FIRE AS A DRIVER OF ABOVE AND BELOWGROUND DYNAMICS IN A LONGLEAF PINE ECOSYSTEM

by

MICHELLE HENSON

(Under the Direction of Anny Chung)

ABSTRACT

Fire regimes play a pivotal role in shaping the structure and function of biotic communities in fire-adapted ecosystems. While fire is well recognized as a driver of plant diversity, the effects of fire regimes on belowground microbial diversity, plant-microbe interactions, and post-fire recovery patterns remain comparatively understudied. This dissertation addresses these gaps by examining how two long-term prescribed fire regimes, annual (1-year fire return interval) and maintenance (1-3-year fire return interval), influence plant communities, soil bacterial and fungal assemblages, plant-soil feedbacks, and post-fire successional trajectories in a longleaf pine-wiregrass savanna. We found that frequent annual fires reduced plant richness and diversity, depleted soil phosphorus, and homogenized fungal communities, indicating that highly frequent prescribed burning may undermine resilience in this ecosystem. Plant-soil feedback experiments revealed that recurrent annual fire likely promoted antagonistic soil biota that inhibited the dominant graminoid in our study, which may have potential complications for future restoration efforts for the species. Moreover, plant and soil microbial communities exhibited divergent recovery trajectories, with both compositional and richness patterns strongly shaped by fire legacy. Annual fire regimes delayed fungal recovery, increased bacterial richness, decreased plant richness, and slowed depth-dependent recovery for fungi, whereas maintenance regimes promoted stronger recovery and greater overall biodiversity retention. Together, these findings underscore that fire regimes fundamentally govern community composition, recovery trajectories, and the long-term stability and function of ecosystems. This work highlights the importance of fire management strategies that account for both above and belowground responses, and the cumulative impacts of repeated burns on ecosystem resilience.

INDEX WORDS: Soil microbes, plant diversity, fire regime, plant-microbe interactions, resilience, prescribed fire

FIRE AS A DRIVER OF ABOVE AND BELOWGROUND DYNAMICS IN A LONGLEAF PINE ECOSYSTEM

by

MICHELLE HENSON

B.S., University of North Carolina at Asheville, 2017

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2025

FIRE AS A DRIVER OF ABOVE AND BELOWGROUND DYNAMICS IN A LONGLEAF PINE ECOSYSTEM

by

MICHELLE HENSON

Major Professor:	Anny Chung
Committee:	Rebecca Abney Lisa Giencke Chris Peterson Nina Wurzburger

Electronic Version Approved:

Ron Walcott
Vice Provost for Graduate Education and Dean of the Graduate School
The University of Georgia
December 2025

ACKNOWLEDGEMENTS

I am grateful to my advisor, Anny Chung, for her support, guidance, and mentorship, and for her patience and encouragement at every stage of this process. Anny fostered my growth as a scientist, and helped me develop independence, perseverance, and problem-solving skills that continue to shape how I navigate both my career and life. I also thank Rebecca Abney, Lisa Giencke, Chris Peterson, and Nina Wurzburger for serving on my dissertation committee and for their impactful and thoughtful input. I am especially appreciative of the members of the Chung lab for their advice, encouragement, and assistance with each chapter of this dissertation. I am particularly grateful to Lisa Giencke for her help with field logistics, her guidance in implementing the experimental design, and for giving me a warm and welcoming introduction to the longleaf pine ecosystem. I also thank the members of the Plant Ecology lab at the Jones Center at Ichauway for their assistance in the field and for making research both productive and fun. I am immensely thankful for the friendships I formed in graduate school, which provided support, care, and perspective during this process. Finally, I am deeply grateful for my family, especially my husband, whose unconditional love, steady encouragement, and support kept me smiling, focused, and motivated to finish this work. I am thankful for my parents, who have supported me not only through this dissertation but throughout my life, encouraging me to pursue my dreams. Lastly, I am incredibly grateful for my two kids, whose curiosity, joy, and belief in me as a “doctor of plants” inspired and motivated me throughout this journey.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Recurrent fire is a fundamental driver of community structure and ecosystem dynamics in fire-adapted biomes, including grasslands, savannas, and coniferous forests (Bond & Parr 2010, Keeley & Pausas 2019). In these pyrogenic systems, fire maintains open-canopy conditions by limiting woody encroachment and facilitating the persistence of fire-adapted vegetation (Bradstock, Tozer, & Keith 1997, Glitzenstein, Platt, & Streng 1995, Keeley & Pausas 2019). Among these, longleaf pine (*Pinus palustris)* savannas of the southeastern United States are globally recognized for their extraordinary levels of plant richness and endemism (Peet & Allard 1993, Noss et al. 2015). These ecosystems are maintained by frequent, low-intensity surface fires that reinforce a dynamic equilibrium between vegetation, fuels, and fire behavior (Mitchell et al. 2009, Loudermilk et al. 2022). Attributes of the fire regime, including the frequency, intensity, seasonality, and severity of fire, shape patterns of species composition and ecological function over space and time (Platt et al. 2016, Glitzenstein, Platt, & Streng 1995), influencing both immediate post-fire conditions and long-term successional dynamics. Fire operates as both a pulse disturbance (abrupt, short-term change) and a press disturbance (sustained, long-term impact), with consequences for ecological resilience, stability, and feedbacks between above and belowground components of an ecosystem (Bender, Case, & Gilpin 1984, Pellegrini & Jackson 2020).

While the effects of fire on aboveground vegetation have been extensively documented, the responses of soil microbial communities remain comparatively understudied. Soil microbial communities play essential roles in nutrient cycling, organic matter decomposition, plant productivity, and ecosystem recovery (Sahu et al. 2017, Van Der Heijden et al. 2008, Graham & Knelman 2023). In recently burned systems, these communities must either persist through the disturbance or reassemble afterward, yet their recovery trajectories and functional responses under varying fire regimes remain underexplored (but see Hu et al. 2023, Fontúrbel et al. 2012, Semenova-Nelsen et al. 2019, Hopkins et al. 2020, Garcia-Pausas et al. 2022). Because microbial communities mediate key plant-soil interactions, they reflect a key but often overlooked dimension of fire ecology.

Plant-soil feedbacks (PSFs) represent a mechanistic pathway through which fire regimes can influence vegetation dynamics and ecosystem function (Kardol et al. 2023). Interactions between plants and the biotic and abiotic components of soil are increasingly recognized as key drivers of plant community assembly and mediators of ecosystem responses to environmental change (van der Putten et al. 2016, Collings, Shoemaker, & Diez 2025). Plants shape microbial community composition and activity through litter deposition, root exudation, and nutrient uptake (Bennett & Klironomos 2019, Bardgett & van der Putten 2014). In turn, soil microbes can exert positive, neutral, or negative effects on plant growth, survival, and competitive interactions, ultimately shaping plant community structure and ecosystem processes (Bever, Platt, & Morton 2012, van der Putten et al. 2013). Fire can disrupt these feedbacks by altering microbial composition and function, thereby shifting the strength and direction of PSFs (Warneke et al. 2023, Hopkins et al. 2024, Senior et al. 2018). These shifts have the potential to modify competitive dynamics, plant community structure, and successional trajectories (van der Putten et al. 2016). However, despite growing recognition of their importance, the explicit integration of fire regimes in plant-soil feedback frameworks remain limited.

Understanding how fire regimes and individual fire events shape both plant and microbial communities is especially critical because these interactions have far-reaching consequences for plant communities, ecosystem function, and global environmental changes (Pellegrini & Jackson 2020, Harvey & Enright 2022). Long-term fire regimes act as selective filters, favoring species that can persist or recover rapidly under recurrent disturbance (Krebs et al. 2010, Pausas & Schwilk 2012). Single fire events, by contrast, cause immediate mortality and trigger succession, with outcomes contingent on fire history (Keeley 2009). Changes in fire frequency or seasonality, as increasingly expected under climate change, may alter successional pathways, disrupt plant-microbe mutualisms, and shift the functional composition of both above and belowground communities (Dooley & Treseder 2012, Rudgers et al. 2020, Neary et al. 1999). These changes, in turn, can modify future fire behavior, generating feedback loops that can either stabilize or destabilize the system (Kelly et al. 2020, Keeley & Pausas 2019, Kardol et al. 2023). Despite growing recognition of the belowground responses to fire, empirical studies that integrate plant and microbial dynamics across fire regimes remain scarce.

In this dissertation, I investigate how fire regimes shape plant and soil microbial communities in the North American Coastal Plain through an aboveground and belowground lens. In Chapter 2, I characterize changes in plant and microbial diversity, community composition, and soil chemistry across varying prescribed fire regimes. In Chapter 3, I investigate plant-soil feedbacks as a mechanistic pathway through which fire regimes may influence plant coexistence and productivity. Finally, in Chapter 4, I explore the resilience and trajectory of these communities, disentangling how a single fire event and fire legacy shape community assembly. Together, these chapters integrate above and belowground perspectives to provide a more comprehensive understanding of how fire regimes influence biodiversity and ecosystem function in one of North America's most diverse and imperiled ecosystems. By incorporating soil microbial processes into the study of fire ecology, this work aims to inform management strategies and advance our understanding of ecological resilience in the face of global change.

References

Bardgett, R. D., & Van Der Putten, W. H. (2014). Belowground biodiversity and ecosystem functioning. *Nature*, *515*(7528), 505-511. https://doi.org/10.1038/nature13855

Bender, E. A., Case, T. J., & Gilpin, M. E. (1984). Perturbation experiments in community ecology: theory and practice. *Ecology*, *65*(1), 1-13. https://doi.org/10.2307/1939452

Bennett, J. A., & Klironomos, J. (2019). Mechanisms of plant–soil feedback: interactions among biotic and abiotic drivers. *New Phytologist*, *222*(1), 91-96. https://doi.org/10.1111/nph.15603

Bever, J. D., Platt, T. G., & Morton, E. R. (2012). Microbial population and community dynamics on plant roots and their feedbacks on plant communities. *Annual Review of Microbiology*, *66*(1), 265-283. https://doi.org/10.1146/annurev-micro-092611-150107

Bond, W. J., & Parr, C. L. (2010). Beyond the forest edge: ecology, diversity and conservation of the grassy biomes. *Biological Conservation*, *143*(10), 2395-2404. https://doi.org/10.1016/j.biocon.2009.12.012

Bradstock, R. A., Tozer, M. G., & Keith, D. A. (1997). Effects of high frequency fire on floristic composition and abundance in a fire-prone heathland near Sydney. *Australian Journal of Botany*, 45(4), 641-655. https://doi.org/10.1071/BT96083

Collings, J. A., Shoemaker, L. G., & Diez, J. M. (2025). Environmental context alters plant–soil feedback effects on plant coexistence. *Ecology*, *106*(8), e70170. https://doi.org/10.1002/ecy.70170

Dooley, S. R., & Treseder, K. K. (2012). The effect of fire on microbial biomass: a meta-analysis of field studies. *Biogeochemistry*, 109(1), 49-61. https://doi.org/10.1007/s10533-011-9633-8

Fontúrbel, M. T., Barreiro, A., Vega, J. A., Martín, A., Jiménez, E., Carballas, T., ... & Díaz-Raviña, M. (2012). Effects of an experimental fire and post-fire stabilization treatments on soil microbial communities. *Geoderma*, 191, 51-60. https://doi.org/10.1016/j.geoderma.2012.01.037

Garcia-Pausas, J., Romanyà, J., & Casals, P. (2022). Post-fire recovery of soil microbial functions is promoted by plant growth. *European Journal of Soil Science*, 73(4), e13290. https://doi.org/10.1111/ejss.13290

Glitzenstein, J. S., Platt, W. J., & Streng, D. R. (1995). Effects of fire regime and habitat on tree dynamics in north Florida longleaf pine savannas. *Ecological Monographs*, *65*(4), 441-476. https://doi.org/10.2307/2963498

Graham, E. B., & Knelman, J. E. (2023). Implications of soil microbial community assembly for ecosystem restoration: patterns, process, and potential. *Microbial Ecology*, *85*(3), 809-819. https://doi.org/10.1007/s00248-022-02155-w

Harvey, B. J., & Enright, N. J. (2022). Climate change and altered fire regimes: impacts on plant populations, species, and ecosystems in both hemispheres. *Plant Ecology*, *223*(7), 699-709. https://doi.org/10.1007/s11258-022-01248-3

Hopkins, J. R., Huffman, J. M., Platt, W. J., & Sikes, B. A. (2020). Frequent fire slows microbial decomposition of newly deposited fine fuels in a pyrophilic ecosystem. *Oecologia*, *193*(3), 631-643. https://doi.org/10.1007/s00442-020-04699-5

Hu, M., Wang, J., Lu, L., Gui, H., & Wan, S. (2023). Global recovery patterns of soil microbes after fire. *Soil Biology and Biochemistry*, 183, 109057. https://doi.org/10.1016/j.soilbio.2023.109057

Kardol, P., Yang, T., Arroyo, D. N., & Teste, F. P. (2023). Plant-soil feedback in the 'real world': how does fire fit into all of this? *Plant and Soil*, 485(1), 91-102. https://doi.org/10.1007/s11104-022-05778-7

Keeley, J. E. (2009). Fire intensity, fire severity and burn severity: a brief review and suggested usage. *International Journal of Wildland Fire*, 18(1), 116-126. https://doi.org/10.1071/WF07049

Keeley, J. E., & Pausas, J. G. (2019). Distinguishing disturbance from perturbations in fire-prone ecosystems. *International Journal of Wildland Fire*, *28*(4), 282-287. https://doi.org/10.1071/WF18203

Kelly, L. T., Giljohann, K. M., Duane, A., Aquilué, N., Archibald, S., Batllori, E., ... & Brotons, L. (2020). Fire and biodiversity in the Anthropocene. *Science*, 370(6519), eabb0355. https://doi.org/10.1126/science.abb0355

Krebs, P., Pezzatti, G. B., Mazzoleni, S., Talbot, L. M., & Conedera, M. (2010). Fire regime: history and definition of a key concept in disturbance ecology. *Theory in Biosciences*, *129*(1), 53-69. https://doi.org/10.1007/s12064-010-0082-z

Loudermilk, E. L., O’Brien, J. J., Goodrick, S. L., Linn, R. R., Skowronski, N. S., & Hiers, J. K. (2022). Vegetation’s influence on fire behavior goes beyond just being fuel. *Fire Ecology*, *18*(1), 9. https://doi.org/10.1186/s42408-022-00132-9

Neary, D. G., Klopatek, C. C., DeBano, L. F., & Ffolliott, P. F. (1999). Fire effects on belowground sustainability: a review and synthesis. *Forest Ecology and Management*, *122*(1-2), 51-71. https://doi.org/10.1016/S0378-1127(99)00032-8

Pausas, J. G., & Schwilk, D. (2012). Fire and plant evolution. *New Phytologist*, *193*(2), 301-303. https://www.jstor.org/stable/newphytologist.193.2.301

Pellegrini, A. F., & Jackson, R. B. (2020). The long and short of it: a review of the timescales of how fire affects soils using the pulse-press framework. *Advances in Ecological Research*, *62*, 147-171. https://doi.org/10.1016/bs.aecr.2020.01.010

Mitchell, R. J., Hiers, J. K., O'Brien, J., & Starr, G. (2009). Ecological forestry in the Southeast: understanding the ecology of fuels. *Journal of Forestry*, 107(8), 391-397. https://doi.org/10.1093/jof/107.8.391

Noss, R. F., Platt, W. J., Sorrie, B. A., Weakley, A. S., Means, D. B., Costanza, J., & Peet, R. K. (2015). How global biodiversity hotspots may go unrecognized: lessons from the North American Coastal Plain. *Diversity and Distributions*, *21*(2), 236-244. https://doi.org/10.1111/ddi.12278

Peet, R. K., & Allard, D. J. (1993, May). Longleaf pine vegetation of the southern Atlantic and eastern Gulf Coast regions: a preliminary classification. In *Proceedings of the Tall Timbers fire ecology conference* (Vol. 18, pp. 45-81).

Pellegrini, A. F., & Jackson, R. B. (2020). The long and short of it: a review of the timescales of how fire affects soils using the pulse-press framework. *Advances in ecological research*, *62*, 147-171. https://doi.org/10.1016/bs.aecr.2020.01.010

Platt, W. J., Ellair, D. P., Huffman, J. M., Potts, S. E., & Beckage, B. (2016). Pyrogenic fuels produced by savanna trees can engineer humid savannas. *Ecological Monographs*, 86(3), 352-372. https://doi.org/10.1002/ecm.1224

Rudgers, J. A., Afkhami, M. E., Bell-Dereske, L., Chung, Y. A., Crawford, K. M., Kivlin, S. N., ... & Nuñez, M. A. (2020). Climate disruption of plant-microbe interactions. *Annual Review of Ecology, Evolution, and Systematics*, *51*(1), 561-586. https://doi.org/10.1146/annurev-ecolsys-011720-090819

Sahu, N., Vasu, D., Sahu, A., Lal, N., & Singh, S. K. (2017). Strength of microbes in nutrient cycling: a key to soil health. In *Agriculturally important microbes for sustainable agriculture: Volume I: Plant-soil-microbe nexus* (pp. 69-86). Singapore: Springer Singapore. https://doi.org/10.1007/978-981-10-5589-8_4

Semenova-Nelsen, T. A., Platt, W. J., Patterson, T. R., Huffman, J., & Sikes, B. A. (2019). Frequent fire reorganizes fungal communities and slows decomposition across a heterogeneous pine savanna landscape. *New Phytologist*, *224*(2), 916-927. https://doi.org/10.1111/nph.16096

Senior, J. K., O'Reilly-Wapstra, J. M., Schweitzer, J. A., Bailey, J. K., & Potts, B. M. (2018). Forest fire may disrupt plant–microbial feedbacks. *Plant Ecology*, 219(5), 497-504. https://doi.org/10.1007/s11258-018-0811-9

Van Der Heijden, M. G., Bardgett, R. D., & Van Straalen, N. M. (2008). The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology letters*, *11*(3), 296-310. https://doi.org/10.1111/j.1461-0248.2007.01139.x

van der Putten, W. H., Bardgett, R. D., Bever, J. D., Bezemer, T. M., Casper, B. B., Fukami, T., ... & Wardle, D. A. (2013). Plant–soil feedbacks: the past, the present and future challenges. *Journal of Ecology*, 101(2), 265-276. https://doi.org/10.1111/1365-2745.12054

Warneke, C. R., Yelenik, S. G., & Brudvig, L. A. (2023). Fire modifies plant–soil feedbacks. *Ecology*, 104(5), e3994. https://doi.org/10.1002/ecy.3994

van der Putten, W. H., Bradford, M. A., Pernilla Brinkman, E., van de Voorde, T. F., & Veen, G. F. (2016). Where, when and how plant–soil feedback matters in a changing world. *Functional Ecology*, *30*(7), 1109-1121. https://doi.org/10.1111/1365-2435.12657

CHAPTER 2

CAN A PYROGENIC ECOSYSTEM EXPERIENCE TOO MUCH FIRE? EXPERIMENTAL MANIPULATION OF A LONG-TERM FIRE REGIME DECREASES PLANT DIVERSITY AND HOMOGENIZES SOIL FUNGAL COMMUNITIES[1]

---

[1] Henson, M.S., Giencke, L.M., Chung, Y.A. To be submitted to *Applied Soil Ecology*.

Abstract

Longleaf pine savannas are among the most biologically diverse ecosystems in temperate North America, with frequent, low-intensity surface fires playing a critical role in maintaining groundcover diversity. While the effects of prescribed fire on aboveground plant communities are well studied, the responses of soil microbial communities to different fire regimes remain poorly understood. In this study, we examined bacterial, fungal, and plant community responses to two experimentally prescribed fire regime treatments after 22 years, annual (1-year fire return interval) and maintenance (1–3-year fire return interval), across three sites in the Coastal Plain of southwestern Georgia. We found that annual fire reduced plant richness and diversity, while bacterial and fungal richness remained relatively resilient and taxon-rich, showing no differences between fire regime treatments. Soil microbial composition responded strongly and shifted in response to fire regime. Fungal communities became more homogenized under annual fire, dominated by saprotrophic and ectomycorrhizal taxa, while maintenance fire communities were associated with more functionally diverse assemblages, such as parasitic, pathogenic, and endophytic taxa. Bacterial communities exhibited greater resilience and compositional stability, which may reflect faster recovery dynamics and broader functional redundancy relative to fungi. Annual fire also led to reduced soil phosphorus, while other soil properties were largely unaffected. Together, these results suggest that frequent fire regimes reshape above and belowground communities in ways that impact long-term resilience and recovery. Resilience in fire-adapted ecosystems depends not just on fire presence, but on the regime itself, reinforcing the importance of fire management strategies that support both biodiversity and functional recovery of aboveground and belowground communities.

Introduction

Fire is a fundamental ecological and evolutionary force that shapes biodiversity in terrestrial ecosystems worldwide (Pausas & Keeley 2009, He et al. 2019). In the southeastern United States, longleaf pine (*Pinus palustris* Mill.) savannas depend on frequent, low-intensity surface fires to maintain their open structure and biologically rich understory (Mitchell et al. 2009, Kirkman et al. 2004). Flammable grasses within the pine-grassland matrix facilitate the spread of recurrent fires, supporting exceptionally high levels of species richness and endemism (Noss et al. 2015; Peet & Allard 1993), making fire a foundational component of longleaf pine ecosystems. Frequent fires promote spatial heterogeneity in fuel loads, substrates, and plant species distribution. This heterogeneity influences fuel accumulation and feeds back into future fire regimes, characterized by fire frequency, intensity, seasonality, and severity (Platt et al. 2016, McLauchlan et al. 2020). Together, these processes highlight the interconnectedness of fire-fuel dynamics in these ecosystems. Given the significant role of fire regimes in shaping plant composition and structure, much of the existing literature has focused on aboveground vegetation (i.e. fuels), while belowground microbial communities, such as bacteria and fungi, have received far less attention.

Soil microbes play a critical role in terrestrial ecosystem function by regulating nutrient cycling, decomposing organic matter, and by driving plant community dynamics (Aislabie et al. 2013, Reynolds et al. 2003, Van Der Heijden et al. 2008). Fire directly alters soil microbial assemblages through heat-induced mortality and indirectly through shifts in soil physical, chemical, or biological properties (Hart et al. 2005, Mataix-Solera et al. 2009). The strength and persistence of these effects largely depend on characteristics of the fire regime (Hu et al. 2023, Bowd et al. 2022). In general, low-intensity fires restructure soil microbial communities (Fox et al. 2024, Dao et al. 2022, Hopkins et al. 2021, Semenova-Nelsen et al. 2019, Oliver et al. 2015), and selectively favor taxa that can withstand heat (Glassman et al. 2016, Owen et al. 2019) or survive in the post-fire environment through stress tolerance, dispersal, nutrient mineralization, or the ability to degrade aromatic hydrocarbon (Fischer et al. 2021, Nelson et al. 2022). Sensitivity to fire varies among microbial guilds, which can lead to substantial impacts on ecosystem functioning (Dooley & Treseder 2012, Holden and Treseder 2013). For example, Semenova-Nelsen et al. (2019) observed a decline in the abundance of fungal saprotrophs in burned plots compared to unburned plots within a pine savanna, resulting in slower fuel decomposition rates in litter. This, in turn, could lead to increased fine fuel accumulation, potentially influencing future fire dynamics in fire-prone ecosystems. Over longer timescales, fire-driven shifts in plant hosts, substrates, and soil nutrient availability may have the greatest indirect effect on soil microbial communities (Hart et al. 2005). Yet, the impacts of fire regimes on belowground communities remain largely underexplored due to the difficulty of experimentally manipulating fire at relevant spatial and temporal scales.

Fire regime, particularly frequency and seasonality of fire, likely influences bacterial and fungal communities and their interactions with plant community dynamics. Plants associate with and drive the assembly of diverse and highly structured soil microbial communities, which in turn influence patterns of plant productivity, diversity, and succession over time (Kardol et al. 2007, Bauer et al. 2015, van der Putten et al. 2013, Schmid et al. 2021). This creates reciprocal feedbacks that drive ecosystem resilience by altering resistance and recovery to environmental change (Rudgers et al. 2025). While previous studies have demonstrated that environmental change such as drought, warming, and altered precipitation patterns can directly or indirectly impact soil microbial communities via shifts in plant communities (Louw et al. 2022, Sayer et al.

2017, Classen et al. 2015), the combined effects of fire regimes on both plants and soil microbes remain less explored, particularly in longleaf pine ecosystems.

Recurrent fires at short intervals select for fast-growing plant species capable of rapid post-fire recovery, while reducing the persistence and recruitment of slower-growing species that require more time to establish and reproduce (Abrahamson et al. 2021, Bond & Keely 2005, He et al. 2019, Miller et al. 2019). In longleaf pine ecosystems, the Most Frequent Fire Hypothesis posits that maintaining the highest fire frequency historically supported by available fuels maximizes plant species richness and maintains an open, herbaceous-dominated ground layer community (Glitzenstein et al. 2012). Frequent fires characteristic of these systems promote the dominance of graminoids and forbs while reducing the density, cover, and biomass of woody species due to mortality from fire-related stress, disruption of seedling establishment, and insufficient time for seed bank replenishment (Brewer 2023, Palmquist et al. 2015). Similarly, high-frequency fires favor microbial taxa adapted to repeated thermal and nutrient stress, reinforcing feedbacks that maintain nutrient cycling and microbial community function under frequent fire (Nelson et al. 2022, Hopkins et al. 2025c). However, if fire intervals become too short, the frequency may exceed the tolerance thresholds of some species, potentially reducing diversity and homogenizing communities rather than promoting heterogeneity (Grupenhoff & Safford 2024, Hopkins et al. 2025c). Additionally, many traits that confer fire persistence are linked to plant phenology, such that shifts beyond these bounds may have impacts on substrate inputs and microbial resource dynamics, with consequences for vegetation recovery and long-term species persistence when fire occurs outside of the historical fire season (Tangney et al. 2022).

In addition to altering biotic communities, fire also influences soil chemical properties by modifying nutrient availability, which can either promote or inhibit plant recolonization and microbial community recovery. Depending on fire frequency and intensity, soil nutrient availability may be depleted through organic matter combustion or temporarily enhanced via ash deposition, which can raise pH and nutrient concentrations (Moghli et al. 2021, Agbeshie et al. 2022, Garcia-Pausas et al. 2022). Despite these clear links among fire, vegetation, and soil processes, studies rarely assess soil chemistry alongside concurrent plant and microbial responses, limiting our understanding of ecosystem responses to shifting fire regimes.

To address these gaps, we leveraged a unique long-term field experiment in a longleaf pine-wiregrass savanna to evaluate how variation in prescribed fire regimes (annual [1-year fire return interval] vs. maintenance [1-3-year fire return interval]) shape soil chemistry, bacterial and fungal communities, and plant community composition. We hypothesized that increased fire frequency would negatively affect both above and belowground biodiversity and alter soil chemical properties. Specifically, we predicted that, relative to maintenance fires, 1) annual fires would negatively affect plant and soil microbial richness and diversity, 2) annual fires would promote an enrichment of fire-adapted microbial taxa capable of tolerating or thriving under repeated thermal and nutrient stress, and 3) annual fires would increase pH and reduce nutrient availability due to combustion losses and limited litter accumulation.

Materials and Methods

Study site and long-term fire regime experiment

We conducted our study at The Jones Center at Ichauway (31.2201° N; 84.4779° W), an 11,500-hectare private ecological reserve in Newton, Georgia, United States, located in the lower

Gulf Coastal Plain, USA. Since the 1930s, The Jones Center property has been managed with frequent fire following the Stoddard-Neel forest management approach, an uneven-aged silvicultural system that integrates selective harvesting with frequent prescribed burning to sustain longleaf pine ecosystems (McIntyre et al. 2008). We utilized a long-term field experiment replicated across three sites at The Jones Center (Figure 2.1): Dan Lilly (DL), 21-Acre (TOA), and Fourth of July (FOJ). All sites occur on upland *Pinus palustris* (Mill.) savanna with an open canopy of widely spaced pines, a sparse shrub layer, and a dense, species-rich groundcover of grasses and forbs. Prescribed fire has been implemented on a 1–3-year interval with varying seasonality since 1990 ("maintenance" regime). In 2003, experimental yearly summer fires ("annual" regime) were added to support wiregrass (*Aristida beyrichiana*) seed production for restoration efforts and later expanded to assess effects on groundcover species richness, composition, and woody stem abundance. In 2012, ten permanent vegetation sampling plots ($1m^2$) were established at each site, where aerial percent cover of individual plant species cover has been annually surveyed. Within each plot, groundcover species were identified using Weakley's (2020) nomenclature. In this study, we used plant cover data surveyed in October 2020 (17 years after the beginning of experimental fire treatments).

Soil sampling

In June 2021, we collected soil samples from each $1m^2$ permanent vegetation plot across all sites and fire treatments described above (n = 60). In each plot, we collected a 2.5 cm diam. by 10 cm depth core from each corner and homogenized the four cores from each plot to form a single sample for each plot (150 g). Samples in the field were kept on ice for transportation and stored at -20°C until further processing.

