	*pityopsis*	forb	0	20	5	6	1
1047	*Bidens bipinata*	forb	2	18	5	2	0

1061	*Bidens bipinata*	forb	12	8	5	0	1
956	*pityopsis*	forb					
1045	*Bidens bipinata*	forb					
876	*pityopsis*	forb					
990	*Bidens bipinata*	forb	11	9	1	2	1
870	*pityopsis*	forb	4	16	2	2	2
1063	*Bidens bipinata*	forb					
1017	*Bidens bipinata*	forb	10	10	1	0	1
1068	*Bidens bipinata*	forb	13	7	3	0	3
875	*pityopsis*	forb	1	19	2	0	1
940	*pityopsis*	forb					
950	*pityopsis*	forb					
965	*Bidens bipinata*	forb	13	7	2	1	0
1003	*Bidens bipinata*	forb	1	19	5	2	0
905	*pityopsis*	forb					
972	*Bidens bipinata*	forb	1	19	5	0	0
881	*pityopsis*	forb	3	17	4	4	0
897	*pityopsis*	forb	6	14	2	0	1
1016	*Bidens bipinata*	forb	12	8	2	0	0
993	*Bidens bipinata*	forb					
1044	*Bidens bipinata*	forb	3	17	5	0	2
1000	*Bidens bipinata*	forb					
868	*pityopsis*	forb					
856	*pityopsis*	forb	0	20	3	4	1
1009	*Bidens bipinata*	forb					
898	*pityopsis*	forb	3	17	4	1	0
981	*Bidens bipinata*	forb					
1062	*Bidens bipinata*	forb					
891	*pityopsis*	forb	2	18	1	11	1
997	*Bidens bipinata*	forb	3	17	6	0	1
1032	*Bidens bipinata*	forb	7	13	6	2	0
1077	*Bidens bipinata*	forb					
978	*Bidens bipinata*	forb	12	8	5	0	0
1015	*Bidens bipinata*	forb					
954	*pityopsis*	forb					
842	*pityopsis*	forb	3	17	5	3	0
1042	*Bidens bipinata*	forb	1	19	3	1	0
1014	*Bidens bipinata*	forb	1	19	15	9	1
914	*pityopsis*	forb	3	17	7	2	0
979	*Bidens bipinata*	forb	1	19	0	1	1
1074	*Bidens bipinata*	forb	8	12	3	0	0
970	*Bidens bipinata*	forb	1	19	7	0	2
906	*pityopsis*	forb	1	19	15	2	0
928	*pityopsis*	forb	1	19	6	4	1
1070	*Bidens bipinata*	forb	0	20	5	4	4

895	*pityopsis*	forb	0	20	4	4	0
999	*Bidens bipinata*	forb	2	18	3	0	1
1066	*Bidens bipinata*	forb	0	20	11	0	5
890	*pityopsis*	forb	1	19	6	1	0
959	*pityopsis*	forb					
986	*Bidens bipinata*	forb	7	13	5	1	0
980	*Bidens bipinata*	forb	12	8	1	0	0
947	*pityopsis*	forb					
910	*pityopsis*	forb	0	20	8	4	2
1071	*Bidens bipinata*	forb	3	17	3	1	0
874	*pityopsis*	forb					
1041	*Bidens bipinata*	forb	2	18	6	2	2
884	*pityopsis*	forb	2	18	6	0	2
886	*pityopsis*	forb	2	18	1	6	0
902	*pityopsis*	forb					
998	*Bidens bipinata*	forb	6	14	5	2	1
945	*pityopsis*	forb	2	18	6	0	0
912	*pityopsis*	forb	0	20	5	9	3
992	*Bidens bipinata*	forb	1	19	5	10	5
944	*pityopsis*	forb	0	20	5	2	1
985	*Bidens bipinata*	forb	12	8	2	1	0
862	*pityopsis*	forb					
866	*pityopsis*	forb	2	18	6	1	0
966	*Bidens bipinata*	forb	8	12	1	1	0
848	*pityopsis*	forb	1	19	3	17	0
865	*pityopsis*	forb					
930	*pityopsis*	forb	0	20	3	9	0
991	*Bidens bipinata*	forb					
1038	*Bidens bipinata*	forb					
1059	*Bidens bipinata*	forb					
1067	*Bidens bipinata*	forb	10	10	6	0	0
1037	*Bidens bipinata*	forb	1	19	7	3	0
843	*pityopsis*	forb	0	20	3	1	1
1001	*Bidens bipinata*	forb	4	16	5	0	0
859	*pityopsis*	forb	0	20	2	18	2
1006	*Bidens bipinata*	forb	12	8	3	0	1
926	*pityopsis*	forb	0	20	3	6	0
935	*pityopsis*	forb					
988	*Bidens bipinata*	forb	12	8	3	0	0
1078	*Bidens bipinata*	forb					
989	*Bidens bipinata*	forb	7	13	4	0	2
976	*Bidens bipinata*	forb					
937	*pityopsis*	forb					
925	*pityopsis*	forb	1	19	6	4	5
1080	*Bidens bipinata*	forb					