Soil chemistry, water content, and microbial characterization

From each sample, we processed soil by sieving through a 2 mm stainless steel mesh sieve to remove large organic debris prior to analysis. A 60 g subsample was sent to Midwest Labs Inc. (Omaha, Nebraska, USA) for standard soil chemical analysis. Organic matter (OM) was determined by loss on ignition, which estimates the proportion of soil mass derived from decomposed plant and microbial residues. Available phosphorus (P1) was measured using the Bray-1 extraction method, representing the pool of phosphorus readily available for plant uptake, whereas total phosphorus (P2) quantifies all forms of soil phosphorus. Exchangeable potassium (K), magnesium (Mg), and calcium (Ca) were extracted using ammonium acetate, reflecting the macronutrient cations available to plants. Soil pH was measured in a 1:1 soil-to-water slurry, providing an index of soil acidity. Cation exchange capacity (CEC) was determined via ammonium acetate saturation, which quantifies the soil's ability to retain and exchange nutrient cations. Nitrate (NO3) was measured colorimetrically following extraction with potassium chloride, representing the primary form of inorganic nitrogen available to plants. Finally, gravimetric water content (GWC) was measured by weighing 10 g of fresh soil, oven-drying at 105°C for 48 h, and reweighing to calculate the proportion of water lost.

Microbial genomic DNA was extracted from 250 mg of each soil sample using a DNEasy PowerSoil Pro Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol and stored at -20°C until PCR amplification. Extracted DNA was quantified using a Nanodrop (ThermoFisher Scientific, Waltham, Massachusetts, USA). The bacterial V4 hyper-variable region of the ribosomal RNA gene (16S) and the fungal Internal Transcribed Spacer (ITS2) were selected due to their high variability and suitability for short paired-end Illumina MiSeq sequencing. Amplicons were generated using 515F and 806R primers (Caporaso et al. 2012) for the bacterial 16S region, and f/gITS7 (Ihrmark et al. 2012) and ITS4 (White et al. 1990) for the fungal ITS2 region. For Illumina sequencing, we added the following overhang adapter sequences to each primer set: 5’-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3’ and 5’-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3’.

Bacterial PCRs were performed in 25 µL volumes: 6.25ul nfH2O, 5ul 5X Q5 Reaction Buffer, 1ul dNTPs (10 µM), 1.25ul 515F primer (10 µM), 1.25ul 806 primer (10 µM), 0.25ul Q5 High Fidelity DNA Polymerase, 5ul Enhancer, and 5 µL of DNA. PCR protocol consisted of an initial denaturing at 98°C for 30s; followed by 10 touchdown cycles consisting of denaturation at 98°C for 20 seconds, annealing at 65°C for 15 seconds (decreasing by 1°C per cycle) and extension at 72°C for 1 minute. Following the touchdown cycles, 25 cycles of denaturation at 98°C for 15 seconds, annealing at 56°C for 15 seconds, extension at 72°C for 1 minute, and a final extension at 72°C for 10 minutes.

Fungal PCRs were performed in 25 µL volumes: 5 µL nfH2O, 1.25 µL f/gITS7 primer (10 µM), ITS4 primer (10 µM), 12.5 µL Phusion Green Hot Start II DNA polymerase, and 5 µL of DNA. The PCR thermocycler conditions were as follows: initial denaturation at 98°C for 30 seconds; followed by 30 cycles of denaturation at 98°C for 10 seconds, annealing at 60°C for 15 seconds, and extension at 72°C for 30 seconds; with a final extension at 72°C for 5 minutes.
All PCR products were visualized on a 1.5% agarose gel to ensure successful amplification of DNA fragments. Negative controls were used throughout the PCR procedures. PCR products were submitted for library preparation and sequencing using Illumina MiSeq PE 300 at the Georgia Genomics and Bioinformatics Core (Athens, Georgia, USA) (RRID: SCR_010994).

Bioinformatics

Sequence data were demultiplexed, downloaded as FASTQ files, and imported into QIIME 2-2022.11. The control of sequence quality and filtering chimeras was achieved using the QIIME2 DADA2 plugin (Callahan et al. 2016). Sequence primers and adapters were trimmed and truncated to the shortest sequence length. The bacterial dataset produced 4,203,225 total sequence reads. After quality control and chimera filtering, a total of 2,637,454 reads were included in the analyses. The fungal dataset produced 4,638,589 total sequence reads. After quality control, the final sequence count was 3,025,568 reads. Taxonomic classification was performed using the QIIME2 feature-classifier plugin using a Naïve Bayes classifier trained on the SILVA 138 database (version 138) for bacteria (Akerele et al. 2022), and the UNITE (version 8) reference database for fungi. Sequences were clustered into species-level amplicon sequence variants (ASVs). We defined bacterial and fungal community composition using ASVs to provide a more refined, higher taxonomic resolution, allowing us to detect the fine-scale shifts in soil microbial communities that may be influenced by fire regimes.

Statistical Analyses

All analyses were conducted in R software version 4.0.2 (R Core Team 2020). We used analysis of variance (ANOVA) to determine if differences in soil chemistry and water content existed between fire regime, sites, and their interaction. Each soil response variable was analyzed using a linear model with fire treatment, site, and their interaction as predictors. ANOVA was followed by *post-hoc* comparisons of group means using estimated marginal means with a Tukey test in the case of significant differences ($p < 0.05$) (emmeans package in R) (Lenth 2024). We also used ANOVAs to test for differences in plant, bacterial, and fungal richness, diversity, and evenness among the fire treatments and sites. We explored time since fire as a categorical variable in the microbial diversity models to ascertain that the effects of fire regimes were not confounded by time since fire at each site (Appendix A: Table A1). To analyze compositional responses, we used the R package vegan (Oksanen et al. 2022) and derived Bray-Curtis distance matrices using vegdist() function. We then used a PERMANOVA model including fire regime, field site, and their interaction with the adonis() function for plants, bacteria, and fungi, and visualized these results using NMDS ordinations. Lastly, we used indicator species analysis to identify microbial indicators of each fire regime using the indicspecies package (De Carceres & Jansen 2016) (Appendix A: Table A2, A3). We also calculated the dispersion of plant and microbial communities (convergence or divergence) to test the homogeneity of multivariate dispersions using the 'betadisper' function in vegan.

Fungal Guilds

We used FungalTraits (Põlme et al. 2020) to assign ASVs to ecological guilds in each fire treatment, then conducted ANOVAs and Tukey's HSD *post-hoc* tests to better understand how specific ecological guilds may differ among the fire treatments. We aggregated fungal taxa into broader functional guilds, specifically saprotrophs (soil, litter, dung, wood, pollen), parasites (myco, animal, algal), and endophytes (root, foliar) to reduce the complexity of the dataset. The four most abundant guilds were saprotrophs, ectomycorrhizal fungi, pathogens, and endophytes. We conducted a Pearson's chi-square test of independence to determine whether fungal guild composition differed between fire treatments.

Results

Plant diversity responds strongly to fire regime, while microbial diversity remains stable

Plant diversity showed stronger responses to the long-term fire regime treatment, whereas microbial diversity showed no detectible change (Figure 2.2). Annual fire significantly decreased plant community richness, evenness, and diversity. Annual fire decreased plant species richness by 14-27% across sites ($\chi^2 = 30.33$, df = 1, $p < 0.0001$), which inherently varied in richness ($\chi^2 = 18.34$, df = 2, $p = 0.0001$) (Figure 2.2A-C). Annual fire also decreased Pielou's evenness in the plant community ($\chi^2 = 5.68$, df = 1, $p = 0.017$), and the effect depended on site (interaction $\chi^2 = 7.25$, df = 2, $p = 0.026$). Altogether, we found that annual fire significantly decreased Shannon diversity ($\chi^2= 27.91$, df = 1, $p < 0.0001$), which also varied among sites ($\chi^2 = 18.38$, df = 2, $p = 0.0001$), but not in their interaction ($\chi^2 = 1.93$, df = 2, $p = 0.380$).

Fire regime treatments did not significantly affect fungal richness ($F_{1,54} = 0.162$, $p = 0.688$), evenness ($F_{1,54} = 3.18$, $p = 0.080$), or diversity ($F_{1,54} = 1.65$, $p = 0.203$). Additionally, there was no evidence of fire × site interactions for richness ($F_{2,54} = 2.70$, $p = 0.076$), diversity ($F_{2,54} = 1.26$, $p = 0.291$) or evenness ($F_{2,54} = 0.288$, $p = 0.750$) (Figure 2.2D-F). Similarly, fire treatments had no significant effect on bacterial richness ($F_{1,54} = 0.0024$, $p = 0.969$), evenness ($F_{1,54} = 0.9594$, $p = 0.3317$), or Shannon diversity ($F_{1,54}=0.3535$, $p = 0.5546$). Additionally, we observed no evidence for fire regime × site interactions for richness ($F_{2,54} = 2.196$, $p = 0.1211$) and Shannon's diversity ($F_{2,54} = 1.664$, $p = 0.1989$) (Figure 2.2G-I). However, site had a significant effect on evenness ($F_{2,54} = 8.00$; $p = 0.0009$). *Post-hoc* comparisons showed significant differences between DL and FOJ (df = 54, $p = 0.043$), and between DL and TOA sites (df = 54, $p = 0.0006$). Field site DL exhibited the highest evenness, followed by FOJ, with TOA having the lowest bacterial evenness.

Fire regime shapes plant, fungal, and bacterial community composition

Across bacterial, fungal, and plant communities, fire regime significantly influenced community composition, with site-specific differences and fire × site interactions. In plant communities, fire regime showed the greatest impact on composition ($F_{1,54} = 6.974$, $p = 0.001$, $R^2 = 0.092$), followed by site (Figure 2.3A) ($F_{2,54} = 5.034$, $p = 0.001$, $R^2 = 0.133$) and fire regime × site interaction ($F_{2,54} = 2.240$, $p = 0.001$, $R^2 = 0.059$). Lastly, there was no significant difference in multivariate dispersion between fire regimes ($F_{1,58} = 1.73$, $p = 0.193$).

For fungal communities, fire regime was a strong structuring force ($F_{1,54} = 3.72$, $p < 0.0001$, $R^2 = 0.057$), while site and fire regime × site interactions contributed similar levels of variation (Figure 2.3B) (site: $F_{2,54} = 1.93$, $p < 0.0001$, $R^2 = 0.059$; interaction: $F_{2,54} = 1.49$, $p < 0.0001$, $R^2 = 0.046$). Fungal communities showed a significant difference in multivariate dispersion between fire regimes ($F_{1,58} = 11.04$, $p = 0.0015$). The average distance to centroid was lower in the annual fire treatment (mean = 0.56) compared to the maintenance treatment (mean = 0.60), indicating that fungal communities were more compositionally homogenous under annual fire. In contrast, bacterial community composition was moderately influenced by fire regime (PERMANOVA: $F_{1,54} = 2.71$, $p = 0.0006$, $R^2 = 0.040$), while site contributed more strongly to variation in composition ($F_{2,54} = 3.89$, $p = 0.0001$, $R^2 = 0.115$), with a significant fire × site interaction effect ($F_{2,54} = 1.50$, $p = 0.021$, $R^2 = 0.044$) (Figure 2.3C). There was no significant difference in multivariate dispersion between the two fire regimes for bacteria ($F_{1,58} = 0.11$, $p = 0.747$), indicating that variability in bacterial communities was similar across fire treatments. These findings highlight that, while all three communities responded significantly to fire regime and site, the magnitude of fire regime effects varied, with the strongest effect observed in plant communities.

Mantel test results indicated that both bacterial and fungal community compositions were significantly correlated with plant community composition. Specifically, there were weak but significant positive correlations between bacterial and plant compositions (r = 0.28, p = 0.001) and between fungal and plant compositions (r = 0.26, p = 0.001). This suggests that while the magnitude of fire regime effects on different communities varied, they are likely still responding in tandem.

Indicator taxa reveal distinct bacterial and fungal responses to fire regimes

To understand which taxa may drive community shifts between fire treatments, we conducted an indicator species analysis (ISA) for both bacteria and fungi. The top ten indicator taxa were identified and grouped based on the indicator values score and p-values across the treatments (Appendix A: Table A1). Additionally, we visualized the total abundance of ISA phyla sequences reads for each fire regime (Figure 2.4A, B), and the relative abundance of the ten most abundant indicator genera for each microbial group across fire regimes (Appendix A: Figure A1).

Fungal indicator analysis revealed 82 indicator ASVs of annual fire and 125 indicator ASVs of maintenance fire. The top three indicator taxa of the annual fire regime were in the phylum Ascomycota, consisting of an unidentified taxon in the order Pleosporales, *Paraphaeosphaeria verruculosa* (Didymosphaeriaceae), and *Periconia lateralis*. Members of the family Didymosphaeriaceae are known to be generally saprophytic, while taxa in the genus *Periconia* have been previously identified as fire-responders in a study performed in a longleaf pine ecosystem (Semenova-Nelsen et al. 2019). Indicator taxa present in maintenance plots were also in the phyla Ascomycota and class Dothideomycetes but consisted of an unidentified taxon in the family Didymellaceae, followed by *Devriesia acadiensis* (Teratosphaeriaceae) and *Sarea resinae*. Members of the family Teratosphaeriaceae are known to contain several plant pathogens and can survive in harsh environments. Compared to indicators for the annual fire regime, more of the maintenance fire regime fungal indicators were in Ascomycota, and less in Basidiomycota and Mucoromycota (Figure 2.4A). *Russula* and *Bifiguratus* were also enriched in annual plots, while *Talaromyces*, *Saitozyma*, *Coniochaeta*, *Paraphaeosphaeria*, and *Cenococcum* were among the dominant indicator taxa in maintenance plots (Appendix A: Figure A1).

For bacteria, we identified 242 indicator ASVs for the annual fire regime and 281 indicator ASVs for the maintenance fire regime. Top bacterial indicators of annual fire plots were in the phylum Firmicutes consisting of bacteria in the genus *Ammoniphilus*, *Bacillus deserti*, followed by a taxon in the genus *Streptomyces* (Appendix A: Table A1). *Firmicutes* contain some of the most thermotolerant bacteria, including members of the genus *Bacillus*, which can produce heat resistant endospores that allow them to survive at soil temperatures up to 100°C (Certini et al. 2021, Galperin 2015). *Bacillus* and *Ammoniphilus* were also the most enriched bacterial genera in annual fire plots based on relative abundance (Appendix A: Figure A2). Maintenance plots, in contrast, were associated with bacterial indicators in the genus *Burkholderia* (Proteobacteria), a member of the family Gemmataceae, and the genus *Gemmata*.

Fire regimes drive shifts in fungal functional guilds

To understand how fire regimes altered the functional composition of fungi, we assigned fungal ASVs to ecological guilds based on their functional roles and ecological niches. Functional guilds were assigned to 34.98% of the identified fungal genera. We found that fire regime significantly altered the proportions of fungal functional guilds ($\chi^2 = 59{,}452$, df = 20, p <

0.001). Parasitic, endophytic, and pathogenic fungi were more relatively abundant under the maintenance fire regime, whereas saprotrophic and ectomycorrhizal fungi were more prevalent in the annual fire regime (Figure 2.5).

Annual fire reduces soil phosphorus while other soil properties vary by site

We found that fire regime and field site significantly affected soil phosphorus, but not their interaction. *Post-hoc* comparisons showed that annual fire decreased both available (P1) ($t = -5.876$, $df = 54$, $p < 0.0001$) and total phosphorus (P2) ($t = -5.676$, $df = 54$, $p < 0.0001$) compared to the maintenance fire regime (Figure 2.6A, B). We did not find significant changes in other abiotic soil characteristics due to fire regime treatments or fire regime × site interactions. However, we found differences in soil moisture (GWC), organic matter (OM), magnesium (Mg), calcium (Ca), pH, and cation exchange capacity (CEC) between TOA-FOJ and DL-FOJ site comparisons ($p < 0.05$) (Table 2.1).

Discussion

Divergent aboveground and belowground diversity responses to fire

Fire regimes had markedly different effects on plants than on microbial communities. While soil microbial diversity and richness remained stable across fire regimes, plant diversity and richness were significantly reduced under the annual fire treatment. These patterns challenge the Most Frequent Fire Hypothesis, which suggests that the highest fire frequency that fuels can tolerate (i.e., annual fire), maximize plant species richness in pyrogenic ecosystems (Glitzenstein et al, 2003, Brewer 2023, Brockway & Lewis 1997). Instead, our findings align more closely with the Intermediate Disturbance Hypothesis (Connell 1978), which posits that diversity peaks under moderate disturbance frequencies and declines when disturbance becomes too rare or too frequent. The reduction in plant diversity under annual fire likely reflects strong environmental filtering, leading to a community dominated by a narrower suite of fire-resilient taxa and eliminated the recruitment and establishment of slower-growing or fire-intolerant species. This suggests trait-based filtering, where annual fire selects for stress-tolerant or disturbance-resilient species, while the maintenance fire regime may allow a mix of ruderal and competitive strategies to coexist (Grime 1977). In contrast, microbial communities, particularly bacteria, appear more resistant to frequent fire due to their rapid turnover, high dispersal capacity, and metabolic plasticity (Weissman et al. 2021, Hopkins et al. 2025b). These contrasting responses reflect fundamental differences in plant and microbial life history strategies and the spatial and temporal scales at which these communities experience disturbance (Bernhardt et al. 2022, Treseder 2023).

Fire regime is a homogenizing force in soil fungal communities

While microbial diversity remained stable across fire regimes, our findings show that fungal community composition became more homogenized under annual fire. Fungal communities exhibited significantly lower multivariate dispersion in annual fire plots, indicating that fungal communities in this fire treatment were more compositionally similar to one another (i.e. lower β diversity) than in the maintenance treatment. This pattern may reflect a reduction in environmental heterogeneity or strong filtering effects associated with the high fire frequency, where repeated fires may restrict niche availability or dispersal opportunities, selecting for fungi that tolerate or thrive in frequently disturbed soils (Nelson et al. 2022, Vellend et al. 2007). In fire-prone systems, such homogenization may constrain ecosystem function by reducing ecological redundancy, thereby limiting the capacity of microbial communities to maintain ecosystem processes under changing conditions. For example, compositional homogenization may lead to the loss of taxa with specialized functions or mutualistic associations, impairing key processes such as organic matter decomposition, nutrient mineralization, and plant-fungal symbioses (Allison and Martiny 2008, Peay et al., 2016). These shifts may ultimately reduce ecosystem resilience to future environmental variability.

In contrast, bacterial communities exhibited relatively stable dispersion and composition across fire regimes, suggesting higher resistance and faster recovery from fire-induced environmental changes. This supports previous research showing that bacteria recover more rapidly following disturbance and exhibit more compositional stability after fire events (Pinto et al. 2023, Soria et al. 2023, Caiafa et a., 2023). These divergent responses highlight fundamental differences in ecological strategies and life histories between fungi and bacteria, where fungi often have slower turnover and narrower niche breadths, while bacteria are generally more metabolically flexible, faster-reproducing, and better adapted to fluctuating environments (Treseder 2023, Hopkins et al. 2025c, Strickland & Rousk 2010).

Fungal functional guilds respond to fire regimes in unexpected ways

Fire regime drove distinct changes in fungal functional guilds and taxa. Contrary to our expectations, annual fire plots showed higher relative abundances of ectomycorrhizal and saprotrophic fungi, whereas maintenance plots were enriched with endophytes, pathogens, and parasitic fungi. This is surprising given that fire exclusion or less frequent fire is typically associated with greater abundance of ectomycorrhizal fungi due to increased woody plant richness and leaf litter (Fox et al. 2024, Pérez-Izquierdo, 2021). However, certain ectomycorrhizal fungi (e.g. *Cenococcum*, *Russula*) are known to produce heat-resistant spores or hyphal fragments and can persist under repeated fire (Kipfer et al. 2010, Glassman et al. 2016). Likewise, saprotrophic fungi may be favored in frequently burned plots due to increased inputs of charred organic material, which can be a resource for specific decomposers. The prevalence of these guilds under annual fire may promote faster litter decomposition and nutrient turnover, reinforcing vegetation dynamics that sustain frequent fire at this interval. In contrast, the higher relative abundance of endophytes, pathogens, and parasitic fungi in maintenance plots may reflect greater host availability and influence plant health, competition, and successional trajectories under less frequent disturbance.

Annual fire regimes showed fire-responsive indicator taxa

Indicator species analysis further revealed that annual fire plots were enriched with known fire-responsive genera such as *Bacillus*, *Russula*, and *Periconia*, which are known for endospore formation or post-fire proliferation (Berkeley & Goodfellow 1981, Semenova-Nelsen et al. 2019, Horton et al. 1998, Oliver et al. 2015). Maintenance fire regimes, in contrast, supported a more diverse array of indicator taxa, such as *Talaromyces*, *Saitozyma*, *Paraphaeophaeria*, *Devriesa*, *Coniochaeta*, and *Cenococcum*. Previous studies have shown that *Cenococcum* (Kipfer et al. 2010) and *Coniochaeta* are known fire responders (Caiafa et al. 2023). *Cenococcum* is an ectomycorrhizal fungus that forms persistent sclerotia and is highly melanized (Obase et al. 2014), while *Coniochaeta* can degrade lignocellulose in woody substrates (Lopez et al. 2007), suggesting that less frequent fires may support a broader fungal community reflective of more diverse substrates and host associations. These patterns emphasize that while overall microbial diversity remained unchanged, community membership shifted in ways consistent with selection for fire-adapted traits, reflecting compositional turnover and trait filtering based on fire regime.

Too much fire? Indications of reduced resilience in a frequently burned system and implications for fire management

Our findings suggest that annual fire regimes may undermine longleaf pine ecosystem resilience by limiting recovery opportunities. In annually burned plots, we observed shifts in community composition and reductions in plant diversity. Notably, we found that soil phosphorus was significantly lower in annual fire plots, indicating potential nutrient depletion from repeated fire events with insufficient recovery intervals (Byre 2006, Kutiel & Naveh 1987). Phosphorus limitation, in turn, may constrain soil microbial growth and suppress primary productivity, undermining the ecological processes that reinforce resilience (Oliverio et al. 2020, Turner et al. 2007, Treseder & Cross 2006). While other soil properties appeared relatively stable, the decline in phosphorus may indicate long-term nutrient depletion that, over time, could restrict plant recovery and soil microbial functioning. Such nutrient loss may slow recovery rates and reduce the capacity of the system to rebound after disturbance (Rastetter et al. 2013), raising concerns about long-term sustainability under annual fire regimes.

While frequent fire may promote compositional turnover without reducing overall diversity in soil communities, it can concurrently reduce aboveground diversity and spatial heterogeneity of fungal communities. Land managers must navigate trade-offs between biodiversity conservation, fuel reduction, and wildfire risk mitigation when implementing prescribed fire regimes (Kelly et al. 2020). However, climate change is increasingly reshaping fire regimes worldwide, altering fire frequency, intensity, seasonality, and spatial extent, often beyond the bounds of historical variability (Sayedi et al. 2024, Kelly et al. 2020). These shifts risk decoupling fire from the ecological processes and recovery dynamics that evolved under past disturbance regimes, particularly in systems already subjected to climatic stress (Harvey and Enright 2022). In this context, adaptive management strategies that prioritize maintaining ecological processes that support resistance, recovery, and reorganization, rather than restoring reference conditions, are becoming increasingly essential (Falk 2017, Gillson et al. 2019). Together, our results underscore the importance of considering both the taxonomic and functional responses of multiple community types to prescribed fire regimes.

Conclusion

Our findings demonstrate that even in fire-adapted ecosystems, there are thresholds beyond which frequent fire reduces biodiversity and ecosystem resilience. While soil microbial richness remained stable, annual fires homogenized fungal communities, altered functional guilds, and reduced plant diversity. These patterns suggest that different fire regimes filter for distinct soil communities with varying functional roles, likely driven by fire history, resource dynamics, and plant community structure. Compositional turnover and functional shifts point to strong environmental filtering, with potential implications for nutrient cycling, disruption of plant-microbe interactions, and long-term ecosystem functioning. We conclude that maintenance fire regimes (1–3-year intervals) sustain higher plant diversity and more functionally diverse fungal assemblages. As climate change intensifies, land managers must consider how fire regimes shape the biological underpinnings of resilience. Prescribed fire strategies that maintain spatial heterogeneity and functional diversity will be critical for sustaining ecosystem function and supporting post-fire recovery in a rapidly changing world.

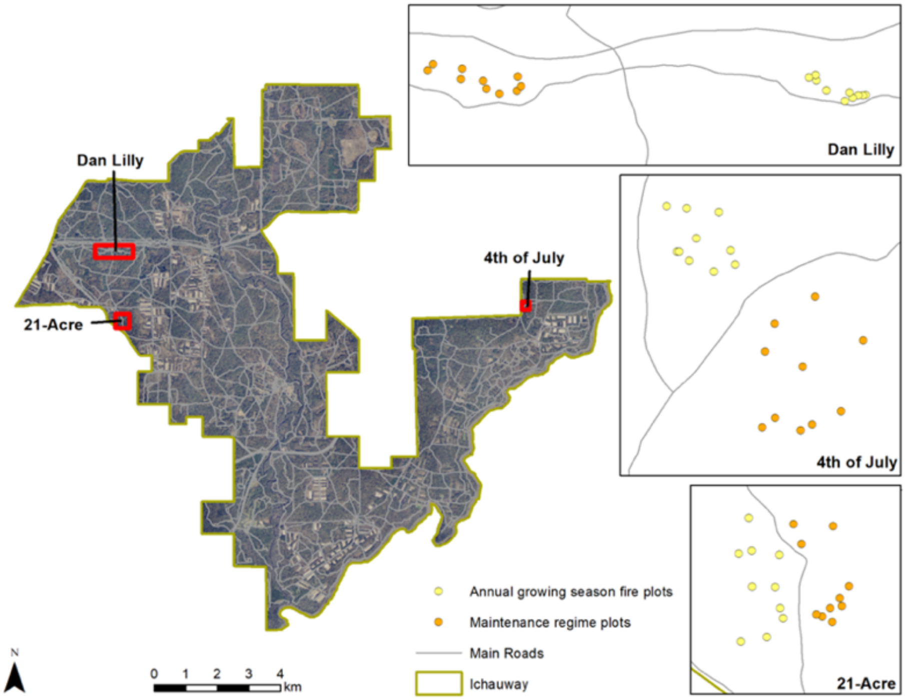

Figure 2.1: Map of burn treatments and field plot design. Map of the three experimental burn sites (Dan Lilly, 21-Acre, Fourth of July) at the Jones Center at Ichauway in Newton, Georgia (USA). Each burn unit includes ten permanent vegetation plots ($1m^2$) exposed to either annual growing season burn (yellow circles), or maintenance regime burn (orange circles). Secondary roads divide each burn unit within each site.

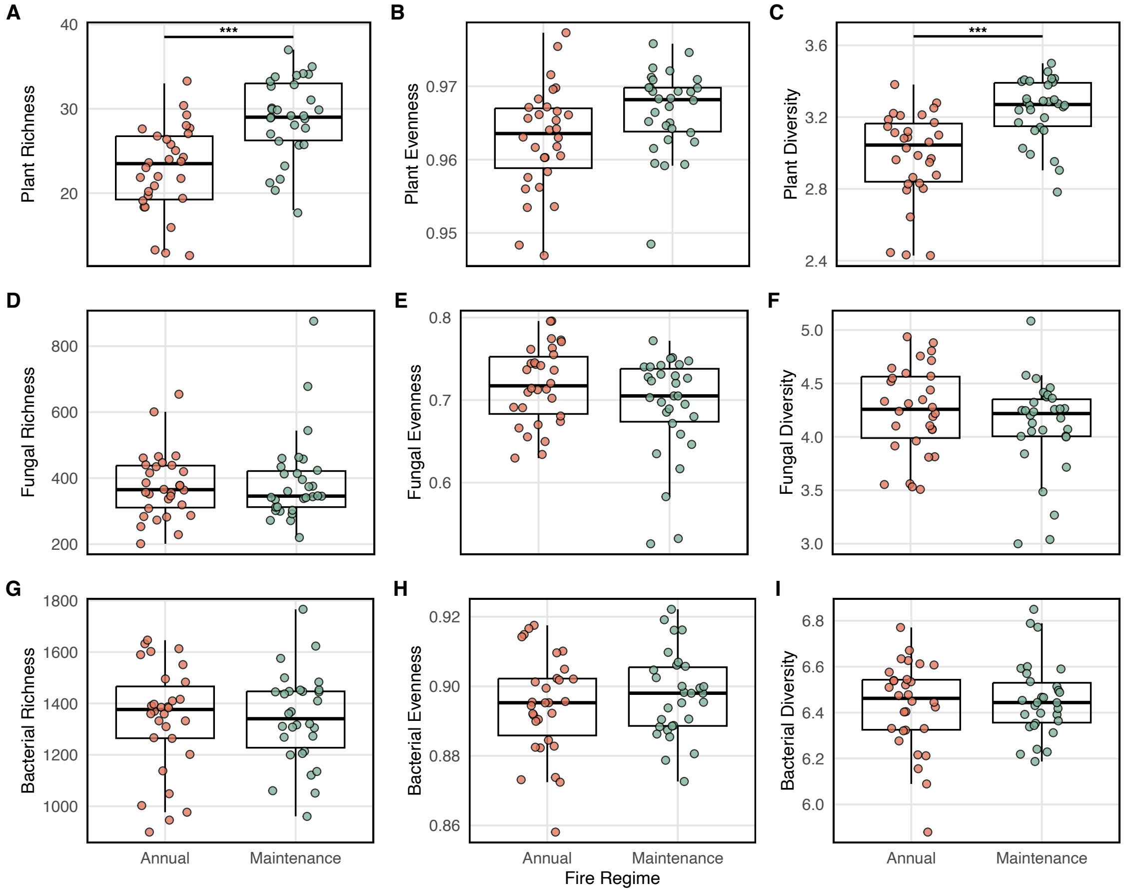

Figure 2.2: Plant, fungal, and bacterial alpha diversity metrics. Mean (± SE) alpha diversity metrics (richness, evenness, diversity) for A-C) plants, D-F) fungi, and G-I) bacteria across fire treatments (red = annual fires, green = maintenance fires). Asterisks (*) indicate significant differences based on *post-hoc* multiple comparisons: $p < 0.05$, $p < 0.001$, $p < 0.0001$.

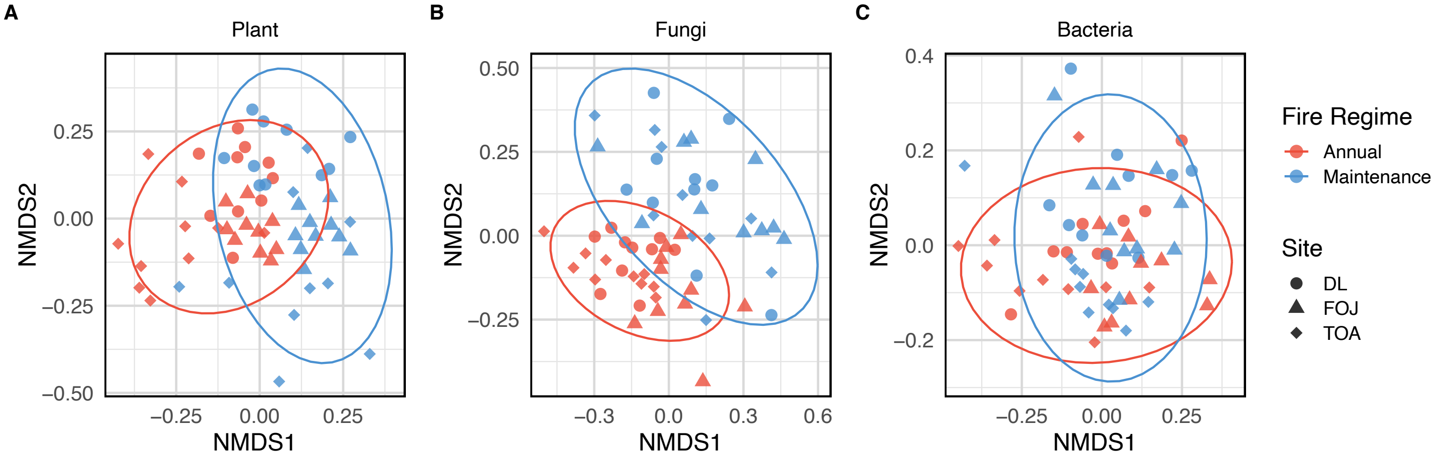

Figure 2.3: NMDS ordinations of plant, fungi, and bacteria across fire treatments and site. NMDS for A) plant (stress = 0.251), B) fungal (stress = 0.236), and C) bacterial (stress = 0.133) communities for fire treatments (red = annual fires, blue = maintenance fires), and field sites (circle = Dan Lilly, triangle = Fourth of July, diamond = Twenty-One Acre).

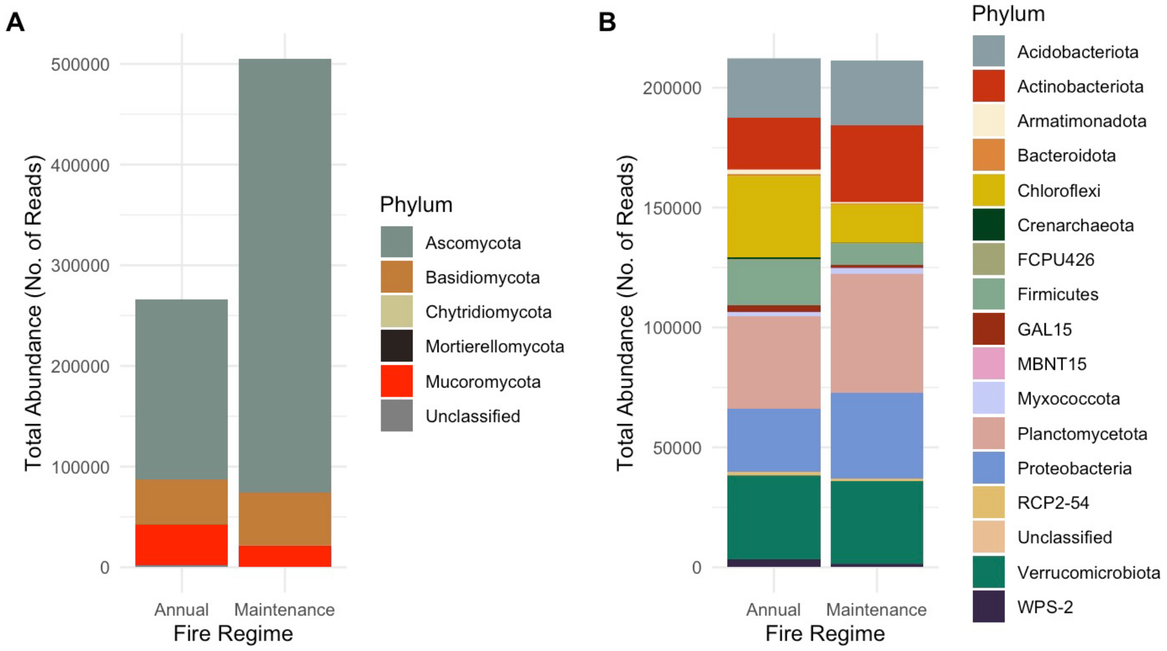

Figure 2.4: Total abundance of fungal and bacterial phyla across fire regimes. Total sequence abundance of indicator taxa identified in the indicator species analysis across fire regime treatments for A) fungi and B) bacteria. All taxa shown were statistically significant ($p < 0.05$).

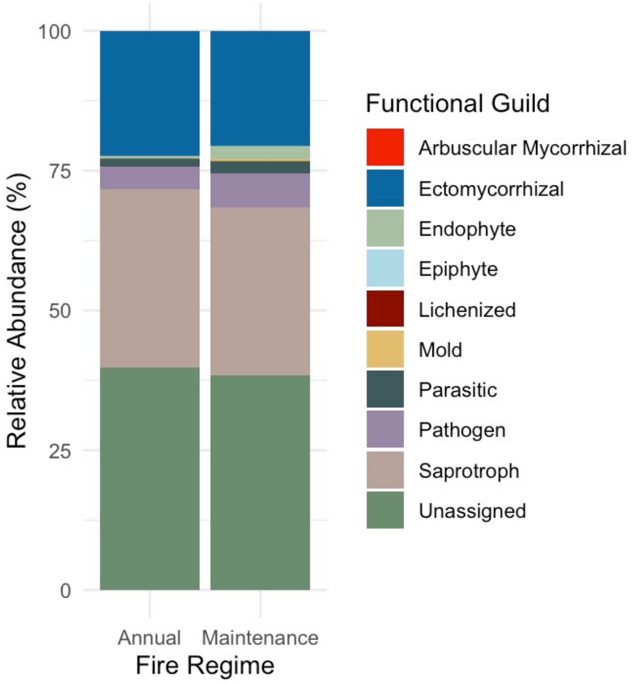

Figure 2.5: Fungal functional guilds across fire regimes. Mean relative abundance (%) of fungal functional guilds across fire treatments. Bars are stacked by functional guild.

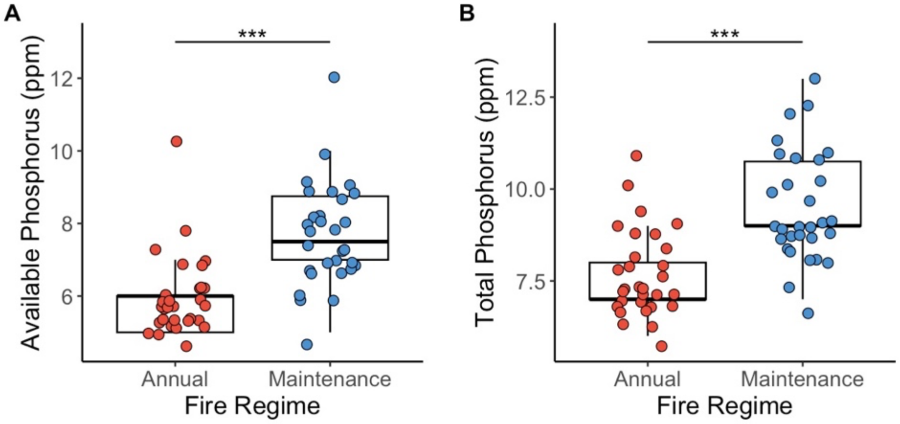

Figure 2.6: Soil phosphorus means across fire regimes. Mean (± SE) of A) available phosphorus and B) total phosphorus across fire treatments (red = annual fires, blue = maintenance fires). Means denoted as different asterisks (***) are significantly different based on a *post-hoc* multiple comparisons of means of fire regime treatments ($p < 0.001$).

Table 2.1: **Summary of soil chemistry means across fire regime and site.** Means and standard deviations of each soil chemistry variable by fire regime treatment and site.

	Treatment	Mean	SD	Fire Regime	Site	Fire Regime x Site
GWC	Annual	8.08	3.4	p = 0.199	**p < 0.001**	p = 0.087
	Maintenance	9.25	4.6			
OM	Annual	3.49	2.35	p = 0.106	**p < 0.001**	p = 0.966
	Maintenance	4.34	2.7			
p1	Annual	5.93	1.11	**p < 0.0001**	**p = 0.014**	p = 0.376
	Maintenance	7.73	1.39			
p2	Annual	7.66	1.18	**p < 0.0001**	**p = 0.024**	p = 0.108
	Maintenance	9.5	1.48			
K	Annual	29.96	10.25	p = 0.156	p = 0.066	p = 0.147
	Maintenance	32.36	8.28			
Mg	Annual	84	40.6	p = 0.102	**p < 0.001**	p = 0.216
	Maintenance	98	36.92			
Ca	Annual	424.23	225.74	p = 0.09	**p < 0.001**	p = 0.231
	Maintenance	492.93	180.05			
pH	Annual	5.45	0.255	p = 0.488	**p < 0.001**	p = 0.447
	Maintenance	5.49	0.255			
CEC	Annual	3.89	1.78	p = 0.058	**p < 0.001**	p = 0.203
	Maintenance	4.53	1.45			
NO3	Annual	1.4	1.49	p = 0.91	p = 0.179	p = 0.172
	Maintenance	1.43	0.62			

References

Abrahamson, W. G., Abrahamson, C. R., & Keller, M. A. (2021). Lessons from four decades of monitoring vegetation and fire: maintaining diversity and resilience in Florida's uplands. *Ecological Monographs*, 91(2), e01444. https://doi.org/10.1002/ecm.1444

Agbeshie, A. A., Abugre, S., Atta-Darkwa, T., & Awuah, R. (2022). A review of the effects of forest fire on soil properties. *Journal of Forestry Research*, 33(5), 1419-1441. https://doi.org/10.1007/s11676-022-01475-4

Aislabie, J., Deslippe, J. R., & Dymond, J. (2013). Soil microbes and their contribution to soil services. *Ecosystem Services in New Zealand–Conditions and Trends*. Manaaki Whenua Press, Lincoln, New Zealand, 1(12), 143-161. https://doi.org/10.7931/DL1MS3

Akerele, G., Al Hakeem, W. G., Lourenco, J., & Selvaraj, R. K. (2022). The effect of necrotic enteritis challenge on production performance, cecal microbiome, and cecal tonsil transcriptome in broilers. *Pathogens*, 11(8), 839. https://doi.org/10.3390/pathogens11080839

Allison, S. D., & Martiny, J. B. (2008). Resistance, resilience, and redundancy in microbial communities. *Proceedings of the National Academy of Sciences*, 105(supplement_1), 11512-11519. https://doi.org/10.1073/pnas.0801925105

Bárcenas-Moreno, G., García-Orenes, F., Mataix-Solera, J., & Mataix-Beneyto, J. (2016). Plant community influence on soil microbial response after a wildfire in Sierra Nevada National Park (Spain). *Science of The Total Environment*, 573, 1265-1274. https://doi.org/10.1016/j.scitotenv.2016.05.013

Bauer, J. T., Mack, K. M., & Bever, J. D. (2015). Plant-soil feedbacks as drivers of succession: evidence from remnant and restored tallgrass prairies. *Ecosphere*, 6(9), 1-12. https://doi.org/10.1890/ES14-00480.1

Berkeley, R. C. W., & Goodfellow, M. (1981). The aerobic endospore-forming bacteria: classification and identification (pp. xv+-373).

Bernhardt, L. T., Smith, R. G., Grandy, A. S., Mackay, J. E., Warren, N. D., Geyer, K. M., & Ernakovich, J. G. (2022). Soil microbial communities vary in composition and functional strategy across soil aggregate size class regardless of tillage. *Elem Sci Anth*, 10(1), 00023. https://dx.doi.org/10.1525/elementa.2022.00023

Bond, W. J., & Keeley, J. E. (2005). Fire as a global 'herbivore': the ecology and evolution of flammable ecosystems. *Trends in Ecology & Evolution*, 20(7), 387-394. https://doi.org/10.1016/j.tree.2005.04.025

Bowd, E. J., Egidi, E., Lindenmayer, D. B., Wardle, D. A., Kardol, P., Cary, G. J., & Foster, C. (2022). Direct and indirect effects of fire on microbial communities in a pyrodiverse dry-sclerophyll forest. *Journal of Ecology*, 110(7), 1687-1703. https://doi.org/10.1111/1365-2745.13903

Brewer, J. S. (2023). Mechanisms of fire-maintained plant species diversity in species-rich wet pine savannas. *Ecosphere*, 14(1), e4387. https://doi.org/10.1002/ecs2.4387

Brockway, D. G., & Lewis, C. E. (1997). Long-term effects of dormant-season prescribed fire on plant community diversity, structure and productivity in a longleaf pine wiregrass ecosystem. *Forest Ecology and Management*, 96(1-2), 167-183. https://doi.org/10.1016/S0378-1127(96)03939-4

Brye, K. R. (2006). Soil physiochemical changes following 12 years of annual burning in a humid–subtropical tallgrass prairie: a hypothesis. *Acta Oecologica*, 30(3), 407-413. https://doi.org/10.1016/j.actao.2006.06.001

Caiafa, M. V., Nelson, A. R., Borch, T., Roth, H. K., Fegel, T. S., Rhoades, C. C., ... & Glassman, S. I. (2023). Distinct fungal and bacterial responses to fire severity and soil depth across a ten-year wildfire chronosequence in beetle-killed lodgepole pine forests. *Forest Ecology and Management*, 544, 121160. https://doi.org/10.1016/j.foreco.2023.121160

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, 13(7), 581-583. https://doi.org/10.1038/nmeth.3869

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., ... & Knight, R. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME Journal*, 6(8), 1621-1624. https://doi.org/10.1038/ismej.2012.8

Certini, G., Moya, D., Lucas-Borja, M. E., & Mastrolonardo, G. (2021). The impact of fire on soil-dwelling biota: A review. *Forest Ecology and Management*, 488, 118989. https://doi.org/10.1016/j.foreco.2021.118989

Classen, A. T., Sundqvist, M. K., Henning, J. A., Newman, G. S., Moore, J. A., Cregger, M. A., ... & Patterson, C. M. (2015). Direct and indirect effects of climate change on soil microbial and soil microbial-plant interactions: What lies ahead? *Ecosphere*, 6(8), 1-21. https://doi.org/10.1890/ES15-00217.1

Connell, J. H. (1978). Diversity in tropical rain forests and coral reefs: high diversity of trees and corals is maintained only in a nonequilibrium state. *Science*, 199(4335), 1302-1310. https://doi.org/10.1126/science.199.4335.1302

Dao, V. Q., Potts, S. E., Johnson, C. N., Sikes, B. A., & Platt, W. J. (2022). Substrate and low intensity fires influence bacterial communities in longleaf pine savanna. *Scientific Reports*, 12(1), 20904. https://doi.org/10.1038/s41598-022-24896-x

De Caceres, M., Jansen, F., & De Caceres, M. M. (2016). Package 'indicspecies'. indicators, 8(1).

Dooley, S. R., & Treseder, K. K. (2012). The effect of fire on microbial biomass: a meta-analysis of field studies. *Biogeochemistry*, 109(1), 49-61. https://doi.org/10.1007/s10533-011-9633-8

Dove, N. C., Klingeman, D. M., Carrell, A. A., Cregger, M. A., & Schadt, C. W. (2021). Fire alters plant microbiome assembly patterns: integrating the plant and soil microbial response to disturbance. *New Phytologist*, 230(6), 2433-2446. https://doi.org/10.1111/nph.17248

Falk, D. A. (2017). Restoration ecology, resilience, and the axes of change1. *Annals of the Missouri Botanical Garden*, 102(2), 201-216. https://doi.org/10.3417/2017006

Fischer, M. S., Stark, F. G., Berry, T. D., Zeba, N., Whitman, T., & Traxler, M. F. (2021). Pyrolyzed substrates induce aromatic compound metabolism in the post-fire fungus, Pyronema domesticum. *Frontiers in Microbiology*, 12, 729289. https://doi.org/10.3389/fmicb.2021.729289

Fox, S., Taylor, M. K., & Jumpponen, A. (2024). Fire-excluded and frequently burned longleaf pine forests have contrasting soil microbial communities. *Forest Ecology and Management*, 551, 121519. https://doi.org/10.1016/j.foreco.2023.121519

Galperin, M. Y. (2016). Genome diversity of spore-forming Firmicutes. *The Bacterial Spore: From Molecules to Systems*, 1-18. https://doi.org/10.1128/9781555819323.ch1

Garcia-Pausas, J., Romanyà, J., & Casals, P. (2022). Post-fire recovery of soil microbial functions is promoted by plant growth. *European Journal of Soil Science*, 73(4), e13290. https://doi.org/10.1111/ejss.13290

Gillson, L., Whitlock, C., & Humphrey, G. (2019). Resilience and fire management in the Anthropocene. *Ecology and Society*, 24(3). https://www.jstor.org/stable/26796998

Glassman, S. I., Levine, C. R., DiRocco, A. M., Battles, J. J., & Bruns, T. D. (2016). Ectomycorrhizal fungal spore bank recovery after a severe forest fire: some like it hot. *The ISME Journal*, 10(5), 1228-1239. https://doi.org/10.1038/ismej.2015.182

Glitzenstein, J. S., Streng, D. R., & Wade, D. D. (2003). Fire frequency effects on longleaf pine (Pinus palustris P. Miller) vegetation in South Carolina and northeast Florida, USA. *Natural Areas Journal*. 23 (1): 22-37. 2003.

Glitzenstein, J. S., Streng, D. R., Masters, R. E., Robertson, K. M., & Hermann, S. M. (2012). Fire-frequency effects on vegetation in north Florida pinelands: another look at the long-term Stoddard Fire Research Plots at Tall Timbers Research Station. *Forest Ecology and Management*, 264, 197-209. https://doi.org/10.1016/j.foreco.2011.10.014

Grime, J. P. (1977). Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *The American Naturalist*, 111(982), 1169-1194. https://doi.org/10.1086/283244

Grupenhoff, A. R., & Safford, H. D. (2024). High fire frequency in California chaparral reduces postfire shrub regeneration and native plant diversity. *Ecosphere*, 15(12), e70128. https://doi.org/10.1002/ecs2.70128

Hart, S. C., DeLuca, T. H., Newman, G. S., MacKenzie, M. D., & Boyle, S. I. (2005). Post-fire vegetative dynamics as drivers of microbial community structure and function in forest soils. *Forest Ecology and Management*, 220(1-3), 166-184. https://doi.org/10.1016/j.foreco.2005.08.012

Harvey, B. J., & Enright, N. J. (2022). Climate change and altered fire regimes: impacts on plant populations, species, and ecosystems in both hemispheres. *Plant Ecology*, 223(7), 699-709. https://doi.org/10.1007/s11258-022-01248-3

He, T., Lamont, B. B., & Pausas, J. G. (2019). Fire as a key driver of Earth's biodiversity. *Biological Reviews*, 94(6), 1983-2010. https://doi.org/10.1111/brv.12544

Holden, S. R., Gutierrez, A., & Treseder, K. K. (2013). Changes in soil fungal communities, extracellular enzyme activities, and litter decomposition across a fire chronosequence in Alaskan boreal forests. *Ecosystems*, 16(1), 34-46. https://doi.org/10.1007/s10021-012-9594-3

Hopkins, J. R., & Bennett, A. E. (2023). Spore traits mediate disturbance effects on arbuscular mycorrhizal fungal community composition and mutualisms. *Ecology*, 104(5), e4016. https://doi.org/10.1002/ecy.4016

Hopkins, J. R., Semenova-Nelsen, T., & Sikes, B. A. (2021). Fungal community structure and seasonal trajectories respond similarly to fire across pyrophilic ecosystems. *FEMS Microbiology Ecology*, 97(1), fiaa219. https://doi.org/10.1093/femsec/fiaa219

Hopkins, J. R., Semenova-Nelsen, T. A., Huffman, J. M., Jones, N. J., Robertson, K. M., Platt, W. J., & Sikes, B. A. (2025a). Fuel accumulation shapes post-fire fuel decomposition through soil heating effects on plants, fungi, and soil chemistry. *Science of The Total Environment*, 961, 178386. https://doi.org/10.1016/j.scitotenv.2025.178386

Hopkins, J. R., Bennett, A. E., & McKenna, T. P. (2025b). Fire Frequency Driven Increases in Burn Heterogeneity Promote Microbial Beta Diversity: A Test of the Pyrodiversity-Biodiversity Hypothesis. *Molecular Ecology*, 34(10), e17756. https://doi.org/10.1111/mec.17756

Hopkins, J. R., Vizzari, G., Bennett, A. E., & Malacrinò, A. (2025c). Fire limits soil microbial dispersal and differentially impacts bacterial and fungal communities. *bioRxiv*, 2025-05. https://doi.org/10.1101/2025.05.02.651892

Horton, T. R., Cázares, E., & Bruns, T. D. (1998). Ectomycorrhizal, vesicular-arbuscular and dark septate fungal colonization of bishop pine (Pinus muricata) seedlings in the first 5 months of growth after wildfire. *Mycorrhiza*, 8(1), 11-18. https://doi.org/10.1007/s005720050205

Hu, M., Wang, J., Lu, L., Gui, H., & Wan, S. (2023). Global recovery patterns of soil microbes after fire. *Soil Biology and Biochemistry*, 183, 109057. https://doi.org/10.1016/j.soilbio.2023.109057

Ihrmark, K., Bödeker, I. T., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., ... & Lindahl, B. D. (2012). New primers to amplify the fungal ITS2 region–evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiology Ecology*, 82(3), 666-677. https://doi.org/10.1111/j.1574-6941.2012.01437.x

Kardol, P., Cornips, N. J., Van Kempen, M. M., Bakx-Schotman, J. T., & Van Der Putten, W. H. (2007). Microbe-mediated plant–soil feedback causes historical contingency effects in plant community assembly. *Ecological Monographs*, 77(2), 147-162. https://doi.org/10.1890/06-0502

Kelly, L. T., Giljohann, K. M., Duane, A., Aquilué, N., Archibald, S., Batllori, E., ... & Brotons, L. (2020). Fire and biodiversity in the Anthropocene. *Science*, 370(6519), eabb0355. https://doi.org/10.1126/science.abb0355

Kipfer, T., Egli, S., Ghazoul, J., Moser, B., & Wohlgemuth, T. (2010). Susceptibility of ectomycorrhizal fungi to soil heating. *Fungal Biology*, 114(5-6), 467-472. https://doi.org/10.1016/j.funbio.2010.03.008

Kirkman, L. K., Goebel, P. C., Palik, B. J., & West, L. T. (2004). Predicting plant species diversity in a longleaf pine landscape. *Ecoscience*, 11(1), 80-93. https://doi.org/10.1080/11956860.2004.11682812

Knelman, J. E., Graham, E. B., Trahan, N. A., Schmidt, S. K., & Nemergut, D. R. (2015). Fire severity shapes plant colonization effects on bacterial community structure, microbial biomass, and soil enzyme activity in secondary succession of a burned forest. *Soil Biology and Biochemistry*, 90, 161-168. https://doi.org/10.1016/j.soilbio.2015.08.004

Kutiel, P., & Naveh, Z. (1987). The effect of fire on nutrients in a pine forest soil. *Plant and Soil*, 104(2), 269-274. https://doi.org/10.1007/BF02372541

Lenth, R. V (2024). Emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.10.1. https://cran.r-project.org/web/packages/emmeans/index.html

Lopez, M. J., del Carmen Vargas-García, M., Suárez-Estrella, F., Nichols, N. N., Dien, B. S., & Moreno, J. (2007). Lignocellulose-degrading enzymes produced by the ascomycete

Coniochaeta ligniaria and related species: application for a lignocellulosic substrate treatment. *Enzyme and Microbial Technology*, 40(4), 794-800. https://doi.org/10.1016/j.enzmictec.2006.06.012

Louw, N., Gherardi, L. A., Sala, O. E., & Chung, Y. A. (2022). Dryland soil mycobiome response to long-term precipitation variability depends on host type. *Journal of Ecology*, 110(12), 2984-2997. https://doi.org/10.1111/1365-2745.14006

Obase, K., Douhan, G. W., Matsuda, Y., & Smith, M. E. (2014). Culturable fungal assemblages growing within Cenococcum sclerotia in forest soils. *FEMS Microbiology Ecology*, 90(3), 708-717. https://doi.org/10.1111/1574-6941.12428

Oliverio, A. M., Bissett, A., McGuire, K., Saltonstall, K., Turner, B. L., & Fierer, N. (2020). The role of phosphorus limitation in shaping soil bacterial communities and their metabolic capabilities. *MBio*, 11(5), 10-1128. https://doi.org/10.1128/mbio.01718-20

Palmquist, K. A., Peet, R. K., & Mitchell, S. R. (2015). Scale-dependent responses of longleaf pine vegetation to fire frequency and environmental context across two decades. *Journal of Ecology*, 103(4), 998-1008. https://doi.org/10.1111/1365-2745.12412

Peay, K. G., Kennedy, P. G., & Talbot, J. M. (2016). Dimensions of biodiversity in the Earth mycobiome. *Nature Reviews Microbiology*, 14(7), 434-447. https://doi.org/10.1038/nrmicro.2016.59

Pinto, R., Ansola, G., Calvo, L., & de Miera, L. E. S. (2023). High resilience of soil bacterial communities to large wildfires with an important stochastic component. *Science of The Total Environment*, 899, 165719. https://doi.org/10.1016/j.scitotenv.2023.165719

Mataix-Solera, J., Guerrero, C., García-Orenes, F., Bárcenas, G. M., Torres, M. P., & Barcenas, M. (2009). *Forest Fire Effects on Soil Microbiology*. Fire effects on soils and restoration strategies, 5, 133-175. https://doi.org/10.1016/j.coesh.2021.100264

McIntyre, R. K., S. B. Jack, R. J. Mitchell, J. K. Hiers, and L. W. Neel. 2008. Multiple Value Management: The Stoddard-Neel Approach to Ecological Forestry in Longleaf Pine Grasslands. *The Jones Center at Ichauway Outreach Catalog*: OP 08-1. 30 p. https://doi.org/10.58497/74641

McLauchlan, K. K., Higuera, P. E., Miesel, J., Rogers, B. M., Schweitzer, J., Shuman, J. K., ... & Watts, A. C. (2020). Fire as a fundamental ecological process: Research advances and frontiers. *Journal of Ecology*, 108(5), 2047-2069. https://doi.org/10.1111/1365-2745.13403

Mitchell, R. J., Hiers, J. K., O'Brien, J. J., Jack, S. B., & Engstrom, R. T. (2006). Silviculture that sustains: the nexus between silviculture, frequent prescribed fire, and conservation of biodiversity in longleaf pine forests of the southeastern United States. *Canadian Journal of Forest Research*, 36(11), 2724-2736. https://doi.org/10.1139/x06-100

Mitchell, R. J., Hiers, J. K., O'Brien, J., & Starr, G. (2009). Ecological forestry in the Southeast: understanding the ecology of fuels. *Journal of Forestry*, 107(8), 391-397. https://doi.org/10.1093/jof/107.8.391

Miller, R. G., Tangney, R., Enright, N. J., Fontaine, J. B., Merritt, D. J., Ooi, M. K., ... & Miller, B. P. (2019). Mechanisms of fire seasonality effects on plant populations. *Trends in Ecology & Evolution*, 34(12), 1104-1117. https://doi.org/10.1016/j.tree.2019.07.009

Moghli, A., Santana, V. M., Baeza, M. J., Pastor, E., & Soliveres, S. (2022). Fire recurrence and time since last fire interact to determine the supply of multiple ecosystem services by

Mediterranean forests. *Ecosystems*, 25(6), 1358-1370. https://doi.org/10.1007/s10021-021-00720-x

Nelson, A. R., Narrowe, A. B., Rhoades, C. C., Fegel, T. S., Daly, R. A., Roth, H. K., ... & Wilkins, M. J. (2022). Wildfire-dependent changes in soil microbiome diversity and function. *Nature Microbiology*, 7(9), 1419-1430. https://doi.org/10.1038/s41564-022-01203-y

Noss, R. F., Platt, W. J., Sorrie, B. A., Weakley, A. S., Means, D. B., Costanza, J., & Peet, R. K. (2015). How global biodiversity hotspots may go unrecognized: lessons from the North American Coastal Plain. *Diversity and Distributions*, 21(2), 236-244. https://doi.org/10.1111/ddi.12278

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., ... & Wagner, H. (2022). vegan: Community Ecology Package. R package version 2.5-7. 2020. Preprint at, 3-1.