1021	*Bidens bipinata*	forb	14	6	3	2	0
888	*pityopsis*	forb	3	17	6	3	4
931	*pityopsis*	forb	0	20	0	0	0
936	*pityopsis*	forb	0	20	5	2	1
920	*pityopsis*	forb					
850	*pityopsis*	forb					
1051	*Bidens bipinata*	forb	0	20	6	3	0
871	*pityopsis*	forb					
1075	*Bidens bipinata*	forb	4	16	7	2	0
938	*pityopsis*	forb					
1031	*Bidens bipinata*	forb	13	7	1	1	0
907	*pityopsis*	forb	3	17	7	2	3
1054	*Bidens bipinata*	forb	14	6	1	0	1
1002	*Bidens bipinata*	forb	11	9	8	0	0
939	*pityopsis*	forb					
857	*pityopsis*	forb	1	19	4	1	1
1065	*Bidens bipinata*	forb	6	14	11	1	1
1058	*Bidens bipinata*	forb	6	14	16	1	2
880	*pityopsis*	forb	3	17	5	7	2
851	*pityopsis*	forb	0	20	7	7	0
903	*pityopsis*	forb	2	18	5	0	2
1057	*Bidens bipinata*	forb	6	14	10	1	0
1049	*Bidens bipinata*	forb	0	20	6	1	0
899	*pityopsis*	forb	6	14	6	2	1
1012	*Bidens bipinata*	forb	3	17	7	1	1
909	*pityopsis*	forb	2	18	11	5	4
893	*pityopsis*	forb	3	17	3	4	1
1050	*Bidens bipinata*	forb	4	16	4	0	0
1018	*Bidens bipinata*	forb	9	11	6	0	0
1011	*Bidens bipinata*	forb					
960	*pityopsis*	forb					
889	*pityopsis*	forb	1	19	8	6	2
1027	*Bidens bipinata*	forb					
845	*pityopsis*	forb	5	15	10	1	2
849	*pityopsis*	forb	1	19	7	3	2
975	*Bidens bipinata*	forb	8	12	9	0	0
921	*pityopsis*	forb	2	18	3	3	0
911	*pityopsis*	forb	0	20	6	1	3
1060	*Bidens bipinata*	forb	2	18	12	2	0
996	*Bidens bipinata*	forb	1	19	14	0	0
1013	*Bidens bipinata*	forb	2	18	5	1	0
987	*Bidens bipinata*	forb					
1076	*Bidens bipinata*	forb	5	15	5	2	2
962	*Bidens bipinata*	forb					
1007	*Bidens bipinata*	forb	5	15	4	2	1