Oliver, A. K., Callaham Jr, M. A., & Jumpponen, A. (2015). Soil fungal communities respond compositionally to recurring frequent prescribed burning in a managed southeastern US forest ecosystem. *Forest Ecology and Management*, 345, 1-9. https://doi.org/10.1016/j.foreco.2015.02.020

Owen, S. M., Patterson, A. M., Gehring, C. A., Sieg, C. H., Baggett, L. S., & Fulé, P. Z. (2019). Large, high-severity burn patches limit fungal recovery 13 years after wildfire in a ponderosa pine forest. *Soil Biology and Biochemistry*, 139, 107616. https://doi.org/10.1016/j.soilbio.2019.107616

Pausas, J. G., & Keeley, J. E. (2009). A burning story: the role of fire in the history of life. *BioScience*, 59(7), 593-601. https://doi.org/10.1525/bio.2009.59.7.10

Peet, R. K., & Allard, D. J. (1993, May). Longleaf pine vegetation of the southern Atlantic and eastern Gulf Coast regions: a preliminary classification. *In Proceedings of the Tall Timbers Fire Ecology Conference* (Vol. 18, pp. 45-81).

Pérez-Izquierdo, L., Clemmensen, K. E., Strengbom, J., Granath, G., Wardle, D. A., Nilsson, M. C., & Lindahl, B. D. (2021). Crown-fire severity is more important than ground-fire severity in determining soil fungal community development in the boreal forest. *Journal of Ecology*, 109(1), 504-518. https://doi.org/10.1111/1365-2745.13529

Platt, W. J., Ellair, D. P., Huffman, J. M., Potts, S. E., & Beckage, B. (2016). Pyrogenic fuels produced by savanna trees can engineer humid savannas. *Ecological Monographs*, 86(3), 352-372. https://doi.org/10.1002/ecm.1224

Põlme, S., Abarenkov, K., Henrik Nilsson, R., Lindahl, B. D., Clemmensen, K. E., Kauserud, H., ... & Tedersoo, L. (2020). FungalTraits: a user-friendly traits database of fungi and fungus-like stramenopiles. *Fungal Diversity*, 105(1), 1-16. https://doi.org/10.1007/s13225-020-00466-2

Rastetter, E. B., Yanai, R. D., Thomas, R. Q., Vadeboncoeur, M. A., Fahey, T. J., Fisk, M. C., ... & Hamburg, S. P. (2013). Recovery from disturbance requires resynchronization of ecosystem nutrient cycles. Ecological Applications, 23(3), 621-642. https://doi.org/10.1890/12-0751.1

R Core Team, R. (2020). R: A language and environment for statistical computing.

Reinhart, K. O., Dangi, S. R., & Vermeire, L. T. (2016). The effect of fire intensity, nutrients, soil microbes, and spatial distance on grassland productivity. *Plant and Soil*, 409(1), 203-216. https://doi.org/10.1007/s11104-016-2957-3

Revillini, D., David, A. S., Menges, E. S., Main, K. N., Afkhami, M. E., & Searcy, C. A. (2022). Microbiome-mediated response to pulse fire disturbance outweighs the effects of fire legacy on plant performance. *New Phytologist*, 233(5), 2071-2082. https://doi.org/10.1111/nph.17689

Reynolds, H. L., Packer, A., Bever, J. D., & Clay, K. (2003). Grassroots ecology: plant–microbe–soil interactions as drivers of plant community structure and dynamics. *Ecology*, 84(9), 2281-2291. https://doi.org/10.1890/02-0298

Rudgers, J. A., Gehring, C. A., Taylor, D. L., Taylor, M. D., & Chung, Y. A. (2025). Integration of plant–soil feedbacks with resilience theory for climate change. *Trends in Ecology & Evolution*. https://doi.org/10.1016/j.tree.2025.05.001

Sayedi, S. S., Abbott, B. W., Vannière, B., Leys, B., Colombaroli, D., Romera, G. G., ... & Daniau, A. L. (2024). Assessing changes in global fire regimes. *Fire Ecology*, 20(1), 1-22. https://doi.org/10.1186/s42408-023-00237-9

Sayer, E. J., Oliver, A. E., Fridley, J. D., Askew, A. P., Mills, R. T., & Grime, J. P. (2017). Links between soil microbial communities and plant traits in a species-rich grassland under long-term climate change. *Ecology and Evolution*, 7(3), 855-862. https://doi.org/10.1002/ece3.2700

Schmid, M. W., van Moorsel, S. J., Hahl, T., De Luca, E., De Deyn, G. B., Wagg, C., ... & Schmid, B. (2021). Effects of plant community history, soil legacy and plant diversity on soil microbial communities. *Journal of Ecology*, 109(8), 3007-3023. https://doi.org/10.1111/1365-2745.13714

Semenova-Nelsen, T. A., Platt, W. J., Patterson, T. R., Huffman, J., & Sikes, B. A. (2019). Frequent fire reorganizes fungal communities and slows decomposition across a heterogeneous pine savanna landscape. *New Phytologist*, 224(2), 916-927. https://doi.org/10.1111/nph.16096

Soria, R., Tortosa, A., Rodríguez-Berbel, N., Lucas-Borja, M. E., Ortega, R., & Miralles, I. (2023). Short-term response of soil bacterial communities after prescribed fires in semi-arid mediterranean forests. *Fire*, 6(4), 145. https://doi.org/10.3390/fire6040145

Strickland, M. S., & Rousk, J. (2010). Considering fungal: bacterial dominance in soils–methods, controls, and ecosystem implications. *Soil Biology and Biochemistry*, 42(9), 1385-1395. https://doi.org/10.1016/j.soilbio.2010.05.007

Tangney, R., Paroissien, R., Le Breton, T. D., Thomsen, A., Doyle, C. A., Ondik, M., ... & Ooi, M. K. (2022). Success of post-fire plant recovery strategies varies with shifting fire seasonality. *Communications Earth & Environment*, 3(1), 126. https://doi.org/10.1038/s43247-022-00453-2

Treseder, K. K., & Cross, A. (2006). Global distributions of arbuscular mycorrhizal fungi. *Ecosystems*, 9(2), 305-316. https://doi.org/10.1007/s10021-005-0110-x

Treseder, K. K. (2023). Ecological strategies of microbes: Thinking outside the triangle. *Journal of Ecology*, 111(9). https://doi.org/10.1111/1365-2745.14115

Turner, B. L., Condron, L. M., Richardson, S. J., Peltzer, D. A., & Allison, V. J. (2007). Soil organic phosphorus transformations during pedogenesis. *Ecosystems*, 10(7), 1166-1181. https://doi.org/10.1007/s10021-007-9086-z

Van Der Heijden, M. G., Bardgett, R. D., & Van Straalen, N. M. (2008). The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters*, 11(3), 296-310. https://doi.org/10.1111/j.1461-0248.2007.01139.x

Van der Putten, W. H., Bardgett, R. D., Bever, J. D., Bezemer, T. M., Casper, B. B., Fukami, T., ... & Wardle, D. A. (2013). Plant–soil feedbacks: the past, the present and future challenges. *Journal of Ecology*, 101(2), 265-276. https://doi.org/10.1111/1365-2745.12054

Vellend, M. A. R. K., Verheyen, K., Flinn, K. M., Jacquemyn, H. A. N. S., Kolb, A., Van Calster, H., ... & Hermy, M. (2007). Homogenization of forest plant communities and weakening of species–environment relationships via agricultural land use. *Journal of Ecology*, 95(3), 565-573. https://doi.org/10.1111/j.1365-2745.2007.01233.x

Weakley, A. S. (2020). Flora of the southern and mid-Atlantic states. University of North Carolina Herbarium, North Carolina Botanical Garden, Chapel Hill, NC, USA. https://ncbg.unc.edu/research/unc-herbarium/flora-apps/ [accessed 23 July 2021].

Weissman, J. L., Hou, S., & Fuhrman, J. A. (2021). Estimating maximal microbial growth rates from cultures, metagenomes, and single cells via codon usage patterns. *Proceedings of the National Academy of Sciences*, 118(12), e2016810118. https://doi.org/10.1073/pnas.2016810118

White, T. J., Bruns, T., Lee, S. J. W. T., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: A guide to methods and applications*, 18(1), 315-322. http://dx.doi.org/10.1016/B978-0-12-372180-8.50042-1

CHAPTER 3

HIGH FREQUENCY FIRE REGIME PROMOTES NEGATIVE PLANT-SOIL FEEDBACKS IN A FIRE-PRONE ECOSYSTEM[2]

---

[2] Henson, M.S., Giencke, L.M., Chung, Y.A. To be submitted to *Oikos*.

Abstract

Fire regimes are key drivers of plant community dynamics in fire-prone systems, yet their influence on the interactions between plants and soil biota remains poorly understood. Plant-soil feedback (PSF) is an important mechanism by which plants modify the biotic and abiotic properties of soil through interactions with microbial communities, and those modifications then feedback to impact plant fitness. While these feedbacks are shaped by environmental conditions, it remains unclear how and when fire alters the strength or direction of PSFs. Here, we examined how two prescribed fire regimes, annual (1-year fire return interval) and maintenance (1–3-year fire return interval), influence PSFs in a longleaf pine savanna by testing the growth responses of three native plant species (wiregrass; *Aristida beyrichiana*, twinflower; *Dyschoriste oblongifolia*, sensitive-briar; *Mimosa microphylla*) in live and sterilized conspecific and heterospecific soils. We found that sensitive-briar performed better in live soils, while live soils reduced wiregrass survival and biomass, indicating that soil microbes drive host-specific patterns in plant performance. Wiregrass showed strong net negative pairwise negative PSFs in live annually burned soils, suggesting the buildup of antagonistic host-specific soil biota under highly frequent fire. PSFs were largely neutral across all species pairs under the maintenance fire regime. These results indicate that highly frequent fire may disrupt beneficial microbial associations and promote soil communities that inhibit dominant graminoids. These patterns have important implications for restoration, suggesting that fire regimes not only influence aboveground plant communities but also shape soil microbial dynamics in ways that affect plant establishment. Managing fire regimes to support beneficial plant-microbe interactions can improve restoration success and enhance long-term ecosystem resilience, and integrating PSFs into fire management

strategies may further promote biodiversity and stability in fire-adapted ecosystems, leading to better restoration outcomes.

Introduction

Fire is a fundamental ecological process that influences community assembly patterns, nutrient cycling, and soil dynamics worldwide (Pausas & Keely 2009, He et al. 2019 Archibald et al. 2018). Frequent fire in fire-prone ecosystems promotes plant species richness and endemism (He et al. 2019), where fire produces environmental heterogeneity across spatial scales, creating biodiversity hotspots (Myers et al. 2000). One such biodiversity hotspot is the longleaf pine (*Pinus palustris* Mill.) ecosystem of the Gulf Coastal Plain in the United States (Noss et al. 2015), which is characterized by an open canopy and a dense, species-rich groundcover community maintained by frequent fire (Walker and Peet 1984, Mulligan et al. 2002, Kirkman et al. 2001). While it is firmly established that recurrent fire drives plant community structure and function, the impact of fire on the important linkages between plants and soil microbes has received less attention (Pressler et al. 2019). In particular, the mechanisms maintaining diversity in the exceptionally species-rich groundcover remain poorly understood, especially regarding the role of plant-soil feedbacks in promoting coexistence under frequent fire.

Plant-soil feedback (PSF) is a mechanism by which plants alter the biotic and abiotic soil environment through root and litter associated soil microbes in a species-specific manner, which can then feedback to impact their own plant growth and performance (Bever et al. 1997). These feedbacks can be positive, where plants accumulate mutualistic soil biota and induce conditions that are beneficial for their growth, or negative, where plants build up host-specific soil pathogens that result in self-limitation (van der Putten et al. 2013, Bever et al. 2012, Warneke et al. 2023). These biotic and abiotic feedbacks influence the spatial and temporal trajectories of plant community composition (Chung 2023), the long-term and large-scale patterns of plant community diversity and coexistence (Chung & Rudgers 2016, Kardol et al. 2023, Crawford et al. 2019), and are key in regulating ecosystem dynamics (van der Putten et al. 2013). Importantly, negative PSFs promote stabilization via conspecific negative frequency dependence and drive the maintenance of diversity in hyperdiverse systems (Bever 1997, Bever et al. 2015, Crawford et al. 2019, Chung & Rudgers 2016). However, PSFs are highly context-dependent and influenced by a range of interacting environmental factors (Smith-Ramesh et al. 2017, De Long et al. 2023). Despite decades of work on PSF, it remains unclear how and when external factors, such as fire, can alter the strength or direction of PSFs.

Fire can alter microbial communities in ways that impact plant growth and performance (Prendergast-Miller et al. 2017), and these effects largely depend on the intensity, seasonality, size, and frequency of the fire. Fire can reduce microbial abundance and diversity through soil heating (Certini et al. 2021), or through changes in the physical, chemical, or biological environment (Certini 2005, Alcaniz et al.2018). Fire associated changes can differentially impact microbial functional guilds, where fire can select for pathogens (Hewitt et al. 2016) or mutualists (Glassman et al. 2016, Dove and Hart 2017, Prendergast-Miller et al. 2017). If these impacts on microbes are specific to plant hosts, then changing fire regimes could alter the magnitude and direction of PSFs, leading to shifts in predicted plant relative abundance and coexistence. For instance, Warneke et al. 2023 showed that a high-intensity wildfire weakened mutualistic interactions and reduced the strength of positive PSFs in two nitrogen-fixing leguminous trees. Alternatively, Hopkins et al. 2024 found that low-intensity fires neutralized negative PSFs in

Schizacharyrium scoparium, a fire-adapted bunchgrass, suggesting that fire removes harmful pathogens that tend to build up in grass-conditioned soil, thus benefiting species dominance. Previous studies have consistently shown that the effect of a single fire event on PSFs is mediated through soil biological communities (Carvalho et al. 2010, Senior et al. 2018, Revillini et al. 2021, Warneke et al. 2023, Hopkins et al. 2024), with significant consequences on plant community assembly dynamics and post-fire succession (Hart et al. 2005, Kardol et al. 2023). However, the effects of fire must be considered within the context of the fire regime, taking the cumulative impacts of fire intensity, frequency, and seasonality into account. To our knowledge, no previous studies have considered PSFs within the context of fire regimes.

This study aimed to investigate how different fire regimes impact plant-soil feedbacks in a longleaf pine savanna. Specifically, we tested the growth responses of three plant species (wiregrass, *Aristida beyrichiana* Trin. & Rupr.; twinflower, *Dyschoriste oblongifolia* (Michx.); sensitive-briar, *Mimosa microphylla* Dryand.) in soils from two prescribed fire regimes: annual (1-year summer fires) vs. maintenance (1–3-year fires). We hypothesized that fire effects on PSFs would vary by plant species. Specifically, we predicted that annual fire would reduce pathogen loads, resulting in positive PSFs for wiregrass and twinflower. Accordingly, we expected these species to perform better in conspecific soils from the annual fire regime compared to the maintenance fire regime. Conversely, since legume typically generate positive PSFS by stimulating N-fixing rhizobia (Cortois et al. 2016, Kulmatiski et al. 2008), we hypothesized that high frequency regimes may weaken rhizobial associations in sensitive-briar, leading to negative PSFs.

Materials and Methods

Study site

We conducted the field portion of our study at The Jones Center at Ichauway (The Jones Center) (Newton, Georgia, USA; 31.2201° N; 84.4779° W). The Jones Center is a 11,500-hectare ecological reserve located in the Coastal Plain region of southern Georgia, and consists of sandy coastal plain deposits, with Ultisols as the dominant soil type (Drew et al. 1998). We utilized an established long-term field experiment testing the effects of different fire regimes on understory vegetation. At our site, one side has experienced a “maintenance” fire regime (burns every 1-3 years) since 1990, and the other side an “annual” fire regime (summer burns every year) since 2003. The ecosystem is an upland longleaf pine-wiregrass savanna, where the ground layer is dominated by fire-tolerant graminoids and forbs, and the canopy consists of a patchy matrix of fire-resistant trees dominated by longleaf pine (Peet et al. 2018). Dominant groundcover plant species at the site include: *Aristida beyrichiana* Trin. & Rupr., *Pityopsis graminifolia* (Michx.) Nutt., *Dyschoriste oblongifolia* (Michx.) Kuntze, *Andropogon virginicus* L., *Schizachyrium tenerum* Nees, and *Mimosa microphylla* Dryand. We chose to test three indicator plant species of remnant longleaf pine savannas (Kirkman et al. 2004) with a range of functional types that are likely to respond differentially to fire-induced soil microbial community change: wiregrass (*Aristida beyrichiana*, graminoid), sensitive-briar (*Mimosa microphylla*, legume), and twinflower (*Dyschoriste oblongifolia*, forb). All target plant species seeds were collected from sites at The Jones Center in summer and fall 2021.

Plant-soil feedback experiment

We conducted a two-phase plant-soil feedback experiment in a climate-controlled greenhouse at the University of Georgia (Athens, GA, USA): a conditioning phase to generate host-specific soil microbial communities, and a feedback phase to test the effects of those communities on con- and heterospecific plants.

During the conditioning phase, we cultivated host-specific soil microbial communities from the general soil microbial species pool present in the field. To do this, we grew each plant species separately in field soil collected from either annual or maintenance fire treatment sides of the site (3 species x 2 fire treatments x 10 reps = 60 pots). Prior to planting, two dry gallons of field soil were collected in March 2022 to a soil depth of 20 cm every ~5 m along a 25 m transect from each fire treatment side. At the time of sampling, the maintenance side had last burned 338 days prior, and the annual side 269 days prior. All two-gallon soil samples were pooled and mixed from each fire regime treatment side to create two common starting soil microbial species pools: annual and maintenance. The soil conditioning phase took 3 months to allow for plants to interact with the soil microbes and condition host-specific communities.

In the feedback phase, we tested the growth response of plant species in conditioned soils using all combinations of feedback and conditioning species. To isolate the microbial effect, half of each soil inoculum from the conditioning phase was sterilized (autoclaved 1 hr twice with a 24 hr rest period) before inoculation. We grew each plant in sterilized potting media (8 parts Pro-mix, 2 parts peat, 2 parts sand, 1 part perlite) inoculated with soil communities generated in the previous phase at 10% by volume in fully factorial combinations. Each conditioning species soil pot from the soil conditioning phase was split to inoculate across the three feedback species, keeping the 10 replicates from the conditioning phase as biological replicates in the PSF phase (3 feedback species x 2 sterilization treatments x 60 inocula from conditioning phase = 360 pots). After 6 months, we harvested all plants and measured dried shoot biomass, root biomass, and survival of all plants. Comparisons between fire regime treatments would demonstrate how changes in the general soil microbial species pool due to fire altered plant performance. Comparisons among conditioning species source would demonstrate presence of plant-soil feedbacks. Finally, comparisons between live/sterilized treatments would inform whether these effects are driven by soil microbes.

Soil chemistry

At the end of each phase of the PSF experiment, we collected soil for soil chemical analyses. After the soil conditioning phase and following soil sterilization, 200 g of soil per sample was sent to Midwest Labs Inc. (Omaha, Nebraska, USA). Analyses included organic matter (OM), weak Bray (P1), strong Bray (P2), potassium (K), magnesium (Mg), calcium (Ca), pH, cation exchange capacity (CEC), and nitrate (NO_3^-). OM was estimated by loss of ignition, reflecting the proportion of soil-derived organic material. Bray P1 and P2 tests extract phosphorus using acidic solutions to approximate the pools of plant-available and total extractable phosphorus, respectively. Exchangeable cations (K, Mg, Ca) were measured using ammonium acetate extraction, which assesses nutrient cations available for plant uptake. Soil pH was determined in a 1:1 soil-to-water slurry. CEC measured via ammonium acetate saturation, estimated soil's capacity to retain and exchange nutrient cations. Nitrate was extracted with potassium chloride, representing the primary inorganic nitrogen form available to plants.

After the feedback phase, 80 g of soil was sent to the Soil, Plant, and Water Analysis Laboratory at the University of Georgia Cooperative Extension (Athens, Georgia, USA). This analysis included phosphorus (P), manganese (Mn), zinc (Zn), K, Mg, Ca, pH, and NO_3^-. We conducted slightly different soil tests using two different laboratories for each phase due to smaller amounts of soil available after the feedback phase. The phosphorus test at the University of Georgia differed from Midwest Lab's Bray test, using a single extraction method suitable for small soil volumes. While not directly comparable to the weak and strong Bray tests, it provides a relative estimate of plant-available P. Minor differences in test methods between the two labs reflect differences in soil quantity and laboratory standard procedures.

Statistical analyses

All analyses were conducted in R software version 4.0.2 (R Core Team 2020). We used linear models to analyze individual soil chemistry response after the soil conditioning phase and feedback phase of the plant-soil feedback experiment. Each soil response variable was analyzed with fire treatment (annual, maintenance), soil sterilization (live, sterilized), species (sensitive-briar, twinflower, wiregrass), and their interactions as predictors. For the feedback phase, we additionally included feedback plant species as a predictor. We applied a reciprocal transformation to the nitrate soil variable ($1/NO_3$), as initial model diagnostics indicated non-normality of residuals. We conducted *post-hoc* comparisons of group means using estimated marginal means (emmeans package in R) (Lenth 2024) with a Tukey test in the case of significant main effects. We used a principal component analysis (PCA) to examine all soil chemistry differences among treatments after the soil conditioning phase of the experiment. We then conducted a permutational multivariate analysis of variance (PERMANOVA) using the vegan package in R (Oksanen et al. 2024) on the Euclidean distance matrix derived from the PCA. This analysis tested the effects of soil sterilization, fire regime, and conditioning species on soil chemistry after the conditioning phase, and the effects of soil sterilization, fire regime, and soil source (the original conditioning species) after the feedback phase and split out each species separately to isolate the effects of each soil source.

We used linear mixed effects models to analyze plant biomass (aboveground biomass), root biomass (belowground biomass), and survival for our three species separately. Predictor variables were the fire treatment, conditioned soil source (each source plant species), soil sterilization, and their two- and three-way interactions. Source plant identity and replicate were used as random effects in the model to account for variation due to the original conditioning source plant individuals, and to account for spatial variation in the greenhouse. To investigate the direction and magnitude of PSFs, we calculated the single species PSF as the natural log-ratio of each species' total biomass in conspecific soil versus each of the two heterospecific soils for each treatment. This demonstrates whether each species performs worse in its own soil (negative PSF) or better in its own soil (positive PSF) compared to another species' soil. We analyzed the PSF ratios for each species separately using linear models with fire regime, soil sterilization, and their interaction as fixed effects. This approach allowed us to assess whether the strength or direction of PSFs varied by each treatment or species for each focal species. We also assessed net pairwise PSF by calculating the pairwise interaction coefficient (I_s) following Bever et al. 1997 for all species pair combinations. This allows inference about PSF-mediated coexistence in the absence of competition, where negative I_s is associated with potential for pairwise coexistence (Bever 2003). We determined if I_s differed by treatment and species pair using ANOVA with the main effects being fire treatment, soil sterilization, species pairs, and their interaction. To assess whether log response ratios and I_s values were significantly different than zero, we fit a linear model without an intercept, treating each treatment-pair combination as a separate group, and conducted one sample t-tests on the estimated coefficients.

Results

Plant-soil feedback changes in soil chemistry

After the conditioning phase, soil OM, P1, K, Mg, Ca, pH, and CEC were higher in the soils originally from the maintenance regime compared to annual regime. Sterilization led to an increase in some soil nutrient levels, with significant increases in P1, P2, K, Mg, Ca, and pH (Appendix B: Table B1). In contrast, sterilization decreased NO_3^-. We found that the first and second axes of the PCA explained 71.6% of the total variation, 48.8% by the first axis and 22.8% of the second axis (Figure 3.1A-C). The first axis had large contributions from Mg, Ca, CEC, K, OM, P2 (in order of eigenvalues), and mainly differentiated soils originally from different fire treatments. The second axis had large contributions from NO_3^-, pH, and P1, and primarily reflected the effects of sterilization. PERMANOVA showed that the difference in soil chemistry composition after the conditioning phases was best explained by fire regime ($R^2 = 0.346$, $F_{1,48} = 56.04$, $p = 0.001$), then soil sterilization ($R^2 = 0.215$, $F_{1,48} = 34.81$, $p = 0.001$), and conditioning species identity explained the smallest amount of variation ($R^2 = 0.037$, $F_{2,48} = 3.01$, $p = 0.017$). Significant interactions were observed between soil sterilization and fire regime ($R^2 = 0.021$, $F_{1,48} = 3.39$, $p = 0.022$), soil sterilization and species ($R^2 = 0.032$, $F_{2,48} = 2.61$, $p = 0.018$), and fire regime, and species ($R^2 = 0.033$, $F_{2,48} = 2.66$, $p = 0.017$). These results indicate that these soil chemical properties are influenced by both the individual and combined effects of soil sterilization, fire regime, and conditioning species identity.

After the feedback phase, soils that received sterilized inocula maintained higher concentrations of P, K, Mg, Ca, Zn, and Mn compared to those that received live soil inocula (Appendix B: Table B2). Zn was the only soil nutrient in the feedback phase that varied with fire regime, with higher concentrations observed in soils from the annual fire regime.

After the feedback phase, the PCA analysis showed that soil chemistry variation was best explained by whether the inocula were sterilized, followed by the identity of the feedback plant species. The first and second axes of the PCA explained 68.2% of the total variation, 50.1% by the first axis and 18.1% of the second axis (Figure 3.1D-F). In order of eigenvalues, the first axis had large contributions from Ca, Mg, K, Zn, P, and with live-inoculated soils usually having higher values of those elements. The second axis had large contributions from pH, Mn, and NO_3^-, with wiregrass-grown soils associated with high values along this axis compared to the other two species. In contrast to the conditioning phase, after the feedback phase, soils from the two fire regime treatments showed strong overlap along the first 2 principal components. PERMANOVA analyses derived from the PCA revealed varying effects of fire regime, soil sterilization, and species identity on soil chemistry across the three conditioning plant species sources. For sensitive-briar and wiregrass, soil sterilization was the only significant factor in explaining variation in soil chemistry ($R^2 = 0.341$, $F_{1,47} = 27.85$, $p = 0.001$, and $R^2 = 0.120$, $F_{1,48} = 7.55$, $p = 0.015$, respectively). For twinflower, both soil sterilization ($R^2 = 0.316$, $F_{1,47} = 31.01$, $p = 0.01$) and conditioning species identity ($R^2 = 0.124$, $F_{2,47} = 6.07$, $p = 0.003$) explained significant variation in soil chemistry. Fire regime and its interactions with other factors were not significant for all three source species.

Presence of soil microbes drives species-specific patterns in survival and biomass

We found significant effects of species ($\chi^2 = 26.39$, df = 2, $p < 0.001$) and soil sterilization ($\chi^2 = 4.36$, df = 1, $p = 0.036$) on survival, but no effects of fire and any interactions. *Post-hoc* comparisons (Tukey-adjusted) showed that the impact of soil sterilization on survival varied by species (Figure 3.2A). Wiregrass generally had the lowest survival, but its survival was

104% higher in sterilized soils compared to live soils ($\chi^2 = 5.80$, df = 1, $p = 0.015$). In comparison, survival of sensitive-briar and twinflower did not differ significantly between live and sterilized soils.

We found significant differences in aboveground biomass among species ($\chi^2 = 126.86$, $p < 0.0001$), which depended on soil sterilization ($\chi^2 = 38.75$, $p < 0.0001$) (Figure 3.2B). For sensitive-briar, biomass was significantly influenced by the soil source treatment ($\chi^2 = 6.39$, $p = 0.041$) and soil sterilization ($\chi^2 = 27.36$, $p < 0.001$). Sensitive-briar in live soils produced 42% more biomass compared to sterilized soils ($t = 5.25$, $p < 0.0001$), suggesting overall mutualistic effects of soil microbes. In contrast, wiregrass aboveground biomass was 39% higher in sterilized soils than in live soils ($t = -2.73$, $p = 0.007$), suggesting overall pathogenic effects of soil microbes.

We found differences in belowground biomass among species ($\chi^2 = 194.64$, df = 2, $p < 0.0001$) and soil sterilization ($\chi^2 = 7.43$, df=1, $p = 0.006$), as well as a species by sterilization interaction ($\chi^2 = 6.09$, df = 2, $p = 0.047$). *Post-hoc* comparisons showed that soil sterilization only affected wiregrass root biomass (Figure 3.2C). Wiregrass plants grown in sterilized soils produced 92% more root biomass compared to live soils ($t = -4.977$, $p < 0.0001$), whereas sensitive-briar and twinflower showed no significant difference between live and sterilized soils ($p = 0.92$, $p = 0.099$, respectively).

Single-species PSFs were mediated by microbes and fire treatment

Log-response ratios of total biomass in conspecific soil compared to heterospecific soil showed little variation across fire and soil sterilization treatments, which was not unexpected given the lack of soil source effects on biomass above. One exception was wiregrass, where we found a significant interaction between fire treatment and soil sterilization ($F_{1,47} = 6.57$, $p = 0.013$). When inoculated with live soils, wiregrass exhibited more negative PSFs under annual fire regimes compared to maintenance fire regimes ($t = -2.33$, $p = 0.024$) (Figure 3.3). Wiregrass exhibited reduced growth in annual live sensitive-briar soil compared to its own soil, resulting in negative PSF ($t = -2.17$, $p = 0.034$), with a similar, though not statistically significant, trend observed in twinflower annual soil ($t = -1.54$, $p = 0.12$). Conversely, wiregrass grew better in annual sterile twinflower soil, resulting in positive PSF ($t = 2.38$, $p = 0.021$). Twinflower also showed improved growth in sterile annual soils conditioned by wiregrass relative to its own soil, resulting in positive PSF ($t = -2.81$, $p = 0.006$). In contrast, sensitive-briar showed neutral PSFs across all treatment combinations.

Net pairwise PSFs were mostly neutral

Net pairwise PSFs in total biomass were largely neutral across plant species pairs (Figure 3.4). However, there was a significant interaction between fire and soil sterilization ($F_{1,77} = 4.23$, $p = 0.042$), and fire and plant species pair ($F_{2,77} = 4.03$, $p = 0.021$) in their impacts on net pairwise PSFs. We detected a significant positive net pairwise PSF between wiregrass and twinflower in sterilized annual soils ($t = 3.77$, $p = 0.0003$), indicating that in the absence of soil microbes, both species performed better in their own soils' abiotic conditions relative to each other's soils, suggesting exclusion. In contrast, there was a significant negative net pairwise PSF between wiregrass and sensitive-briar, where the feedback was more negative in annual live soils compared to maintenance live soils, suggesting microbe-mediated coexistence ($t = -2.12$, $p = 0.037$). Notably, the two significant pairwise PSFs we observed were all in annual fire treatments, which builds on the significant feedback ratios found in the previous section.

Discussion

Plant biomass was best predicted by the presence of soil microbes, regardless of fire regime or prior plant conditioning legacy

Plant performance in our study was more strongly influenced by soil sterilization than by fire regime legacy. Wiregrass exhibited significantly greater survival and biomass in sterilized soils across all soil sources, suggesting that live soils harbored microbial antagonists that suppressed its growth. Although sterilization elevated concentrations of several nutrients (e.g. P, K, Ca, Mg), likely due to nutrient release from microbial lysis during autoclaving (Berns et al. 2008), the observed decline in NO_3^- and the limited evidence for nutrient limitation in driving plant performance suggest that nutrient availability alone does not explain the observed growth responses. The improved performance of wiregrass in sterilized soils aligns with previous studies showing that pathogen removal can enhance plant growth (Semchenko et al. 2018, Van Grunsven et al. 2007), reinforcing the idea that microbial antagonists constrained this species' success. In contrast, sensitive-briar produced greater biomass in live soils than in sterilized soils. Like many legumes, this species forms mutualistic associations with nitrogen-fixing rhizobia (Andrews & Andrews 2017), and sterilization likely disrupted these mutualisms, limiting its growth. Despite elevated mineral nutrients in sterilized soils, the loss of microbial partners essential for nitrogen acquisition appears to have outweighed any nutrient benefit. These contrasting responses between a dominant graminoid and a leguminous forb highlight the species-specific nature of plant-microbe interactions and demonstrate that microbial communities can shape plant performance independently of fire regime legacy effects.

Fire regime impacts on PSFs were subtle, but strongest for the pyrogenic dominant wiregrass

Our results did not support our hypothesis that annual fire would promote positive conspecific PSFs for wiregrass and twinflower by reducing host-specific pathogen abundance and enhancing host-specific mutualists. Instead, wiregrass exhibited strongly negative PSFs in live soils from the annual fire regime, and all species showed neutral PSFs in soils from the maintenance fire regime, regardless of soil source or sterilization. Wiregrass, a late successional species dominant in longleaf pine savannas (Laucevicius et al. 2021, Baruzzi et al. 2022), is tightly coupled to frequent, low-intensity fire regimes characteristic of this ecosystem. Late successional species, such as wiregrass, are hypothesized to promote positive PSFs through strong interactions with soil mutualists (Bauer et al. 2015, Kardol, Bezemer & Van der Putten 2006). This was supported by Baruzzi et al. (2022), who observed reduced wiregrass growth in non-native soil ecotypes, suggesting susceptibility to antagonistic interactions outside of conspecific soil. Similarly, Hopkins et al. (2024) found that fire reduced negative PSFs and promoted conspecific growth in another dominant bunchgrass, *Schizochyrium scoparum*, showing that low-intensity fire benefits grass dominance in savanna ecosystems. However, these studies focused on soil edaphic variation and the presence or absence of fire, whereas our study evaluated differences in fire regimes. Contrary to these patterns, wiregrass in our experiment performed worse in its own soil under annual fire, suggesting that high frequency fire promotes the buildup of antagonistic soil biota for this species, undermining potential mutualist benefits and shifting PSFs from neutral to negative. Furthermore, while pairwise feedbacks were neutral across most species' combinations, we observed a negative net pairwise feedback between wiregrass and sensitive-briar in live soils from the annual fire regime. These trends suggest that soil microbes may promote coexistence between these species by causing these species to limit themselves more than they limit the other species (Bever 1997).

Implications for fire-driven plant-soil feedbacks for longleaf pine restoration

Fire management is central to restoration in longleaf pine ecosystems (Mitchell et al. 2006, Wolcott et al. 2007), and a key component of this process is promoting vegetation-fire feedbacks (Beckage et al. 2011, Fill et al. 2015). Our findings show that variation in fire regimes can influence plant-soil feedbacks with implications for plant community diversity, establishment, and the spatial distribution of dominant species (Kardol et al. 2023). While frequent, low-intensity fires are known to promote graminoid recruitment and maintain open structure (Glitzenstein et al. 2003, Bond & Woodward 2003), burning too frequently may foster the accumulation of antagonistic soil biota that suppresses key graminoid species. Thus, the negative PSFs we observed in wiregrass biomass under the annual fire regime may reduce fine fuel continuity and alter the spatial heterogeneity of fire intensity in subsequent prescribed fire events. In contrast, prescribed burning under a 1–3-year maintenance regime may help prevent pathogen buildup for the species and support the persistence or recovery of beneficial mutualists. Allowing longer recovery periods between burns may give mutualistic soil microbes time to reestablish, thereby strengthening plant-microbe interactions and enhancing ecosystem resilience and restoration outcomes.

Nuances in plant-soil feedback outcomes

While our results highlight the importance to incorporating fire regime into PSF frameworks, the patterns we observed are nuanced. The negative conspecific PSFs identified in annually burned soils may act as a stabilizing mechanism that promotes coexistence by reducing wiregrass performance when locally abundant (Chesson 2000, Chung & Rudgers 2016). However, this self-limiting effect appears to contrast with our field observations, where wiregrass showed greater dominance under the annual regime relative to the maintenance regime. This discrepancy may, in part, reflect our field sampling design. Soils from the annual regime were collected in areas dominated by wiregrass, while the maintenance regime supported a more diverse groundcover, likely representing a broader mix of plant-conditioned soils. As a result, the general soil species pool may be more reflective of wiregrass-specific pathogens to begin with and thus, lead to more negative PSFs for wiregrass in annual fire soils. Additionally, we sampled ~9 months after fire in the annual regime, and ~11 months post-fire in the maintenance regime. Although fire imposes lasting legacies on soil microbial communities (Perez-Valera et al. 2018), soil microbial abundance and composition can shift as time since fire increases through post-fire recovery (Hart et al. 2005, Fortúrbel et al. 2012). Thus, the feedbacks we observed may reflect both plant-microbe interactions and the temporal dynamics of microbial succession, which are often most pronounced shortly after fire as microbial communities undergo recovery and reassembly (Barreiro & Díaz-Raviña 2021).

Conclusion

Our findings demonstrate that plant-soil feedbacks are both species-specific and fire regime-dependent, underscoring the complex role of soil microbial communities in mediating plant performance under different disturbance regimes. As fire activity intensifies globally due to climate change and altered land use, it is increasingly important to contextualize PSFs within dynamic environmental conditions. Integrating PSFs into fire ecology frameworks can improve our understanding of plant community assembly and resilience. Moreover, explicitly incorporating soil biotic interactions into fire management and restoration strategies may enhance biodiversity, support the reestablishment of key species, and promote long-term stability in fire-adapted ecosystems.

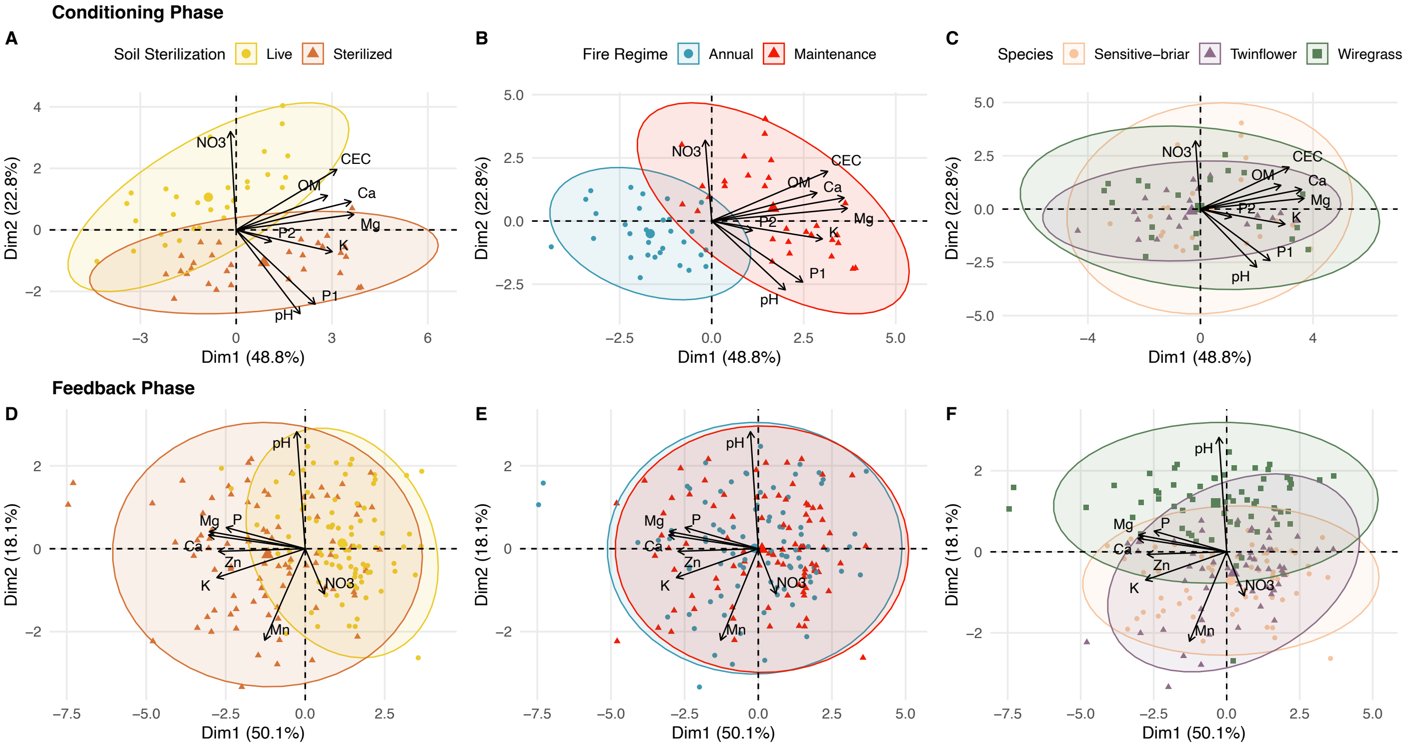

Figure 3.1: Principal component analysis of soil variables among treatments. Biplot diagrams from the Principal Component Analysis (PCA) of all soil response variables across soil sterilization treatments (live, sterilized), fire regime treatments (annual, maintenance) and species identity (sensitive-briar, twinflower, wiregrass). Panels A-C represent the conditioning phase, showing all responses by A) soil sterilization, B) fire regime, and C) conditioning species. Panels D-F represent the feedback phase, showing soil responses by D) soil sterilization, E) fire regime, and F) plant species identity, referring to the species growing in each pot. Soil nutrient projections on the plane are defined by principal components axes (PC) 1 and 2.

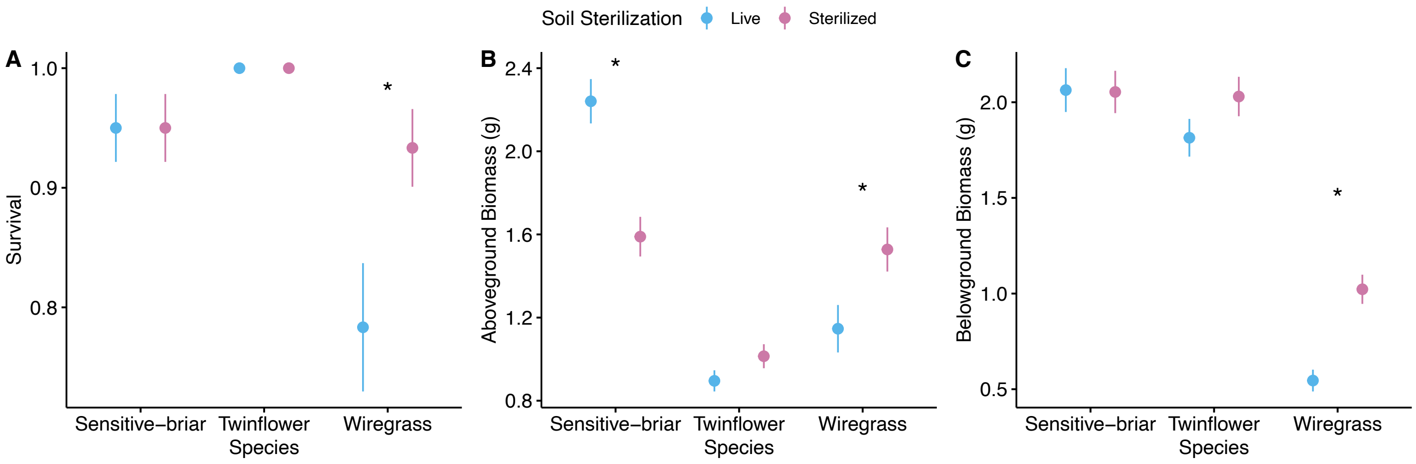

Figure 3.2: Survival and biomass of three focal species across sterilization treatments. Mean (± SE) of A) survival, B) aboveground biomass (g), and C) belowground biomass (g) across sensitive-briar, twinflower, and wiregrass. Significant *post-hoc* comparisons are indicated by an asterisk (*).

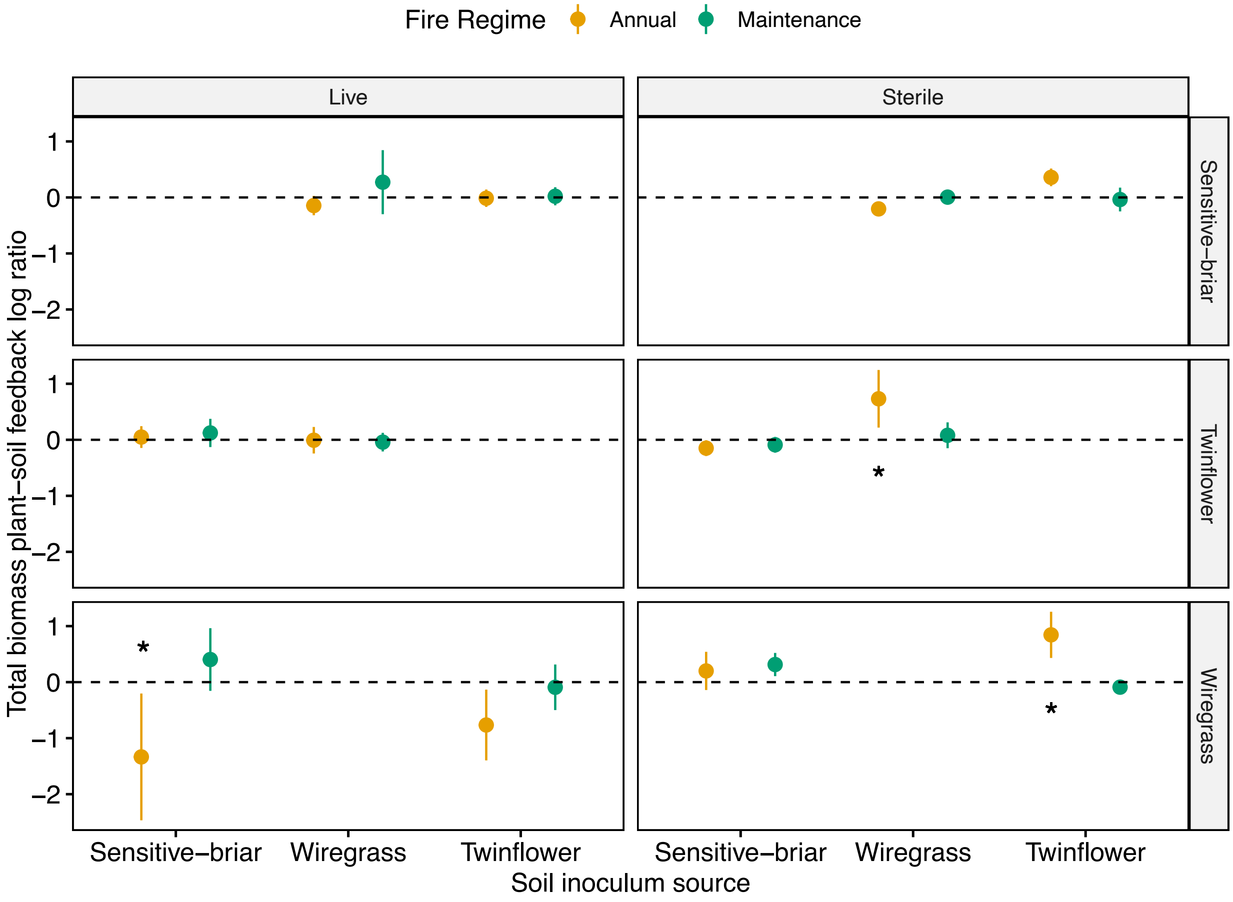

Figure 3.3: **Total biomass feedback ratios.** Feedback ratio (±SE) for each species' for total biomass across the three soil environments. Asterisks (*) denotes ratios significantly different from zero.

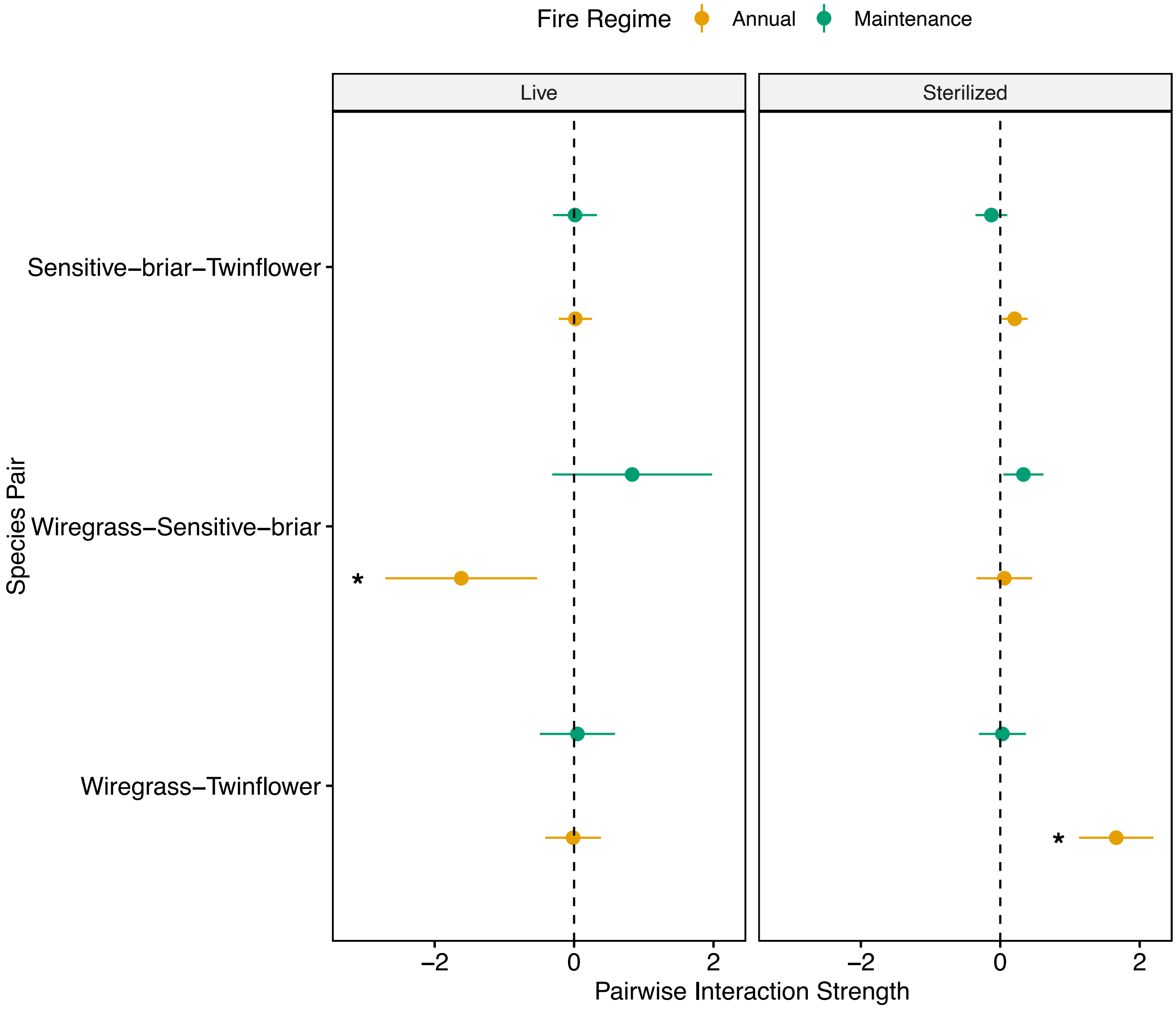

Figure 3.4: **Pairwise interaction strengths for total biomass.** Pairwise interaction strengths (±SE) for total biomass for all three plant species across the three soil environments. Asterisks (*) denotes ratios significantly different from zero.

References

Andrews, M., & Andrews, M. E. (2017). Specificity in legume-rhizobia symbioses. *International Journal of Molecular Sciences*, 18(4), 705. https://doi.org/10.3390/ijms18040705

Alcañiz, M., Outeiro, L., Francos, M., & Úbeda, X. (2018). Effects of prescribed fires on soil properties: A review. *Science of the Total Environment*, 613, 944-957. https://doi.org/10.1016/j.scitotenv.2017.09.144

Archibald, S., Lehmann, C. E., Belcher, C. M., Bond, W. J., Bradstock, R. A., Daniau, A. L., ... & Zanne, A. E. (2018). Biological and geophysical feedbacks with fire in the Earth system. *Environmental Research Letters*, 13(3), 033003. https://doi.org/10.1088/1748-9326/aa9ead

Bauer, J. T., Mack, K. M., & Bever, J. D. (2015). Plant-soil feedbacks as drivers of succession: evidence from remnant and restored tallgrass prairies. *Ecosphere*, 6(9), 1-12. https://doi.org/10.1890/ES14-00480.1

Barreiro, A., & Díaz-Raviña, M. (2021). Fire impacts on soil microorganisms: Mass, activity, and diversity. *Current Opinion in Environmental Science & Health*, 22, 100264. https://doi.org/10.1016/j.coesh.2021.100264

Baruzzi, C., Medina-Irizarry, N., Armstrong, M. N., & Crandall, R. M. (2022). Fire season and canopy cover interact to affect reproduction of a pyrogenic bunchgrass, Aristida beyrichiana. *Plant Ecology*, 223(3), 263-272. https://doi.org/10.1007/s11258-021-01204-7

Beckage, B., Gross, L. J., & Platt, W. J. (2011). Grass feedbacks on fire stabilize savannas. *Ecological Modelling*, 222(14), 2227-2233. https://doi.org/10.1016/j.ecolmodel.2011.01.015

Berns, A. E., Philipp, H., Narres, H. D., Burauel, P., Vereecken, H., & Tappe, W. (2008). Effect of gamma-sterilization and autoclaving on soil organic matter structure as studied by solid state NMR, UV and fluorescence spectroscopy. *European Journal of Soil Science*, 59(3), 540-550. https://doi.org/10.1111/j.1365-2389.2008.01016.x

Bever, J. D., Westover, K. M., & Antonovics, J. (1997). Incorporating the soil community into plant population dynamics: the utility of the feedback approach. *Journal of Ecology*, 561-573. https://doi.org/10.2307/2960528

Bever, J. D., Platt, T. G., & Morton, E. R. (2012). Microbial population and community dynamics on plant roots and their feedbacks on plant communities. *Annual Review of Microbiology*, 66(1), 265-283. https://doi.org/10.1146/annurev-micro-092611-150107

Bever, J. D., Mangan, S. A., & Alexander, H. M. (2015). Maintenance of plant species diversity by pathogens. *Annual Review of Ecology, Evolution, and Systematics*, 46(1), 305-325. https://doi.org/10.1146/annurev-ecolsys-112414-054306

Bond, W. J., Midgley, G. F., & Woodward, F. I. (2003). The importance of low atmospheric CO2 and fire in promoting the spread of grasslands and savannas. *Global Change Biology*, 9(7), 973-982. https://doi.org/10.1046/j.1365-2486.2003.00577.x

Carvalho, L. M., Antunes, P. M., Martins-Loução, M. A., & Klironomos, J. N. (2010). Disturbance influences the outcome of plant–soil biota interactions in the invasive Acacia longifolia and in native species. *Oikos*, 119(7), 1172-1180. https://doi.org/10.1111/j.1600-0706.2009.18148.x

Certini, G. (2005). Effects of fire on properties of forest soils: a review. *Oecologia*, 143(1), 1-10. https://doi.org/10.1007/s00442-004-1788-8

Certini, G., Moya, D., Lucas-Borja, M. E., & Mastrolonardo, G. (2021). The impact of fire on soil-dwelling biota: A review. *Forest Ecology and Management*, 488, 118989. https://doi.org/10.1016/j.foreco.2021.118989

Chesson, P. (2000). Mechanisms of maintenance of species diversity. *Annual Review of Ecology and Systematics*, 31(1), 343-366. https://doi.org/10.1146/annurev.ecolsys.31.1.343

Chung, Y. A. (2023). The temporal and spatial dimensions of plant–soil feedbacks. *New Phytologist*, 237(6), 2012-2019. https://doi.org/10.1111/nph.18719

Chung, Y. A., & Rudgers, J. A. (2016). Plant–soil feedbacks promote negative frequency dependence in the coexistence of two aridland grasses. *Proceedings of the Royal Society B: Biological Sciences*, 283(1835), 20160608. https://doi.org/10.1098/rspb.2016.0608

Cortois, R., Schröder-Georgi, T., Weigelt, A., van der Putten, W. H., & De Deyn, G. B. (2016). Plant–soil feedbacks: role of plant functional group and plant traits. *Journal of Ecology*, 104(6), 1608-1617. https://doi.org/10.1111/1365-2745.12643

Crawford, K. M., Bauer, J. T., Comita, L. S., Eppinga, M. B., Johnson, D. J., Mangan, S. A., ... & Bever, J. D. (2019). When and where plant-soil feedback may promote plant coexistence: a meta-analysis. *Ecology Letters*, 22(8), 1274-1284. https://doi.org/10.6084/m9.figshare.7985195.v1

De Long, J. R., Heinen, R., Heinze, J., Morriën, E., Png, G. K., Sapsford, S. J., ... & Fry, E. L. (2023). Plant-soil feedback: incorporating untested influential drivers and reconciling terminology. *Plant and Soil*, 485(1), 7-43. https://doi.org/10.1007/s11104-023-05908-9

Dove, N. C., & Hart, S. C. (2017). Fire reduces fungal species richness and in situ mycorrhizal colonization: a meta-analysis. *Fire Ecology*, 13(2), 37-65. https://doi.org/10.4996/fireecology.130237746

Drew, M. B., Kirkman, L. K., & Gholson Jr, A. K. (1998). The vascular flora of Ichauway, Baker County, Georgia: a remnant longleaf pine/wiregrass ecosystem. *Castanea*, 1-24. https://www.jstor.org/stable/4034053

Fill, J. M., Platt, W. J., Welch, S. M., Waldron, J. L., & Mousseau, T. A. (2015). Updating models for restoration and management of fiery ecosystems. *Forest Ecology and Management*, 356, 54-63. https://doi.org/10.1016/j.foreco.2015.07.021

Fontúrbel, M. T., Barreiro, A., Vega, J. A., Martín, A., Jiménez, E., Carballas, T., ... & Díaz-Raviña, M. (2012). Effects of an experimental fire and post-fire stabilization treatments on soil microbial communities. *Geoderma*, 191, 51-60. https://doi.org/10.1016/j.geoderma.2012.01.037

Glassman, S. I., Levine, C. R., DiRocco, A. M., Battles, J. J., & Bruns, T. D. (2016). Ectomycorrhizal fungal spore bank recovery after a severe forest fire: some like it hot. *The ISME Journal*, 10(5), 1228-1239. https://doi.org/10.1038/ismej.2015.182

Glitzenstein, J. S., Streng, D. R., & Wade, D. D. (2003). Fire frequency effects on longleaf pine (Pinus palustris P. Miller) vegetation in South Carolina and northeast Florida, USA. *Natural Areas Journal*. 23 (1): 22-37. 2003.