869	*pityopsis*	forb					
1024	*Bidens bipinata*	forb	5	15	4	1	0
1072	*Bidens bipinata*	forb	2	18	5	5	4
917	*pityopsis*	forb					
877	*pityopsis*	forb					
994	*Bidens bipinata*	forb	6	14	4	0	0
887	*pityopsis*	forb	3	17	5	2	4
1010	*Bidens bipinata*	forb	1	19	6	2	1
1035	*Bidens bipinata*	forb	2	18	6	1	1
957	*pityopsis*	forb					
1043	*Bidens bipinata*	forb	1	19	7	3	1
974	*Bidens bipinata*	forb	14	6	2	0	0
984	*Bidens bipinata*	forb	3	17	9	5	4
995	*Bidens bipinata*	forb					
952	*pityopsis*	forb					
1033	*Bidens bipinata*	forb	6	14	6	1	0
916	*pityopsis*	forb					
855	*pityopsis*	forb	0	20	7	14	0
983	*Bidens bipinata*	forb	2	18	16	1	3
934	*pityopsis*	forb					
896	*pityopsis*	forb	2	18	2	3	3
900	*pityopsis*	forb	8	12	1	0	2
1028	*Bidens bipinata*	forb	1	19	7	2	1
1036	*Bidens bipinata*	forb	7	13	4	1	0
873	*pityopsis*	forb	3	17	6	2	1
951	*pityopsis*	forb	4	16	7	0	2
879	*pityopsis*	forb	2	18	4	2	2
883	*pityopsis*	forb	8	12	3	0	1

Table 18: Ectomycorrhizal fungi colonization data for chapter 4

Number	Species	Infected	Non-infected	Infected %
510	P. taeda	75	28	0.72815534
750	P. palustris	53	62	0.460869565
586	P. taeda	83	66	0.55704698
762	P. palustris	39	39	0.5
511	P. taeda	73	47	0.608333333
580	P. taeda	71	35	0.669811321
792	P. palustris	58	75	0.436090226
521	P. taeda	87	54	0.617021277
736	P. palustris	61	58	0.512605042
515	P. taeda	72	46	0.610169492
836	P. palustris	55	65	0.458333333
562	P. taeda	21	39	0.35
828	P. palustris	77	64	0.546099291

512	P. taeda	74	44	0.627118644
819	P. palustris	66	58	0.532258065
829	P. palustris	62	77	0.446043165
812	P. palustris	70	73	0.48951049
837	P. palustris	77	53	0.592307692
529	P. taeda	68	60	0.53125
530	P. taeda	62	48	0.563636364
591	P. taeda	53	72	0.424
725	P. palustris	78	96	0.448275862
584	P. taeda	8	11	0.421052632
753	P. palustris	31	75	0.29245283
556	P. taeda	68	52	0.566666667
520	P. taeda	50	29	0.632911392
730	P. palustris	28	57	0.329411765
551	P. taeda	49	50	0.494949495
497	P. taeda	78	64	0.549295775
780	P. palustris	65	112	0.367231638
759	P. palustris	44	65	0.403669725
553	P. taeda	82	46	0.640625
525	P. taeda	67	55	0.549180328
811	P. palustris	50	54	0.480769231
593	P. taeda	72	61	0.541353383
832	P. palustris	34	59	0.365591398
597	P. taeda	63	76	0.45323741
540	P. taeda	61	50	0.54954955
791	P. palustris	71	85	0.455128205
505	P. taeda	65	42	0.607476636
572	P. taeda	47	33	0.5875
723	P. palustris	56	55	0.504504505
821	P. palustris	83	78	0.51552795
490	P. taeda	56	73	0.434108527
767	P. palustris	50	92	0.352112676
758	P. palustris	54	65	0.453781513
808	P. palustris	72	78	0.48
541	P. taeda	55	75	0.423076923
749	P. palustris	84	65	0.563758389
783	P. palustris	45	46	0.494505495
734	P. palustris	45	73	0.381355932
781	P. palustris	54	70	0.435483871
563	P. taeda	49	61	0.445454545
834	P. palustris	34	72	0.320754717
727	P. palustris	60	94	0.38961039
825	P. palustris	31	92	0.25203252
815	P. palustris	65	63	0.5078125
486	P. taeda	50	61	0.45045045