Hart, S. C., DeLuca, T. H., Newman, G. S., MacKenzie, M. D., & Boyle, S. I. (2005). Post-fire vegetative dynamics as drivers of microbial community structure and function in forest soils. *Forest Ecology and Management*, 220(1-3), 166-184. https://doi.org/10.1016/j.foreco.2005.08.012

He, T., Lamont, B. B., & Pausas, J. G. (2019). Fire as a key driver of Earth's biodiversity. *Biological Reviews*, 94(6), 1983-2010. https://doi.org/10.1111/brv.12544

Hewitt, R. E., Hollingsworth, T. N., Stuart Chapin III, F., & Lee Taylor, D. (2016). Fire-severity effects on plant–fungal interactions after a novel tundra wildfire disturbance: implications for arctic shrub and tree migration. *BMC Ecology*, 16(1), 25. https://doi.org/10.1186/s12898-016-0075-y

Hopkins, J. R., & Bennet, A. E. (2024). Fire effects on soil biota alter the strength and direction of plant-soil feedbacks between Schizachyrium scoparium (Michx.) Nash and Rudbeckia hirta L. *Plant and Soil*, 1-15. https://doi.org/10.1007/s11104-024-06585-y

Kardol, P., Martijn Bezemer, T., & Van Der Putten, W. H. (2006). Temporal variation in plant–soil feedback controls succession. *Ecology Letters*, 9(9), 1080-1088. https://doi.org/10.1111/j.1461-0248.2006.00953.x

Kardol, P., Yang, T., Arroyo, D. N., & Teste, F. P. (2023). Plant-soil feedback in the 'real world': how does fire fit into all of this?. *Plant and Soil*, 485(1), 91-102. https://doi.org/10.1007/s11104-022-05778-7

Kirkman, L. K., Mitchell, R. J., Helton, R. C., & Drew, M. B. (2001). Productivity and species richness across an environmental gradient in a fire-dependent ecosystem. *American Journal of Botany*, 88(11), 2119-2128. https://doi.org/10.1002/ecy.1456

Kulmatiski, A., Beard, K. H., Stevens, J. R., & Cobbold, S. M. (2008). Plant–soil feedbacks: a meta-analytical review. *Ecology Letters*, 11(9), 980-992. https://doi.org/10.1111/j.1461-0248.2008.01209.x

Laucevicius Jr, A. M., Robertson, K. M., Means, D. B., Mitchell, T. R., & Taylor, P. B. (2021). Expansion and population structure of transplanted Aristida beyrichiana (wiregrass) tussocks: results of a 37-year study. *Restoration Ecology*, 29(5), e13404. https://doi.org/10.1111/rec.13404

Lenth, R. V (2024). Emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.10.1. https://cran.r-project.org/web/packages/emmeans/index.html

Mitchell, R. J., Hiers, J. K., O'Brien, J. J., Jack, S. B., & Engstrom, R. T. (2006). Silviculture that sustains: the nexus between silviculture, frequent prescribed fire, and conservation of biodiversity in longleaf pine forests of the southeastern United States. *Canadian Journal of Forest Research*, 36(11), 2724-2736. https://doi.org/10.1139/x06-100

Mulligan, M. K., Kirkman, L. K., & Mitchell, R. J. (2002). Aristida beyrichiana (wiregrass) establishment and recruitment: implications for restoration. *Restoration Ecology*, 10(1), 68-76. https://doi.org/10.1046/j.1526-100X.2002.10107.x

Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, 403(6772), 853-858. https://doi.org/10.1038/35002501

Noss, R. F., Platt, W. J., Sorrie, B. A., Weakley, A. S., Means, D. B., Costanza, J., & Peet, R. K. (2015). How global biodiversity hotspots may go unrecognized: lessons from the North American Coastal Plain. *Diversity and Distributions*, 21(2), 236-244. https://doi.org/10.1111/ddi.12278

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., ... & Wagner, H. (2022). vegan: Community Ecology Package. R package version 2.5-7. 2020. Preprint at, 3-1.

Pausas, J. G., & Keeley, J. E. (2009). A burning story: the role of fire in the history of life. *BioScience*, 59(7), 593-601. https://doi.org/10.1525/bio.2009.59.7.10

Peet, R. K., Platt, W. J., & Costanza, J. K. (2018). Fire-maintained pine savannas and woodlands of the southeastern United States coastal plain. In *Ecology and recovery of eastern old-*

growth forests (pp. 39-62). Washington, DC: Island Press/Center for Resource Economics. https://doi.org/10.5822/978-1-61091-891-6_3

Pérez-Valera, E., Verdu, M., Navarro-Cano, J. A., & Goberna, M. (2018). Resilience to fire of phylogenetic diversity across biological domains. *Molecular Ecology*, 27(13), 2896-2908. https://doi.org/10.1111/mec.14729

Prendergast-Miller, M. T., De Menezes, A. B., Macdonald, L. M., Toscas, P., Bissett, A., Baker, G., ... & Thrall, P. H. (2017). Wildfire impact: Natural experiment reveals differential short-term changes in soil microbial communities. *Soil Biology and Biochemistry*, 109, 1-13. https://doi.org/10.1016/j.soilbio.2017.01.027

Pressler, Y., Moore, J. C., & Cotrufo, M. F. (2019). Belowground community responses to fire: meta-analysis reveals contrasting responses of soil microorganisms and mesofauna. *Oikos*, 128(3), 309-327. https://doi.org/10.1111/oik.05738

R Core Team, R. (2020). R: A language and environment for statistical computing.

Revillini, D., David, A. S., Menges, E. S., Main, K. N., Afkhami, M. E., & Searcy, C. A. (2022). Microbiome-mediated response to pulse fire disturbance outweighs the effects of fire legacy on plant performance. *New Phytologist*, 233(5), 2071-2082. https://doi.org/10.1111/nph.17689

Semchenko, M., Leff, J. W., Lozano, Y. M., Saar, S., Davison, J., Wilkinson, A., ... & Bardgett, R. D. (2018). Fungal diversity regulates plant-soil feedbacks in temperate grassland. *Science Advances*, 4(11), eaau4578. https://doi.org/10.1126/sciadv.aau4578

Senior, J. K., O'Reilly-Wapstra, J. M., Schweitzer, J. A., Bailey, J. K., & Potts, B. M. (2018). Forest fire may disrupt plant–microbial feedbacks. *Plant Ecology*, 219(5), 497-504. https://doi.org/10.1007/s11258-018-0811-9

Smith-Ramesh, L. M., & Reynolds, H. L. (2017). The next frontier of plant–soil feedback research: unraveling context dependence across biotic and abiotic gradients. *Journal of Vegetation Science*, 28(3), 484-494. https://doi.org/10.1111/jvs.12519

Van der Putten, W. H., Bardgett, R. D., Bever, J. D., Bezemer, T. M., Casper, B. B., Fukami, T., ... & Wardle, D. A. (2013). Plant–soil feedbacks: the past, the present and future challenges. *Journal of Ecology*, 101(2), 265-276. https://doi.org/10.1111/1365-2745.12054

Van Grunsven, R. H., Van Der Putten, W. H., Bzemer, T. M., Tamis, W. L., Berendse, F., & Veenendaal, E. M. (2007). Reduced plant-soil feedback of plant species expanding their range as sompared to natives. *Journal of Ecology*, 1050-1057. https://www.jstor.org/stable/4496058

Walker, J., & Peet, R. K. (1984). Composition and species diversity of pine-wiregrass savannas of the Green Swamp, North Carolina. *Vegetatio*, 55(3), 163-179. https://doi.org/10.1007/BF00045019

Warneke, C. R., Yelenik, S. G., & Brudvig, L. A. (2023). Fire modifies plant–soil feedbacks. *Ecology*, 104(5), e3994. https://doi.org/10.1002/ecy.3994

Wolcott, L., O'Brien, J. J., & Mordecai, K. (2007). A survey of land managers on wildland hazardous fuels issues in Florida: A technical note. *Southern Journal of Applied Forestry*, 31(3), 148-150. https://doi.org/10.1093/sjaf/31.3.148

CHAPTER 4

LONG-TERM FIRE REGIME AND SOIL DEPTH DRIVE DIVERGENT PLANT AND SOIL MICROBIAL POST-FIRE RECOVERY TRAJECTORIES IN A LONGLEAF PINE SAVANNA[3]

---

[3] Henson, M.S., Giencke, L.M., Mishra, A., Chung, Y.A. To be submitted to *New Phytologist*.

Abstract

Fire is a critical ecological process that shapes biological communities and ecosystem structure. Soil bacteria and fungi play essential roles in post-fire recovery and regeneration by driving biogeochemical cycling and influencing ecosystem productivity. However, we lack insight into post-fire soil microbial successional trajectories compared to their pre-fire state, and how long-term fire regimes alter such trajectories. Here, we examined plant, fungal, and bacterial community responses to a single low-intensity prescribed fire across two fire regimes (annual, 1-year fire return interval; maintenance, 1–3-year fire return interval) and two soil depths (0-3 and 3-5 cm) from pre-fire to 1-year post-fire in a longleaf pine savanna in southwestern Georgia. Our results showed that fire regime imposed a strong legacy effect on plant and soil microbial communities. Specifically, annual fires reduced plant and fungal diversity, increased bacterial richness, and led to slower depth-dependent fungal recovery from a single fire event. Interestingly, subsurface soils were less resistant to fire, and fungi did not recover, suggesting dispersal limitation in community reassembly. Immediately post-fire, endophytic and ectomycorrhizal fungi increased, while pathogens, parasites, and saprotrophs declined relative to pre-fire conditions. We also identified fire-responsive and fire-sensitive taxa across fungal and bacterial groups that showed different recovery strategies under varying fire regimes. Overall, our results reveal that annual fire regimes reduce richness, delay recovery, and weaken resilience, while maintenance fire regimes more effectively support biodiversity, particularly for plants and fungi.

Introduction

Fire is a major ecological and evolutionary force that shapes biodiversity, species interactions, and ecosystem function in terrestrial ecosystems (Krebs et al. 2010, McLauchlan et al. 2020, Pausas & Keeley 2009, He, Lamont, & Pausas 2019). As climate change continues to alter historical patterns of fire frequency, seasonality, extent, and severity (hereafter fire regime), it is increasingly important to understand how variation in fire regimes influence biological community structure and function (Knelman et al. 2015, Kelly et al. 2020). Fire drives major changes in ecosystem structure and function (Wasserman & Mueller 2023, Hagmann et al. 2021), leading to altered successional trajectories, which in turn can feedback to influence future fire behavior by modifying plant recolonization and soil microbial community structure and function (Hopkins et al. 2021, Knelman et al. 2015). Post-fire plant recolonization is shaped by well-characterized mechanisms such as dispersal limitation, environmental filtering, and biotic interactions (Pulido-Chavez et al. 2023, Caifa et al. 2023, Yang et al. 2020, Salo & Kouki 2018, Knelman et al. 2015, Greenwood et al. 2023). However, it remains unclear whether similar processes govern the reassembly and successional dynamics of soil microbial communities.

Soil microbial responses to fire are influenced by multiple interacting factors, including ecosystem type (Dooley & Treseder 2012), time since fire (Pulido-Chavez et al. 2021, Caifa et al. 2023), fire regime (Pressler et al. 2019), and soil depth (Fox et al. 2024, Barbour et al. 2022). Of these, the impacts of soil depth and fire regime remain the most enigmatic. Variation in fire severity and heat penetration with depth can create post-fire niches, shaping microbial turnover and recovery, yet is seldom explicitly studied. In fire-prone ecosystems, both plant and soil microbial communities have evolved traits that enhance persistence and recolonization after fire, such as heat resistance or tolerance, rapid colonization, dormancy, and regeneration from seed or spore banks (Crowther et al. 2014, Glassman et al. 2016). Pyrophilous microbes exhibit such adaptations, such as heat resistance (Peay et al. 2009), fast growth rates (Whitman et al. 2019), rapid dispersal into newly burned areas (Barbour et al. 2023), post-fire nutrient acquisition (Fischer et al. 2021), and survival in harsh post-fire conditions (Hopkins and Bennett 2023), driving successional shifts over weeks to years (Pulido-Chavez et al. 2021, Hopkins et al. 2021, Dove et al. 2022). These traits likely vary with soil depth, as surface microbial communities experience the most intense heating and may favor resistance or spore-forming strategies, whereas microbes in deeper soils may avoid lethal temperatures but depend on fast growth and upward colonization to explore post-fire resource pulses near the surface (Bruns et al. 2020, Hopkins et al. 2025, Johnson et al. 2023, Palmer et al. 2023). Recolonization of deeper layers may also occur more slowly if recovery is primarily driven by aerial deposition rather than in situ survival or vertical dispersal. However, the ways in which fire regime and soil depth interact to shape microbial succession remain poorly understood, leaving a critical gap in our understanding of post-fire ecosystem recovery.

Plant and soil microbial community structure is shaped not only by the immediate effects of a single fire event, but also by the long-term legacy of fire regimes (Pellegrini and Jackson 2020). A single high severity "pulse" fire can reset successional trajectories by disrupting existing community structure and opening niche space (Keeley 2009). In contrast, fire legacy acts as a "press" disturbance, exerting strong selective pressure on life-history strategies that influence population persistence and the broader environmental context in which succession plays out (Johnstone et al. 2016). For plants, a pulse fire often leads to rapid regrowth and resprouting, favoring species with traits that recover quickly, and temporarily results in transient shifts in community composition with an eventual return to pre-fire states (Pellegrini and

Jackson 2020). However, the legacy of fire regimes can reshape plant communities over time, acting as a persistent ecological filter that promotes fire-adapted traits and excludes fire-sensitive taxa (Glitzenstein et al. 2012). Meanwhile, soil microbial communities respond on much faster timescales. A single fire event can lead to abrupt reductions in microbial biomass and richness, particularly in surface layers directly exposed to heat, followed by rapid recovery through recolonization by heat-tolerant taxa or activation of dormant propagules (Whitman et al. 2019, Pulido-Chavez, 2021, Enright et al. 2023). Unlike plants, soil microbial recovery is driven largely by dispersal and high reproductive rates than demographic turnover, and their high functional redundancy can buffer ecosystem processes when composition changes (Allison & Martiny 2008). With repeated burning, however, soil microbial communities may undergo longer-term restructuring, such as declines in sensitive or slow-growing taxa and shifts in key functional groups, that can alter their capacity to respond to subsequent fires (Dooley & Treseder 2012). Despite this, few studies have considered the interactive effects of both pulse fire and fire legacy on soil microbial communities. However, Revillini et al. (2022) found that the immediate responses of soil microbiomes to a pulse fire strongly influenced plant performance, whereas legacy fire history had a smaller effect. Conversely, Revillini et al. (2025) showed that long-term fire legacy alters microbial functional gene expression for carbon and nutrient cycling, modifying how communities respond to subsequent fires. Collectively, these findings suggest that while pulse fires drive short-term microbial restructuring, fire legacy preconditions the functional potential of soil communities, shaping the magnitude, direction, and recovery trajectory of post-fire responses. Ultimately, these patterns raise broader questions about how pulse and legacy disturbances interact to shape plant and microbial community resilience, the timescales over which communities recover, and the role of microbes in mediating ecosystem processes after fire. Thus, integrating these disturbance types is essential for understanding ecosystem responses and predicting the impacts of global change (Pellegrini and Jackson 2020). Given the central role of microbes in post-disturbance recovery and ecosystem function, understanding their spatial and temporal dynamics is critical for predicting biodiversity and ecosystem resilience in fire-prone systems under increasingly variable fire regimes (Yang et al. 2020, Johnston et al. 2016).

In this study, we conducted a field experiment using fine-scale temporal sampling to characterize plant and soil microbial community recovery dynamics across multiple time points before and after a single prescribed fire event in an imperiled longleaf pine ecosystem. We surveyed soil bacterial and fungal communities across two soil depths (0-3 cm and 3-5 cm), and all communities at nine time points spanning pre-fire (day of fire) and post-fire (1 hr, 2 wk, 1 mo, 3 mo, 6 mo, 9 mo, 12 mo). Our site differed in prescribed fire regime (annual and maintenance burns), allowing us to assess how legacy effects influence community trajectories. We asked the following questions: 1) How do fire regimes influence plant and soil microbial community recovery trajectories following a single fire event? 2) How do soil microbial community responses vary by soil depth? 3) Do microbial taxa exhibit distinct successional trajectories following fire, and how are these patterns shaped by fire regime and time since fire? We predicted that: 1) annual fires would immediately reduce plant and soil microbial richness, and recovery of richness would occur over 12 months under the maintenance regime but would remain suppressed under annual fire. We also hypothesized that community composition would shift rapidly post-fire and remain distinct by fire regime across the entire time series, 2) soil microbial communities would experience larger declines in the surface soil (0-3 cm) compared to the subsurface soil depth (3-5 cm). Specifically, we predicted that the influence of time since fire would be the strongest in the topmost soil depth and show a more muted response in the subsurface soil, and 3) certain microbial taxa and functional guilds would increase in abundance post-fire, while most others (pathogens, ectomycorrhizal fungi, and saprotrophs) would decline. Additionally, we anticipated these patterns would vary by fire regime, with more extreme shifts for taxa under annual fire compared to maintenance.

Materials and Methods

Study site and experimental design

We conducted our study at The Jones Center at Ichauway, which is an ecological reserve located in the southern Coastal Plain region of Georgia (31.2201° N; 84.4779° W) (Newton, Georgia, USA). There, we utilized one field site (21-Acre) from a long-term field experiment, which consists of two burn units, where each burn unit has been exposed to a unique single experimental fire regime. Since 1990, fire has been applied every 1-3 years with varying seasonality as part of the "maintenance" fire regime, which is believed to be the historical fire regime in this ecosystem. In 2003, experimental yearly fires were implemented in late spring/early summer (growing season) as part of the "annual" fire regime. The site is in an upland longleaf pine-grassland savanna, characterized by an open canopy and a dense understory of bunchgrasses and forbs. The burn units at the site are divided by a secondary road. In February 2023, we established ten 1m^2 plots 6 m apart within a 30 m x 10 m area within each fire treatment side. Our study was conducted using a split-plot design, with fire treatments applied at the whole plot-level and 1m^2 subplots serving as replicates. While this design introduces potential pseudoreplication due to limited independence among subplots (Hurlbert 1984), such constraints can be common in fire ecology field studies where replicating disturbance at relevant spatial scales is logistically challenging (Van Mantgem et al. 2001, Davies & Gray 2015). Prior work suggests spatial independence of surface fuels at scales beyond 0.5 m^2 (Hiers et al. 2009), supporting our use of subplots as replicate units. During our sampling year (2023), the "maintenance" side was burned in March, and the "annual fire" side was burned in May using standard prescribed fire methods.

Soil and plant sampling

To fully characterize soil microbial community dynamics in response to a prescribed fire event, we collected soil samples the day of the prescribed fire before ignition, and at 1 hr, 24 hr, 2 weeks, 1 mo, and every 3 mos post-fire for ~1 year (9 time points: pre-fire, 1 hr post-fire, 24 hr, 2 wk, 1 mo, 3 mo, 6 mo, 9 mo, 12 mo). At each time point, we sampled each plot using six soil cores at 0-3 cm and 3-5 cm soil depths to characterize any spatial differences in soil microbe composition from the effects of fire. Keeping the depths separate, soil cores from each plot were pooled and homogenized, then subset for 45 g of soil for amplicon sequencing analyses. Temperature sensors (Thermochron iButtons, Dallas, Texas, USA) were buried in the center of each 1m^2 plot at 3 cm and 5 cm soil depth to measure soil temperature over the duration of the prescribed fire event. To assess simultaneous plant community compositional changes across time, we measured understory vegetation cover by visually assessing the relative area (%) covered by each plant species and total vegetation cover, bare ground, litter, and woody debris in each plot at each soil sampling time point. We identified plant species using Weakley's (2020) nomenclature.

Soil DNA extraction and PCR

Microbial genomic DNA was extracted from 250 mg of soil from each soil sample using the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol and stored at -20°C until PCR amplification. DNA concentration was measured using a Nanodrop spectrophotometer (ThermoFisher Scientific, Waltham, Massachusetts, USA) before downstream amplification. The bacterial 16S ribosomal RNA gene (V4 hypervariable region) and the fungal internal transcribed spacer (ITS2) region were targeted for their high variability and suitability for short paired-end Illumina NextSeq sequencing platform. Amplicons were generated using the 515F/806R primer pair for the bacterial 16S region (Caporaso et al. 2012) and fITS7 (Ihrmark et al. 2012) and ITS4 (White et al. 1990) for the fungal ITS2 region. To facilitate Illumina sequencing, the following overhang adapter sequences were appended to each primer set: 5’-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3’ and 5’-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3’.

Bacterial PCRs were conducted in 25 µL reaction volumes containing 6.25 µL nfH_2O, 5 µL 5X Q5 Reaction Buffer, 1 µL dNTPs (10 µM), 1.25 µL 515F primer (10 µM), 1.25 µL 806 primer (10 µM), 0.25 µL Q5 High-Fidelity DNA Polymerase, 5 µL Enhancer, and 5 µL DNA. The PCR protocol included an initial denaturation at 98°C for 30 seconds, followed by 10 touchdown cycles: denaturation at 98°C for 20 seconds, annealing at 65°C for 15 seconds (decreasing by 1°C per cycle), and extension at 72°C for 1 minute. This was followed by 25 cycles of denaturation at 98°C for 15 seconds, annealing at 56°C for 15 seconds, extension at 72°C for 1 minute, and a final extension at 72°C for 10 minutes. Fungal PCRs were carried out in 25 µL reaction volumes comprising 5 µL nfH_2O, 1.25 µL f/gITS7 primer (10 µM), 1.25 µL ITS4 primer (10 µM), 12.5 µL Phusion Green Hot Start II DNA Polymerase, and 5 µL DNA.

The thermocycler conditions were as follows: an initial denaturation at 98°C for 30 seconds, followed by 30 cycles of denaturation at 98°C for 10 seconds, annealing at 60°C for 15 seconds, and extension at 72°C for 30 seconds, with a final extension at 72°C for 5 minutes. All PCR products were visualized on a 1.5% agarose gel to confirm successful DNA amplification. Negative controls were included throughout the PCR procedures. The PCR products were then submitted for library preparation and sequencing on the Illumina NextSeq 2000 at the Georgia Genomics and Bioinformatics Core (Athens, Georgia, USA) (RRID:SCR_010994).

Bioinformatics

Paired-end sequences were demultiplexed, downloaded as FASTQ files, and imported into QIIME 2-2023.7 for downstream analyses. Sequence quality control and chimera filtering were performed using the QIIME2 DADA2 plugin (Callahan et al. 2016). The bacterial dataset generated 67,241,756 total reads, with forward reads truncated to 204 bp to exclude low-quality regions while maintaining sufficient length for taxonomic assignment. After quality filtering, 56,615,895 reads remained for analysis, and sequences were rarefied to the lowest sequencing depth of 47,419. The fungal dataset yielded 97,364,478 total reads, with forward reads truncated to 215 bp. Following quality control, 73,174,122 reads were retained, and sequences were rarefied to the lowest sequencing depth of 120,545. After performing rarefaction, one sample was excluded due to an exceptionally high number of reads. Taxonomic classification was conducted using the QIIME2 feature-classifier plugin with a Naïve Bayes classifier trained on the SILVA 138 SSU database (version 138) for bacteria and the UNITE (version 10) reference database for fungi. Sequences were clustered into amplicon sequence variants (ASVs) at 100% identity to facilitate the detection of fine-scale shifts in soil microbial communities over time.

Statistical Analyses

All analyses were conducted in R software version 4.0.2 (R Core Team 2020). We used linear mixed models using the *lme4* package in R (Bates et al. 2015) to test for differences in plant, bacterial, and fungal richness in response to the fire regime, soil depth, time point, and their interactions, with plot as a random factor to control for repeated measures. ANOVA was followed by *post-hoc* comparisons of group means using estimated marginal means with a Tukey test in the case of significant effects from the original model ($p < 0.05$). To analyze microbial and plant composition, we used the R package vegan (Oksanen et al. 2019) and derived Bray-Curtis distance matrices using vegdist() function. We ran PERMANOVA using the adonis() function to examine the effects of fire regime and depth on community composition, including pairwise comparisons between each post-fire time point and the pre-fire time points to assess community shifts over time. We used FungalTraits (Põlme et al. 2020) to assign ASVs to ecological guilds in each fire regime, time point, and depth. We aggregated fungal taxa into broader functional guilds, specifically saprotrophs (soil, litter, dung, wood, pollen), parasites (myco, animal, algal), and endophytes (root, foliar) to reduce the complexity of the dataset. The five most abundant guilds were saprotrophs, ectomycorrhizal fungi, parasites, pathogens, and endophytes. We conducted a Pearson's chi-square test of independence to determine whether fungal guild composition differed between fire, time, and depth treatments. To assess changes in fungal and bacterial taxa across time, we identified the top 10 most abundant genera at the pre-fire time point by summing the relative abundance of each genus across all pre-fire samples. We then filtered the dataset to retain only these top ten genera and calculated the mean relative abundance for each genus at each subsequent time point. Percent change in relative abundance was then calculated relative to the pre-fire baseline for each genus and time point.

Results

Post-fire soil temperature

Following fire ignition, soil temperature increased by approximately 5.7°C at 0-3 cm depth and 4.8°C at 3-5 cm depth in the maintenance fire regime. Under the annual fire treatment, temperature increases were larger, with rises of about 9.8°C at 0-3 cm and 8.6°C at 3-5 cm depths. In both treatments, elevated temperatures persisted for several hours post-fire (Appendix C: Figure C1).

Plants, fungi, and bacteria richness have divergent responses to fire disturbance and fire history

Fungal, bacterial, and plant richness diverged in their responses to long-term fire regime and time since single fire event. Fungal richness declined post-fire and recovery was dependent on soil depth and fire regime, whereas bacterial richness remained relatively stable, increasing slightly after fire and fully recovering by 12 months. In contrast, plant richness declined in plots that experienced the long-term annual fire regime but increased in plots that have experienced the long-term maintenance regime at 12 months. Below, we present the results for each community type in more depth.

Fungal richness

Fungal ASV richness differed significantly across fire regimes, soil depths, and time points (Table 4.1). Richness was consistently higher in the maintenance fire regime plots compared to annual fire plots ($t = -2.34$, $p = 0.03$) and higher in the surface soil layer compared to the subsurface layer ($t = 5.02$, $p < 0.0001$). We observed temporal variation in fungal richness with significant pairwise differences in both the immediate and longer-term post-fire response

(Table 4.2). Overall, fungal richness decreased by 12% at 1-hour post-fire and remained 6% lower after 12 months, and these fluctuations depended on fire regime and depth. In the annual fire regime plots, fungal richness declined by 9% at 1-hour post-fire (Figure 4.1A). Richness reached its lowest point at 6 months post-fire, then began to recover. By 9 months, richness approached pre-fire values but did not fully recover by 12 months. Richness was lower in subsurface soils compared to surface soils across time, with a more pronounced post-fire decline of 16% observed immediately after the fire. In the maintenance fire regime plots, fungal richness exhibited a stronger immediate decline, with a 16% reduction at 1-hour post-fire. This effect was more pronounced in the subsurface soil layer, which showed a 20% decrease. Richness increased 9 months post-fire, returning to near pre-fire levels before dipping slightly again at 12 months. Overall, fungal richness under the maintenance regime showed greater sensitivity to the immediate effects of fire, particularly in subsurface soils. However, it also followed a more complete recovery trajectory than the annual fire regime, though full recovery was not observed at the 12-month mark.

Bacterial richness

In contrast to fungi, bacterial richness exhibited relatively stable temporal trends, with statistically significant differences observed only between pre-fire to 3 months, and pre-fire to 9 months (Table 4.2). Overall, bacterial richness increased by 4% at 1-hour post-fire and by 4% after 12 months, though these increases were not statistically significant (Figure 4.1B). Bacterial richness was higher in the annual fire regime ($t = 3.84$, $p = 0.001$), and higher in the surface soil layer (0-3 cm) ($t = 10.52$, $p < 0.0001$).

Plant richness

Plant species richness differed significantly between fire regimes and throughout time (Table 4.1). The maintenance regime supported 45% higher plant richness than the annual fire regime ($t = -3.84$, $p = 0.001$). Plant richness also varied between several pairwise time points (Table 4.2). We observed contrasting trends in plant richness through time since fire for plots in the annual and maintenance fire history regimes (Figure 4.1C). In the annual fire regime, richness declined by 15% from pre-fire to 1-month post-fire, followed by a modest increase at 3 months, but then exhibited a consistent decline over time, with richness levels remaining below pre-fire levels through 12 months. In contrast, the maintenance fire regime showed a 15% increase in richness from pre to 1-month post-fire. Richness remained elevated for much of the year, but declined at 9 months post-fire, likely due to seasonal winter effects. By 12 months post-fire, richness rebounded, reaching recovery levels 14% above pre-fire.

Soil microbe and plant community composition

Bacteria, fungi, and plant community composition significantly differed between plots in annual and maintenance fire regimes (Table 4.3) (Figure 4.2). This was most notable for the plant community, where annual fire regime communities were dominated by *Aristida* spp., and maintenance communities were dominated by *Paspalum* spp. Fungal community composition differed significantly between fire regimes at all time points. In contrast, significant differences in fungal composition between soil depths were detected at only four time points: 24 hours, 2 weeks, 1 month, and 9 months post-fire. Bacterial community composition differed significantly between fire regimes and between soil depths at every time point. In plant communities, fire regime significantly influenced composition at all six time points: pre, 1 month, 3 months, 6 months, 9 months, and 12 months post-fire.

To evaluate how communities changed over time, we compared each post-fire time point to the pre-fire compositional baseline. Fungal community composition was significantly influenced by time since fire (time), fire regime, and depth at 1-hour post-fire. At 24 hours, fire and depth remained significant, and by 2 weeks post-fire, time, fire regime, depth, and their interaction (time × fire) all significantly contributed to the observed variation throughout the 12-month post-fire period. In contrast, bacterial communities did not exhibit a temporal shift until 3 months post-fire (and thereafter), suggesting that fungi responded more rapidly to fire disturbance than bacteria, or that other seasonal factors instead of fire were stronger drivers of soil bacterial composition.

Plant community composition at 1-month post-fire was significantly influenced by both time and fire regime, showing an early shift in composition. At 3, 6, 9, and 12 months, fire regime remained a significant driver of community structure, but plant composition was no longer distinguishable to pre-fire composition. These results suggest that plant communities respond rapidly to fire with differences between fire regimes over time, and exhibit fast recovery to pre-fire composition structure after the first month.

Post-fire enrichment of fungal and bacterial taxa

Of the top ten fungal genera, *Umbelopsis* exhibited the strongest early enrichment, increasing by 62% within 1-hour post-fire (Figure 4.3). Under the annual fire regime, it increased by 77% and remained elevated at 12 months. In contrast, under the maintenance regime, it increased by 56% initially but declined by 24 hours and remained suppressed. *Cenococcum* responded positively overall, with a 32% increase at 1-hour post-fire, continuing to rise at 6 months and remaining elevated relative to pre-fire conditions. Under the annual regime, it increased by 46% initially, dropped sharply after 24 hours, then gradually recovered and peaked at 6 months, remaining enriched at 12 months. Conversely, in the maintenance regime, it declined by 47% initially, increased after 24 hours, and remained elevated through the final sampling point.

Interestingly, *Tympanidaceae* exhibited contrasting post-fire trajectories between fire regimes. *Tympanidaceae* has been identified as a fungal endophyte with demonstrated heat resistance in burned sites, including a symbiont of the fire-adapted adapted *Pinus pungens* (Dowd 2023). In the annual fire treatment, its relative abundance increased by 85%, whereas in the maintenance regime, it rose modestly by 12% before declining over time and failing to recover.

Subgroup 2 bacteria exhibited the most rapid response, increasing by 34% at 1-hour post-fire under the maintenance regime, but subsequently declining below pre-fire levels by 12 months (Figure 4.4). In contrast, it decreased by 10% under the annual regime and gradually recovered by the end of the sampling period. *Acidibacter* increased slightly following fire in both regimes, then declined before rebounding above pre-fire levels at 12 months. *Conexibacter* showed similar trajectories in both treatments, with only a slight increase at 1-hour but a substantial recovery to 75% above pre-fire levels by 12 months, particularly under the maintenance regime. *Acidothermus* responded differently across regimes, where it increased by 9% at 1-hour under the annual regime, peaked at 6-months, and then dropped 5% below pre-fire levels. Under the maintenance regime, it declined immediately post-fire and remained suppressed until recovering slightly above pre-fire levels after 12 months.

Fire-sensitive taxa and limited post-fire recovery

Conversely, several bacterial and fungal taxa exhibited strong sensitivity to fire, showing substantial declines immediately post-fire and little to no recovery after 12 months. Among fungi, *Cladosporium* showed an 80% decrease immediately post-fire and remained 77% below pre-fire levels after 12 months. It was particularly reduced in the annual fire regime, though declines were severe in both fire treatments. *Clavulinopsis* dropped 42% post-fire and declined further to 98% below pre-fire levels, with greater sensitivity in the maintenance treatment. *Trechispora* decreased by 72% initially and fell to 86% below pre-fire levels after a year. Among bacteria, *Aquisphaera* decreased by 15% immediately following the fire and remained 19% below pre-fire levels after 12 months. While it showed some recovery in the annual fire treatment, it remained suppressed under the maintenance regime. *Candidatus Udaeobacter* also declined immediately post-fire and showed minimal recovery, remaining below pre-fire levels at 12 months in both regimes. Its abundance was 17% lower under maintenance fire, while under annual fire it was only 6% below pre-fire levels, indicating a more pronounced suppression under the maintenance regime. *Candidatus Xiphinematobacter* experienced a 5% decline and stayed 20% below pre-fire abundance, with lower recovery under maintenance fire.

Fungal guild responses depend on fire history, pulse disturbance, and soil depth

We found significant differences in the proportions of fungal functional guilds among fire regimes ($\chi^2 = 1{,}916{,}050$, df = 9, $p < 0.0001$), soil depth ($\chi^2 = 212{,}717$, df = 9, $p < 0.0001$), and time points ($\chi^2 = 585{,}168$, df = 72, $p < 0.0001$).

Saprotrophic fungi were the most dominant functional guild, followed by ectomycorrhizal fungi, pathogenic fungi, endophytes, and parasitic fungi. One hour following fire, endophytes, ectomycorrhizal fungi, and mold increased, while epiphytes, lichenized fungi, parasitic fungi, and pathogens declined (Figure 4.5).

Ectomycorrhizal fungi were enriched in plots under the annual fire regime but showed a stronger immediate post-fire increase under the maintenance regime. In contrast, saprotrophic fungi declined immediately under the maintenance regime and did not recover to pre-fire levels. Under the annual regime, saprotrophs initially increased slightly but declined over time, falling below pre-fire levels. Endophytic fungi increased rapidly under the maintenance fire regime, but this increase was transient, returning to pre-fire levels by 12 months. Pathogenic fungi decreased under both fire regimes, however, while they remained suppressed in the annual regime, they rebounded above pre-fire levels in the maintenance regime by the end of the year. Parasitic fungi declined in both regimes and did not recover.

In terms of soil depth, endophytes, pathogens, saprotrophs, and parasitic fungi were slightly more abundant in the surface soil layer compared to the subsurface soil layer (Figure 4.5). Endophytes in the surface soil were strongly enriched at 1-hour post-fire and remained elevated relative to pre-fire levels over time. Ectomycorrhizal fungi increased markedly in the subsurface soil depth immediately after fire and surpassed pre-fire levels by 12 months. In contrast, pathogenic fungi in the surface layer decreased slightly and did not recover, while in the subsurface layer, they declined initially and recovered above pre-fire levels by 12 months. Saprotrophic fungi declined more drastically in the surface soil layer and did not recover to pre-fire levels in either soil depth. Parasitic fungi similarly decreased in surface soils and failed to recovery by the end of the study.

Discussion

Annual fire regime weakens resilience to fire in fungal and plant communities

Soil microbial and plant communities exhibited divergent recovery trajectories, with both compositional and richness patterns strongly shaped by fire legacy and time since fire. These taxon-specific responses suggest that fire regimes act as a selective filter, structuring communities based on functional traits and recolonization strategies (Hollingsworth et al. 2013). Fungal communities responded rapidly to fire, with significant compositional differences between annual and maintenance fire regimes detected as early as 1-hour post-fire. Fungal richness declined sharply immediately after fire, and recovered slowly compared to bacteria, likely due to the lower thermal tolerance of fungi (Neary et al. 1999) and their reliance on plant hosts, which are often altered or damaged by fire (Holden et al. 2013). Declines in fungal richness were even more pronounced under the annual fire regime, where richness did not recover within 12 months. These trends likely suggest that the annual fire regime weakens the resilience of soil fungal richness by imposing significant demands on fungal populations for survival, requiring rapid growth and reproduction within short recovery intervals, and favoring disturbance-tolerant taxa while filtering out slower-growing or host-dependent fungi (Cho et al. 2016).

In contrast to fungi, bacterial richness increased immediately following fire, particularly under the annual fire regime. Composition differed by fire regime and soil depth at all time points, but significant temporal shifts did not emerge until three months post-fire, potentially suggesting a short-term buffering effect, possibly due to deeper soil refuge or fire-adaptive traits. Across both fire regimes, bacterial richness surpassed pre-fire values in the early post-fire period, reflecting adaptation to fire disturbance. This pattern aligns with previous research showing that bacteria generally exhibit greater resilience to fire than fungi (Glassman et al. 2023, Whitman et al. 2019). For example, post-fire environments may favor fire-responsive bacterial taxa with traits such as with high 16S rRNA gene copy numbers, which are associated with rapid proliferation in response to increased nutrient availability and reduced microbial competition (Whitman et al. 2019, Johnson et al. 2023). These fast-growing bacterial taxa can dominate post-fire soils and demonstrate strong functional resilience (Johnson et al. 2023). The loss of fire-sensitive organisms likely opens ecological niches that favor bacteria, where fire-induced changes in soil chemistry and structure further select for taxa adapted to these altered conditions (Dao et al. 2022).

Plant communities also responded rapidly, with composition shifts between fire regimes detected at one-month post-fire. However, unlike fungi, plant communities returned to a composition similar to the pre-fire baseline by 3 months post-fire, indicating a high capacity for recovery, likely driven by resprouting, seed bank recruitment, and fire-adapted life history traits (Keely et al. 2011). Richness patterns, however, showed strong differences between fire regimes. Under annual fires, plant richness declined and showed no recovery by 12 months, whereas maintenance fire significantly enhanced richness at one month and continued to support increased richness at 12 months. These results suggest that maintenance fire regimes could contribute to higher plant diversity, potentially through reduced competitive exclusion and enhanced resource availability. This pattern is consistent with previous research demonstrating that 1 to 3-year fire return intervals maintain savanna structure and biodiversity (Glitzenstein et al. 2003, Robertson et al. 2019), as well as dendrochronological studies that document historical fire regimes within this range (Stambaugh et al. 2011, Bale 2009). Our data reinforce this, demonstrating that overly frequent, annual growing season fires may disrupt these dynamics and suppress both plant and fungal richness. While annual fire may serve targeted management goals, such as rapid fuel reduction or control of woody species (Glitzenstein et al. 2012), our results suggest it is less effective at promoting long-term diversity and resilience in plant and fungal communities.

Subsurface fungal communities are more negatively impacted than surface communities

Soil depth influences post-fire microbial dynamics (Neary et al. 1999), and soil microbial recolonization depends on dispersal pathways. In our study, both bacterial and fungal richness remained highest in surface soils, consistent with previous findings that microbial diversity and activity are higher near the soil surface (Blume et al. 2002). However, contrary to our expectations, fungal richness declined more sharply in subsurface soils immediately following fire and remained suppressed. Higher aboveground temperatures and greater surface soil moisture availability leads to more pronounced impacts on microbial communities (Barbour et al. 2022). Because of this, we anticipated that the deeper subsurface soil would act as a thermal buffer from heat exposure and serve as a reservoir for microorganisms. This unexpected decline suggests that thermal effects alone are not the most important drivers of soil fungal response to prescribed fire. Instead, limited dispersal into the subsurface soil layers may constrain fungal recovery in our study. Fungi typically disperse via spores or hyphal fragmentation, many of which are deposited aerially or carried across surface layers by wind, water, or animals (Borgmann-Winter et al. 2023, Chaudhary et al. 2022). The removal of vegetation and litter by fire may increase surface exposure and reduce physical barriers to aerial deposition into surface layers immediately post-fire, which has been shown to be the most important source of soil fungal dispersal after wildfire (Barbour et al. 2023). In contrast, recolonization of subsurface soils depends on downward or local migration from the surface or regrowth from belowground propagules (Meyer et al. 2022, Baar et al. 1999), processes that may be slower and limited if fungal propagules were destroyed by fire.

Fire-induced heterogeneity can create a mosaic of burn severities, or pyrodiversity (Blomdahl et al. 2019). Within this mosaic, unburned or lightly burned patches may act as a refugia for microbial communities, which can support microbial persistence and serve as important sources for recolonization of adjacent or nearby burned soils (Bruns et al. 2020, Adkins et al. 2020, Birch et al. 2025). Dispersal from these patches can shape long-term recovery through priority effects, with colonizing taxa often maintaining distinct compositions in the altered post-fire soils (Adkins et al. 2020, Barbour et al. 2023). Consequently, priority effects and selective filtering may limit which taxa establish in the subsurface zones, particularly those with poor dispersal capacity or obligate plant associations. While fire-induced mortality likely contributed to initial declines, our findings suggest that fungal recovery in the deeper subsurface soils is further constrained by dispersal limitations.

Microbial genera exhibit distinct temporal patterns

In our study, fire enriched members of the fungal community, including *Cenococcum*, *Umbelopsis*, and *Talaromyces*. Previous research suggests that *Cenococcum*, an ectomycorrhizal fungus, exhibits fire resistance and can survive soil heating as mycelia within the soil matrix (Kipfer et al. 2010, Fox et al. 2022). Post-fire enrichment of this fungus has been documented, notably in longleaf pine habitats (Semenova-Nelsen et al. 2019, Oliver at al. 2015). Consistent with these findings, we observed a gradual and sustained increase in *Cenococcum* across post-fire time points. *Umbelopsis*, a fungal endophyte, has similarly been observed to increase in abundance following fire events (Hopkins et al. 2021, Orumaa et al. 2022, Greenwood et al. 2023, Philpott et al. 2025). In our study, *Umbelopsis* showed the most immediate enrichment, increasing by over 60% within 1-hour post-fire and remaining elevated under the annual fire regime through 12 months. This pattern suggests a strong capacity for rapid colonization and persistence under high-frequency disturbance, particularly in surface soils where early enrichment reached 75%. However, under the maintenance regime, *Umbelopsis* declined after 24 hours and remained suppressed, underscoring that fire regime modulates its recovery trajectory. *Talaromyces*, recognized as a rapid post-fire colonizer (Sharma 1981) and can produce heat resistant ascospores (Yilmaz et al. 2014), has also been identified as an indicator species in longleaf pine savannas (Hopkins et al. 2021). In our study, *Talaromyces* showed a sharp peak at one month in both regimes but only recovered under the annual regime.

In contrast, several fungal taxa displayed pronounced sensitivity to fire. *Cladosporium*, a saprotrophic fungus, declined by 80% immediately post-fire and remained well below pre-fire levels at 12 months. It is a genus common in bioaerosols and has been found to be frequently observed in post-fire smoke air (Kobziar et al. 2022, Mims et al. 2004). *Clavulinopsis,* a saprotroph, and *Trechispora*, a soil-inhabiting basidiomycete, also exhibited severe and prolonged declines. While bacteria did not respond strongly to fire in richness or composition, we uncovered specific genera that were fire-responders. *Acidothermus* and *Conexibacter* were enriched post-fire, aligning with known traits enabling survival under extreme soil conditions. *Acidothermus* is a genus of thermophilic and acidophilic bacteria that are capable of surviving relatively high temperatures (optimal 55°C) (Mohagheghi et al. 1986, Normand et al. 2015) and acidic conditions (optimal pH of 5.5). This genus has been shown to increase in abundance shortly after fire events (Kapoor et al. 2023, Arunrat et al. 2023, Rai et al. 2023, Arunrat et al.

2024). Similarly, *Conexibacter,* a gram-positive bacterium capable of nitrate reduction via denitrification, and *Candidatus Udaeobacter*, have been found to be enriched post-fire, exhibiting significant increases in abundance following fire (Arunrat et al. 2024, Pulido-Chavez et al. 2022, Arunrat et al. 2023). In contrast, *Candidatus Udaeobacter* and *Candidatus Xiphinematbacter* declined sharply and remained suppressed, consistent with a previous study showing large declines post-fire (Arunrat et al. 2024), indicating possible sensitivity to heat or the post-fire environment.

Fire regime and soil depth drive contrasting responses among fungal guilds

We found that endophytes and ectomycorrhizal fungi increased substantially within 1-hour post-fire, suggesting early resilience or opportunism. This contrasts with prior studies in longleaf pine ecosystems that report post-fire declines in ectomycorrhizal fungi under repeated burning (Semenova-Nelsen et al. 2019, Fox et al. 2023). However, in other systems, some ectomycorrhizal taxa have demonstrated heat-tolerance or can rapidly recolonize from resistant spores or propagules (Glassman et al. 2016, Taylor & Bruns, 1999, Kipfer et al. 2010), which may explain their persistence and enrichment in our study, particularly under the annual fire regime. Consistent with previous work in longleaf pine forests, saprotrophic fungi declined under both fire regimes and did not recover to pre-fire levels (Semenova-Nelsen et al. 2019, Fox et al. 2023). This persistent loss of fungal saprotrophs suggests that repeated fire may disrupt decomposition and nutrient cycling processes in these ecosystems (Semenova-Nelsen et al. 2019). Interestingly, relative abundance of endophytic fungi nearly doubled in the surface soils within 1-hour post-fire, likely due to transient enrichment from aerosols or nearby vegetation (Huang et al. 2016), but this increase was short-lived, with abundance returning to pre-fire levels over time. In contrast, pathogenic fungi were suppressed under both fire regimes but recovered and exceeded pre-fire levels under the maintenance regime in subsurface soils, suggesting greater resilience with longer fire-return intervals. Overall, these findings highlight depth and regime-specific trajectories among fungal guilds and underscore the importance of fire regime and time since fire in shaping belowground functional recovery.

Conclusion

Our results demonstrate that fire regimes and soil depth shape distinct recovery trajectories for bacterial, fungal, and plant communities in longleaf pine ecosystems. We found that maintenance fire regimes better support biodiversity, particularly for plants, fungi, and certain microbial groups, while annual fires reduce richness and delay recovery. Contrary to our predictions, subsurface fungal communities were more vulnerable than surface communities, highlighting the importance of dispersal limitation and the role of microbial refugia. We also identified fire-responsive and fire-sensitive taxa under low-intensity and pulse fire events. Together, these findings emphasize the ecological significance of fire legacy and spatial heterogeneity in shaping post-fire biodiversity, reinforcing the value of prescribed fire regimes for sustaining resilient and functionally diverse ecosystems.

Table 4.1. **Model summary of fire, time, depth, and their interaction on species richness.** Model summary results of fire regime (annual vs. maintenance), time (time since fire), depth (0-3cm vs. 3-5cm), and their interactions for fungal, bacterial, and plant species richness. n/a indicates predictors that were not applicable to the particular response community.

	Fungi		**Bacteria**		**Plant**	
	χ^2	Pr ($>\chi^2$)	χ^2	Pr ($>\chi^2$)	χ^2	Pr ($>\chi^2$)
Fire Regime	5.48	**0.019**	14.77	**0.0001**	14.8	**0.0001**
Time	58.62	**< 0.0001**	25.24	**0.001**	179.2	**< 0.0001**
Depth	28.88	**< 0.0001**	110.66	**< 0.0001**	n/a	n/a
Fire Regime × Time	16.62	**0.03**	11.96	0.15	100.93	**< 0.0001**
Fire Regime × Depth	0.25	0.61	9.9	**0.001**	n/a	n/a
Time × Depth	7.17	0.51	2.69	0.95	n/a	n/a
Fire Regime × Time × Depth	4.43	0.81	3.07	0.92	n/a	n/a

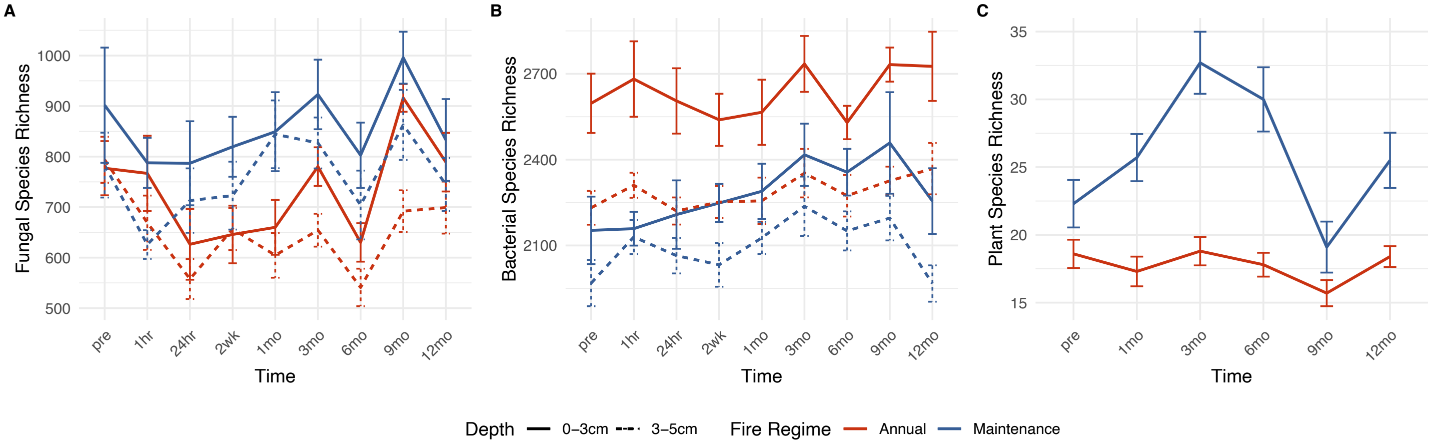

Figure 4.1: Fungal, bacterial, and plant species richness across time and fire regime. Mean species richness between annual (red) and maintenance (blue) fire regimes for A) fungi, B) bacteria, and C) plant species richness across the time scale. Solid lines represent the 0-3cm surface soil, and dashed lines represent the 3-5cm subsurface soil depth for fungi and bacteria. Error bars represent the standard error of the mean.

Table 4.2: **Significant pairwise comparisons of richness across time**. Pairwise comparisons of fungal, bacterial, and plant richness between time points. Only contrasts with statistically significant differences ($p < 0.05$) are shown.

Group	Contrast	Estimate	t-ratio	p-value
Fungi	pre - 24 hr	142.9	4.08	0.001
	pre - 6 mo	144.4	4.13	0.001
	1 hr - 9 mo	-154.2	-4.41	0.0005
	24 hr - 3 mo	-125.2	-3.5	0.01
	24 hr - 9 mo	-195.7	-5.59	< 0.0001
	2 wk - 9 mo	-155.4	-4.44	0.0004
	1 mo - 9 mo	-127.1	-3.63	0.009
	3 mo - 6 mo	126.7	3.62	0.01
	6 mo - 9 mo	-197.2	-5.63	< 0.0001
Bacteria	pre - 3 mo	197.8	3.68	0.008
	pre - 9 mo	193.5	3.57	0.01
Plant	pre - 3 mo	-5.3	-7.75	< 0.0001
	pre - 6 mo	-3.45	-5.05	< 0 .0001
	pre - 9 mo	3.05	4.46	0.0003
	1 mo - 3 mo	-4.69	-6.94	< 0.0001
	1 mo - 6 mo	-2.84	-4.2	0.0008
	3 mo - 9 mo	8.35	12.22	< 0.0001
	3 mo - 12 mo	-3.8	-5.5	< 0.0001
	6 mo - 9 mo	6.5	9.51	< 0.0001
	9 mo - 12 mo	4.55	6.66	< 0.0001

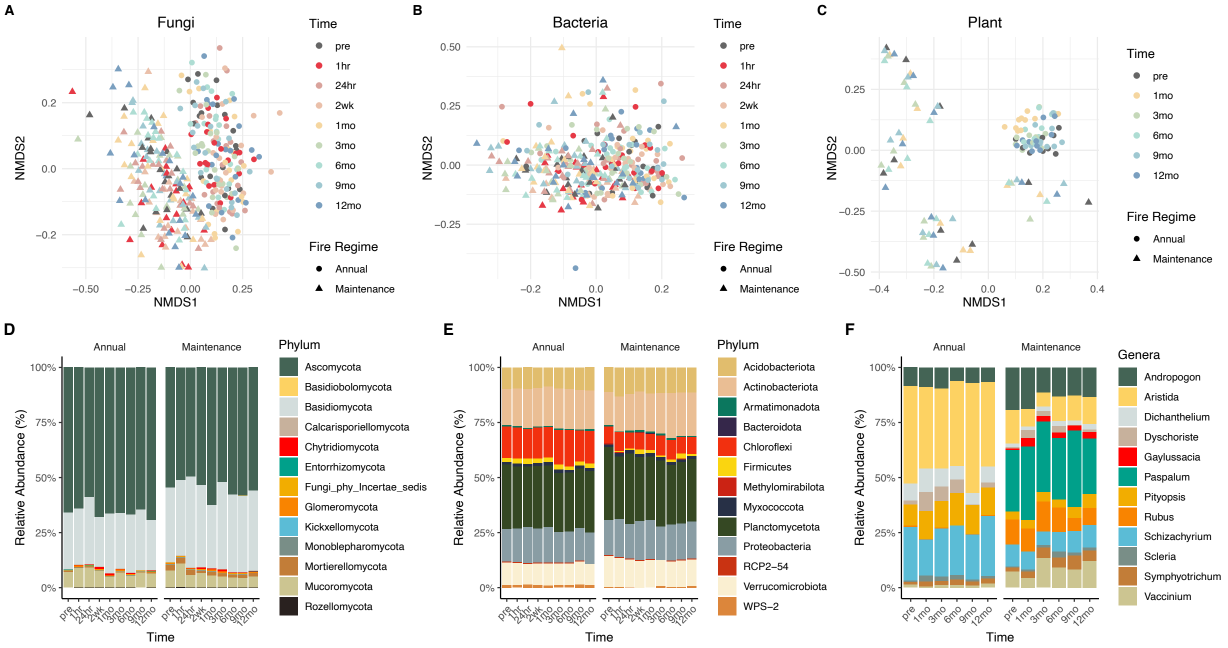

Figure 4.2. NMDS ordinations and relative abundance of fungi, bacteria, and plants. Nonmetric multidimensional scaling (NMDS) ordinations for community composition of A) fungi (stress = 0.229), B) bacteria (stress = 0.180), and C) plants (stress = 0.130). Colors denote time and shape denotes fire regime (annual or maintenance). Stacked bar plots showing the relative abundance (%) of the top D) fungal phyla, E) bacterial phyla, and F) plant genera across fire regimes (annual vs. maintenance) and time since fire.