775	P. palustris	39	84	0.317073171
554	P. taeda	49	39	0.556818182
509	P. taeda	74	65	0.532374101
493	P. taeda	33	46	0.417721519
500	P. taeda	48	57	0.457142857
724	P. palustris	63	67	0.484615385
565	P. taeda	45	53	0.459183673
776	P. palustris	36	64	0.36
576	P. taeda	50	47	0.515463918
574	P. taeda	38	45	0.457831325
534	P. taeda	66	29	0.694736842
756	P. palustris	32	87	0.268907563
794	P. palustris	44	76	0.366666667
588	P. taeda	53	39	0.576086957
800	P. palustris	39	33	0.541666667
803	P. palustris	20	65	0.235294118
579	P. taeda	63	42	0.6
786	P. palustris	29	52	0.358024691
542	P. taeda	29	43	0.402777778
557	P. taeda	80	46	0.634920635
532	P. taeda	49	65	0.429824561
527	P. taeda	43	42	0.505882353
537	P. taeda	40	58	0.408163265
592	P. taeda	71	37	0.657407407
545	P. taeda	60	61	0.495867769
798	P. palustris	47	50	0.484536082
822	P. palustris	43	52	0.452631579
752	P. palustris	36	46	0.43902439
555	P. taeda	67	38	0.638095238
583	P. taeda	49	61	0.445454545
502	P. taeda	57	63	0.475
558	P. taeda	47	32	0.594936709
755	P. palustris	23	81	0.221153846
485	P. taeda	49	66	0.426086957
761	P. palustris	40	84	0.322580645
543	P. taeda	48	40	0.545454545
484	P. taeda	46	38	0.547619048
578	P. taeda	19	22	0.463414634
801	P. palustris	30	65	0.315789474
595	P. taeda	65	41	0.613207547
741	P. palustris	28	54	0.341463415
739	P. palustris	58	51	0.532110092
746	P. palustris	33	36	0.47826087
824	P. palustris	38	45	0.457831325
594	P. taeda	23	29	0.442307692

835	P. palustris	19	48	0.28358209
766	P. palustris	59	44	0.572815534
757	P. palustris	34	49	0.409638554
496	P. taeda	53	46	0.535353535
817	P. palustris	39	36	0.52
568	P. taeda	56	54	0.509090909
747	P. palustris	10	50	0.166666667
577	P. taeda	51	30	0.62962963
802	P. palustris	52	60	0.464285714
524	P. taeda	66	42	0.611111111
506	P. taeda	60	46	0.566037736
491	P. taeda	57	29	0.662790698
590	P. taeda	38	31	0.550724638
738	P. palustris	43	46	0.483146067
559	P. taeda	33	20	0.622641509
729	P. palustris	37	58	0.389473684
795	P. palustris	57	47	0.548076923
754	P. palustris	38	57	0.4
516	P. taeda	44	31	0.586666667
535	P. taeda	71	54	0.568
779	P. palustris	47	83	0.361538462
772	P. palustris	76	64	0.542857143
514	P. taeda	57	46	0.553398058
721	P. palustris	46	68	0.403508772
587	P. taeda	26	30	0.464285714
567	P. taeda	42	39	0.518518519
582	P. taeda	50	35	0.588235294
745	P. palustris	21	48	0.304347826
726	P. palustris	32	44	0.421052632
787	P. palustris	64	43	0.598130841
827	P. palustris	63	49	0.5625
770	P. palustris	44	62	0.41509434
810	P. palustris	43	66	0.394495413
482	P. taeda	48	51	0.484848485
526	P. taeda	58	38	0.604166667
733	P. palustris	46	48	0.489361702
805	P. palustris	18	17	0.514285714
771	P. palustris	54	53	0.504672897
538	P. taeda	63	45	0.583333333
760	P. palustris	36	37	0.493150685
546	P. taeda	68	50	0.576271186
777	P. palustris	59	54	0.522123894
503	P. taeda	26	34	0.433333333
573	P. taeda	27	22	0.551020408
728	P. palustris	22	76	0.224489796

494	P. taeda	43	60	0.417475728
823	P. palustris	23	89	0.205357143
513	P. taeda	36	62	0.367346939
501	P. taeda	27	58	0.317647059
744	P. palustris	15	68	0.180722892
807	P. palustris	61	88	0.409395973
596	P. taeda	36	58	0.382978723
489	P. taeda	62	63	0.496
799	P. palustris	48	104	0.315789474
564	P. taeda	20	26	0.434782609
531	P. taeda	44	47	0.483516484
522	P. taeda	70	64	0.52238806
569	P. taeda	21	39	0.35
818	P. palustris	43	77	0.358333333
504	P. taeda	45	60	0.428571429
748	P. palustris	39	76	0.339130435
814	P. palustris	47	87	0.350746269
519	P. taeda	48	64	0.428571429
838	P. palustris	31	67	0.316326531
813	P. palustris	73	103	0.414772727
544	P. taeda	59	60	0.495798319
507	P. taeda	41	72	0.362831858
523	P. taeda	63	62	0.504
585	P. taeda	71	53	0.572580645
575	P. taeda	56	67	0.455284553
765	P. palustris	27	99	0.214285714
581	P. taeda	29	41	0.414285714
517	P. taeda	35	55	0.388888889
561	P. taeda	45	57	0.441176471
735	P. palustris	48	97	0.331034483
495	P. taeda	66	99	0.4
778	P. palustris	32	50	0.390243902
804	P. palustris	41	82	0.333333333
809	P. palustris	35	71	0.330188679
571	P. taeda	51	57	0.472222222
589	P. taeda	44	59	0.427184466
488	P. taeda	33	87	0.275
764	P. palustris	60	86	0.410958904
743	P. palustris	31	86	0.264957265
566	P. taeda	47	56	0.45631068
518	P. taeda	49	58	0.457943925
539	P. taeda	49	62	0.441441441
552	P. taeda	14	35	0.285714286
751	P. palustris	35	46	0.432098765
785	P. palustris	36	80	0.310344828

790	P. palustris	13	68	0.160493827
548	P. taeda	56	52	0.518518519
740	P. palustris	52	90	0.366197183
498	P. taeda	33	71	0.317307692
788	P. palustris	44	59	0.427184466
570	P. taeda	56	60	0.482758621
737	P. palustris	39	62	0.386138614
599	P. taeda	51	50	0.504950495
533	P. taeda	71	58	0.550387597
549	P. taeda	48	34	0.585365854
820	P. palustris	33	79	0.294642857
492	P. taeda	41	79	0.341666667
499	P. taeda	1	3	0.25
763	P. palustris	37	92	0.286821705
598	P. taeda	48	69	0.41025641
816	P. palustris	19	92	0.171171171
830	P. palustris	51	79	0.392307692
722	P. palustris	56	54	0.509090909
826	P. palustris	55	93	0.371621622
508	P. taeda	36	37	0.493150685
806	P. palustris	62	66	0.484375
481	P. taeda	53	53	0.5
774	P. palustris	20	78	0.204081633
547	P. taeda	46	41	0.528735632
483	P. taeda	50	68	0.423728814
839	P. palustris	44	59	0.427184466
833	P. palustris	34	80	0.298245614
560	P. taeda	54	59	0.477876106
536	P. taeda	49	63	0.4375
773	P. palustris	30	97	0.236220472
731	P. palustris	42	81	0.341463415
550	P. taeda	42	37	0.53164557
793	P. palustris	24	60	0.285714286
782	P. palustris	44	45	0.494382022
831	P. palustris	31	89	0.258333333
528	P. taeda	73	58	0.557251908
769	P. palustris	28	96	0.225806452
768	P. palustris	29	114	0.202797203
840	P. palustris	19	59	0.243589744