Table 4.3. PERMANOVA analysis of fire regime, soil depth, and their interaction. PERMANOVA results testing effects of fire regime, soil depth, and their interaction on plant and microbial community composition across time points. Significant P-values are bolded.

		Fungi			Bacteria			Plant		
Time	**Term**	**F Statistic**	**R²**	**Pr(>F)**	**F Statistic**	**R²**	**Pr(>F)**	**F Statistic**	**R²**	**Pr(>F)**
Pre	Fire Regime	3.073	0.076	**0.001** ***	4.309	0.096	**0.001** ***	6.726	0.272	**0.001** ***
	Depth	1.054	0.026	0.343	3.791	0.085	**0.001** ***	n/a	n/a	n/a
	Fire Regime × Depth	0.531	0.013	1.000	0.708	0.016	0.813	n/a	n/a	n/a
1 hr	Fire Regime	3.312	0.081	**0.001** ***	3.515	0.080	**0.001** ***	n/a	n/a	n/a
	Depth	1.151	0.028	0.215	3.525	0.081	**0.002** **	n/a	n/a	n/a
	Fire Regime × Depth	0.574	0.014	0.990	0.723	0.017	0.794	n/a	n/a	n/a
24 hr	Fire Regime	2.738	0.067	**0.001** ***	3.744	0.084	**0.001** ***	n/a	n/a	n/a
	Depth	1.362	0.034	**0.044** *	3.981	0.090	**0.001** ***	n/a	n/a	n/a
	Fire Regime × Depth	0.490	0.012	1.000	0.592	0.013	0.938	n/a	n/a	n/a
2 wk	Fire Regime	3.437	0.082	**0.001** ***	3.181	0.071	**0.002** **	n/a	n/a	n/a
	Depth	1.812	0.043	**0.003** **	5.290	0.117	**0.001** ***	n/a	n/a	n/a
	Fire Regime × Depth	0.699	0.017	0.961	0.596	0.013	0.930	n/a	n/a	n/a
1 mo	Fire Regime	3.683	0.087	**0.001** ***	3.724	0.083	**0.001** ***	6.089	0.252	**0.001** ***
	Depth	1.793	0.043	**0.008** **	4.219	0.094	**0.001** ***	n/a	n/a	n/a
	Fire Regime × Depth	0.665	0.016	0.966	0.734	0.016	0.789	n/a	n/a	n/a
3 mo	Fire Regime	4.181	0.100	**0.001** ***	4.464	0.098	**0.001** ***	7.777	0.307	**0.001** ***
	Depth	1.212	0.029	0.142	4.508	0.099	**0.001** ***	n/a	n/a	n/a
	Fire Regime × Depth	0.565	0.013	0.993	0.572	0.013	0.940	n/a	n/a	n/a
6 mo	Fire Regime	2.719	0.067	**0.001** ***	4.439	0.098	**0.001** ***	7.394	0.291	**0.001** ***
	Depth	1.134	0.028	0.227	4.309	0.095	**0.001** ***	n/a	n/a	n/a
	Fire Regime × Depth	0.450	0.011	1.000	0.574	0.013	0.941	n/a	n/a	n/a
9 mo	Fire Regime	3.214	0.076	**0.001** ***	3.420	0.076	**0.001** ***	8.626	0.323	**0.002** **
	Depth	2.215	0.052	**0.001** ***	4.854	0.108	**0.001** ***	n/a	n/a	n/a
	Fire Regime × Depth	0.777	0.018	0.908	0.794	0.018	0.716	n/a	n/a	n/a

		Fungi			Bacteria			Plant		
Time	**Term**	**F Statistic**	**R²**	**Pr(>F)**	**F Statistic**	**R²**	**Pr(>F)**	**F Statistic**	**R²**	**Pr(>F)**
12 mo	Fire Regime	2.590	0.064	**0.001 *****	2.651	0.062	**0.003 ****	5.684	0.240	**0.001 *****
	Depth	1.247	0.031	0.109	3.626	0.085	**0.001 *****	n/a	n/a	n/a
	Fire Regime × Depth	0.435	0.011	1.000	0.417	0.010	0.999	n/a	n/a	n/a

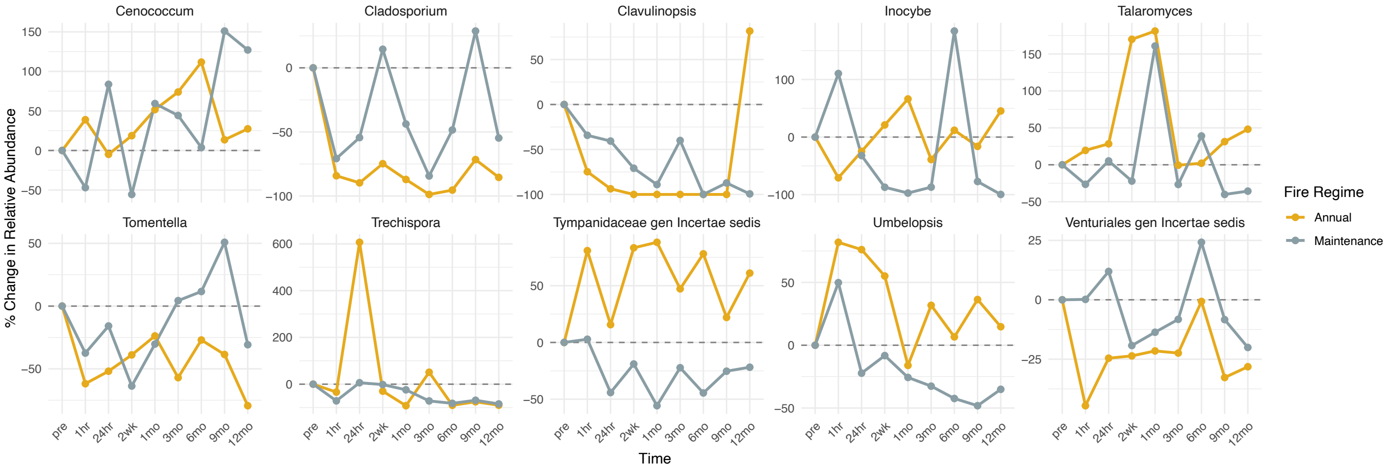

Figure. 4.3: Percent change in mean relative abundance of fungi. Percent change in mean relative abundance of the top ten fungal genera over time following fire. Genera were selected based on the highest mean relative abundance at the pre-fire time point. Each panel shows one genus, with points and lines representing mean percent change compared to the pre-fire baseline across fire regimes (yellow = annual, light blue = maintenance). Note the variation in y-axis scales among panels.

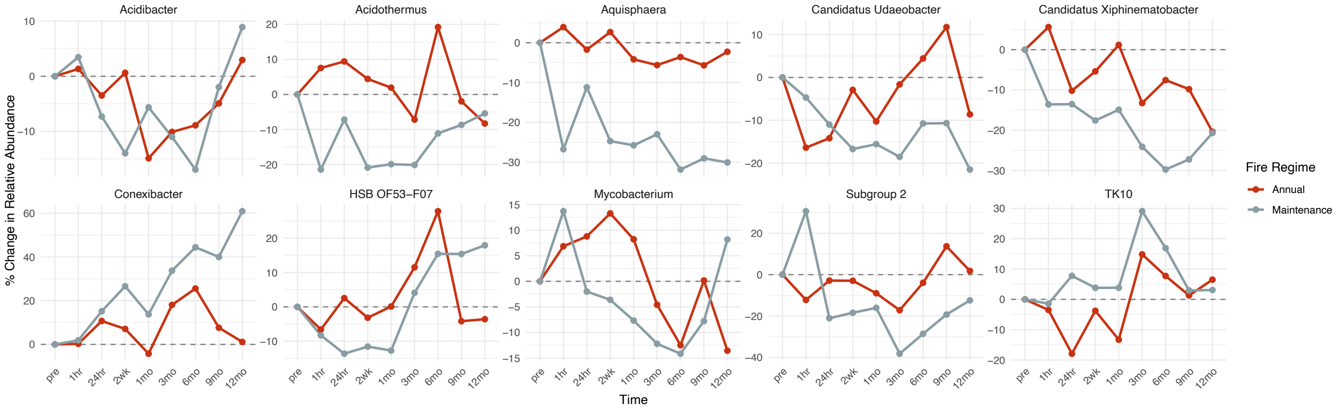

Figure 4.4: Percent change in mean relative abundance of bacteria. Percent change in mean relative abundance of the top ten bacterial genera over time following fire. Genera were selected based on the highest mean relative abundance at the pre-fire time point. Each panel shows one genus, with points and lines representing mean percent change compared to the pre-fire baseline across fire regimes (red = annual, light blue = maintenance). Note the variation in y-axis scales among panels.

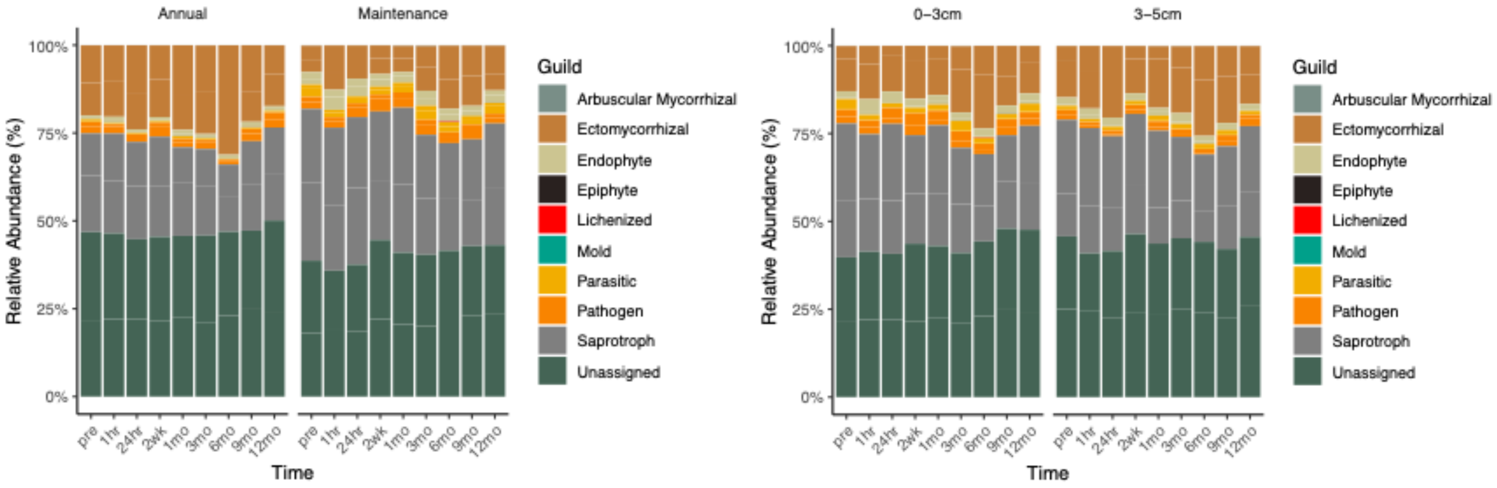

Figure 4.5. Relative abundance of fungal guilds by fire regime and soil depth over time. Stacked bar plots showing the relative abundance (%) of fungal guilds for each fire regime and soil depth across time.

References

Adkins, J., Docherty, K. M., Gutknecht, J. L., & Miesel, J. R. (2020). How do soil microbial communities respond to fire in the intermediate term? Investigating direct and indirect effects associated with fire occurrence and burn severity. *Science of the Total Environment*, 745, 140957. https://doi.org/10.1016/j.scitotenv.2020.140957

Allison, S. D., & Martiny, J. B. (2008). Resistance, resilience, and redundancy in microbial communities. *Proceedings of the National Academy of Sciences*, 105(supplement_1), 11512-11519. https://doi.org/10.1073/pnas.0801925105

Arunrat, N., Sereenonchai, S., Sansupa, C., Kongsurakan, P., & Hatano, R. (2023). Effect of rice straw and stubble burning on soil physicochemical properties and bacterial communities in central Thailand. *Biology*, 12(4), 501. https://doi.org/10.3390/biology12040501

Arunrat, N., Sansupa, C., Sereenonchai, S., Hatano, R., & Lal, R. (2024). Fire-induced changes in soil properties and bacterial communities in rotational shifting cultivation fields in Northern Thailand. *Biology*, 13(6), 383. https://doi.org/10.3390/biology13060383

Baar, J., Horton, T. R., Kretzer, A. M., & Bruns, T. D. (1999). Mycorrhizal colonization of Pinus muricata from resistant propagules after a stand-replacing wildfire. *New Phytologist*, 143(2), 409-418. https://doi.org/10.1046/j.1469-8137.1999.00452.x

Bale, A. M. (2009). Fire effects and litter accumulation dynamics in a montane longleaf pine ecosystem. University of Missouri-Columbia, ProQuest Dissertations & Theses, 1481436,

Barbour, K. M., Weihe, C., Allison, S. D., & Martiny, J. B. (2022). Bacterial community response to environmental change varies with depth in the surface soil. *Soil Biology and Biochemistry*, 172, 108761. https://doi.org/10.1016/j.soilbio.2022.108761

Barbour, K. M., Weihe, C., Walters, K. E., & Martiny, J. B. (2023). Testing the contribution of dispersal to microbial succession following a wildfire. *Msystems*, 8(5), e00579-23. https://doi.org/10.1128/msystems.00579-23

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R. H. B., Singmann, H., ... & Bolker, M. B. (2015). Package 'lme4'. convergence, 12(1), 2.

Birch, J. D., Lutz, J. A., Dickinson, M. B., Franklin, J., Larson, A. J., Swanson, M. E., & Miesel, J. R. (2025). Small-scale fire refugia increase soil bacterial and fungal richness and increase community cohesion nine years after fire. *Science of The Total Environment*, 966, 178677. https://doi.org/10.1016/j.scitotenv.2025.178677

Blomdahl, E. M., Kolden, C. A., Meddens, A. J., & Lutz, J. A. (2019). The importance of small fire refugia in the central Sierra Nevada, California, USA. *Forest Ecology and Management*, 432, 1041-1052. https://doi.org/10.1016/j.foreco.2018.10.038

Blume, E., Bischoff, M., Reichert, J. M., Moorman, T., Konopka, A., & Turco, R. F. (2002). Surface and subsurface microbial biomass, community structure and metabolic activity as a function of soil depth and season. *Applied Soil Ecology*, 20(3), 171-181. https://doi.org/10.1016/S0929-1393(02)00025-2

Borgmann-Winter, B. W., Stephens, R. B., Anthony, M. A., Frey, S. D., D'Amato, A. W., & Rowe, R. J. (2023). Wind and small mammals are complementary fungal dispersers. *Ecology*, 104(6), e4039. https://doi.org/10.1002/ecy.4039

Bruns, T. D., Chung, J. A., Carver, A. A., & Glassman, S. I. (2020). A simple pyrocosm for studying soil microbial response to fire reveals a rapid, massive response by Pyronema species. *PLoS One*, 15(3), e0222691. https://doi.org/10.1371/journal.pone.0222691

Caiafa, M. V., Nelson, A. R., Borch, T., Roth, H. K., Fegel, T. S., Rhoades, C. C., ... & Glassman, S. I. (2023). Distinct fungal and bacterial responses to fire severity and soil depth across a ten-year wildfire chronosequence in beetle-killed lodgepole pine forests. *Forest Ecology and Management*, 544, 121160. https://doi.org/10.1016/j.foreco.2023.121160

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, 13(7), 581-583. https://doi.org/10.1038/nmeth.3869

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., ... & Knight, R. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME journal*, 6(8), 1621-1624. https://doi.org/10.1038/ismej.2012.8

Cho, H., Kim, M., Tripathi, B., & Adams, J. (2017). Changes in soil fungal community structure with increasing disturbance frequency. *Microbial Ecology*, 74(1), 62-77. https://doi.org/10.1007/s00248-016-0919-1

Chaudhary, V. B., Aguilar-Trigueros, C. A., Mansour, I., & Rillig, M. C. (2022). Fungal dispersal across spatial scales. *Annual Review of Ecology, Evolution, and Systematics*, 53(1), 69-85. https://doi.org/10.1146/annurev-ecolsys-012622-021604

Crowther, T. W., Maynard, D. S., Crowther, T. R., Peccia, J., Smith, J. R., & Bradford, M. A. (2014). Untangling the fungal niche: the trait-based approach. *Frontiers in Microbiology*, 5, 579. https://doi.org/10.3389/fmicb.2014.00579

Dao, V. Q., Potts, S. E., Johnson, C. N., Sikes, B. A., & Platt, W. J. (2022). Substrate and low intensity fires influence bacterial communities in longleaf pine savanna. *Scientific Reports*, 12(1), 20904. https://doi.org/10.1038/s41598-022-24896-x

Davies, G. M., & Gray, A. (2015). Don't let spurious accusations of pseudoreplication limit our ability to learn from natural experiments (and other messy kinds of ecological monitoring). *Ecology and Evolution*, 5(22), 5295-5304. https://doi.org/10.1002/ece3.1782

Dooley, S. R., & Treseder, K. K. (2012). The effect of fire on microbial biomass: a meta-analysis of field studies. *Biogeochemistry*, 109(1), 49-61. https://doi.org/10.1007/s10533-011-9633-8

Dove, N. C., Taş, N., & Hart, S. C. (2022). Ecological and genomic responses of soil microbiomes to high-severity wildfire: linking community assembly to functional potential. *The ISME Journal*, 16(7), 1853-1863. https://doi.org/10.1038/s41396-022-01232-9

Dowd, A. C. (2023). Are Fungal Endophytes of Fire Adapted Plants Also Fire Adapted? Chancellor’s Honors Program Projects. https://trace.tennessee.edu/utk_chanhonoproj/2533

Enright, D. J., Frangioso, K. M., Isobe, K., Rizzo, D. M., & Glassman, S. I. (2022). Mega-fire in redwood tanoak forest reduces bacterial and fungal richness and selects for pyrophilous taxa that are phylogenetically conserved. *Molecular Ecology*, 31(8), 2475-2493. https://doi.org/10.1111/mec.16399

Fischer, M. S., Patel, N. J., de Lorimier, P. J., & Traxler, M. F. (2023). Prescribed fire selects for a pyrophilous soil sub-community in a northern California mixed conifer forest.

Environmental Microbiology, 25(11), 2498-2515. https://doi.org/10.1111/1462-2920.16475

Fischer, M. S., Stark, F. G., Berry, T. D., Zeba, N., Whitman, T., & Traxler, M. F. (2021). Pyrolyzed substrates induce aromatic compound metabolism in the post-fire fungus, Pyronema domesticum. *Frontiers in Microbiology*, 12, 729289. https://doi.org/10.3389/fmicb.2021.729289

Fontúrbel, M. T., Barreiro, A., Vega, J. A., Martín, A., Jiménez, E., Carballas, T., ... & Díaz-Raviña, M. (2012). Effects of an experimental fire and post-fire stabilization treatments on soil microbial communities. *Geoderma*, 191, 51-60. https://doi.org/10.1016/j.geoderma.2012.01.037

Fox, S., Sikes, B. A., Brown, S. P., Cripps, C. L., Glassman, S. I., Hughes, K., ... & Jumpponen, A. (2022). Fire as a driver of fungal diversity—A synthesis of current knowledge. *Mycologia*, 114(2), 215-241. https://doi.org/10.1080/00275514.2021.2024422

Fox, S., Taylor, M. K., & Jumpponen, A. (2024). Fire-excluded and frequently burned longleaf pine forests have contrasting soil microbial communities. *Forest Ecology and Management*, 551, 121519. https://doi.org/10.1016/j.foreco.2023.121519

Glassman, S. I., Levine, C. R., DiRocco, A. M., Battles, J. J., & Bruns, T. D. (2016). Ectomycorrhizal fungal spore bank recovery after a severe forest fire: some like it hot. *The ISME Journal*, 10(5), 1228-1239. DOI: 10.1038/ismej.2015.182

Glassman, S. I., Randolph, J. W., Saroa, S. S., Capocchi, J. K., Walters, K. E., Pulido-Chavez, M. F., & Larios, L. (2023). Prescribed versus wildfire impacts on exotic plants and soil microbes in California grasslands. *Applied Soil Ecology*, 185, 104795. https://doi.org/10.1016/j.apsoil.2022.104795

Glitzenstein, J. S., Streng, D. R., & Wade, D. D. (2003). Fire frequency effects on longleaf pine (Pinus palustris P. Miller) vegetation in South Carolina and northeast Florida, USA. *Natural Areas Journal*. 23 (1): 22-37. 2003.

Glitzenstein, J. S., Streng, D. R., Masters, R. E., Robertson, K. M., & Hermann, S. M. (2012). Fire-frequency effects on vegetation in north Florida pinelands: another look at the long-term Stoddard Fire Research Plots at Tall Timbers Research Station. *Forest Ecology and Management*, 264, 197-209. https://doi.org/10.1016/j.foreco.2011.10.014

Goberna, M., García, C., Insam, H., Hernández, M. T., & Verdú, M. (2012). Burning fire-prone Mediterranean shrublands: immediate changes in soil microbial community structure and ecosystem functions. *Microbial Ecology*, 64(1), 242-255. https://doi.org/10.1007/s00248-011-9995-4

Greenwood, L., Nimmo, D. G., Egidi, E., Price, J. N., McIntosh, R., & Frew, A. (2023). Fire shapes fungal guild diversity and composition through direct and indirect pathways. *Molecular Ecology*, 32(17), 4921-4939. https://doi.org/10.1111/mec.17068

Hagmann, R. K., Hessburg, P. F., Prichard, S. J., Povak, N. A., Brown, P. M., Fulé, P. Z., ... & Waltz, A. E. M. (2021). Evidence for widespread changes in the structure, composition, and fire regimes of western North American forests. *Ecological Applications*, 31(8), e02431. https://doi.org/10.1002/eap.2431

He, T., Lamont, B. B., & Pausas, J. G. (2019). Fire as a key driver of Earth's biodiversity. *Biological Reviews*, 94(6), 1983-2010. https://doi.org/10.1111/brv.12544

Hiers, J. K., O'Brien, J. J., Mitchell, R. J., Grego, J. M., & Loudermilk, E. L. (2009). The wildland fuel cell concept: an approach to characterize fine-scale variation in fuels and fire in frequently burned longleaf pine forests. *International Journal of Wildland Fire*, 18(3), 315-325. https://doi.org/10.1071/WF08084

Holden, S. R., Gutierrez, A., & Treseder, K. K. (2013). Changes in soil fungal communities, extracellular enzyme activities, and litter decomposition across a fire chronosequence in Alaskan boreal forests. *Ecosystems*, 16(1), 34-46. DOI:10.1007/s10021-012-9594-3

Hollingsworth, T. N., Johnstone, J. F., Bernhardt, E. L., & Chapin III, F. S. (2013). Fire severity filters regeneration traits to shape community assembly in Alaska's boreal forest. *PloS One*, 8(2), e56033. https://doi.org/10.1371/journal.pone.0056033

Hopkins, J. R., Semenova-Nelsen, T., & Sikes, B. A. (2021). Fungal community structure and seasonal trajectories respond similarly to fire across pyrophilic ecosystems. *FEMS Microbiology Ecology*, 97(1), fiaa219. https://doi.org/10.1093/femsec/fiaa219

Hopkins, J. R., & Bennett, A. E. (2023). Spore traits mediate disturbance effects on arbuscular mycorrhizal fungal community composition and mutualisms. *Ecology*, 104(5), e4016. https://doi.org/10.1002/ecy.4016

Hopkins, J. R., Vizzari, G., Bennett, A. E., & Malacrinò, A. (2025). Fire limits soil microbial dispersal and differentially impacts bacterial and fungal communities. bioRxiv, 2025-05. https://doi.org/10.1101/2025.05.02.651892

Huang, Y. L., Devan, M. N., U'Ren, J. M., Furr, S. H., & Arnold, A. E. (2016). Pervasive effects of wildfire on foliar endophyte communities in montane forest trees. *Microbial Ecology*, 71(2), 452-468. https://doi.org/10.1007/s00248-015-0664-x

Hurlbert, S. H. (1984). Pseudoreplication and the design of ecological field experiments. *Ecological Monographs*, 54(2), 187-211. https://doi.org/10.2307/1942661

Ihrmark, K., Bödeker, I. T., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., ... & Lindahl, B. D. (2012). New primers to amplify the fungal ITS2 region–evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiology Ecology*, 82(3), 666-677. https://doi.org/10.1111/j.1574-6941.2012.01437.x

Johnson, D. B., Woolet, J., Yedinak, K. M., & Whitman, T. (2023). Experimentally determined traits shape bacterial community composition one and five years following wildfire. *Nature Ecology & Evolution*, 7(9), 1419-1431. https://doi.org/10.1038/s41559-023-02135-4

Johnstone, J. F., Allen, C. D., Franklin, J. F., Frelich, L. E., Harvey, B. J., Higuera, P. E., ... & Turner, M. G. (2016). Changing disturbance regimes, ecological memory, and forest resilience. *Frontiers in Ecology and the Environment*, 14(7), 369-378. https://doi.org/10.1002/fee.1311

Kapoor, B., Onufrak, A., Klingeman III, W., DeBruyn, J. M., Cregger, M. A., Willcox, E., ... & Hadziabdic, D. (2023). Signatures of prescribed fire in the microbial communities of Cornus florida are largely undetectable five months post-fire. *PeerJ*, 11, e15822. https://doi.org/10.7717/peerj.15822

Keeley, J. E. (2009). Fire intensity, fire severity and burn severity: a brief review and suggested usage. *International Journal of Wildland Fire*, 18(1), 116-126. https://doi.org/10.1071/WF07049

Kelly, L. T., Giljohann, K. M., Duane, A., Aquilué, N., Archibald, S., Batllori, E., ... & Brotons, L. (2020). Fire and biodiversity in the Anthropocene. *Science*, 370(6519), eabb0355. DOI: 10.1126/science.abb0355

Kipfer, T., Egli, S., Ghazoul, J., Moser, B., & Wohlgemuth, T. (2010). Susceptibility of ectomycorrhizal fungi to soil heating. *Fungal Biology*, 114(5-6), 467-472. https://doi.org/10.1016/j.funbio.2010.03.008

Knelman, J. E., Graham, E. B., Trahan, N. A., Schmidt, S. K., & Nemergut, D. R. (2015). Fire severity shapes plant colonization effects on bacterial community structure, microbial biomass, and soil enzyme activity in secondary succession of a burned forest. *Soil Biology and Biochemistry*, 90, 161-168. https://doi.org/10.1016/j.soilbio.2015.08.004

Kobziar, L. N., Vuono, D., Moore, R., Christner, B. C., Dean, T., Betancourt, D., ... & Gullett, B. (2022). Wildland fire smoke alters the composition, diversity, and potential atmospheric function of microbial life in the aerobiome. *ISME Communications*, 2(1), 8. https://doi.org/10.1038/s43705-022-00089-5

Krebs, P., Pezzatti, G. B., Mazzoleni, S., Talbot, L. M., & Conedera, M. (2010). Fire regime: history and definition of a key concept in disturbance ecology. *Theory in Biosciences*, 129(1), 53-69. https://doi.org/10.1007/s12064-010-0082-z

Mantgem, P. V., Schwartz, M., & Keifer, M. (2001). Monitoring fire effects for managed burns and wildfires: coming to terms with pseudoreplication. *Natural Areas Journal*, 21(3), 266-273.

Meyer, J., Zakhary, S., Larocque, M., & Lazar, C. S. (2022). From surface to subsurface: diversity, composition, and abundance of sessile and endolithic bacterial, archaeal, and eukaryotic communities in sand, clay and rock substrates in the Laurentians (Quebec, Canada). *Microorganisms*, 10(1), 129. https://doi.org/10.3390/microorganisms10010129

Mims, S. A., & Mims III, F. M. (2004). Fungal spores are transported long distances in smoke from biomass fires. *Atmospheric Environment*, 38(5), 651-655. https://doi.org/10.1016/j.atmosenv.2003.10.043

Mino, L., Kolp, M. R., Fox, S., Reazin, C., Zeglin, L., & Jumpponen, A. (2021). Watershed and fire severity are stronger determinants of soil chemistry and microbiomes than within-watershed woody encroachment in a tallgrass prairie system. *FEMS Microbiology Ecology*, 97(12), fiab154. https://doi.org/10.1093/femsec/fiab154

McLauchlan, K. K., Higuera, P. E., Miesel, J., Rogers, B. M., Schweitzer, J., Shuman, J. K., ... & Watts, A. C. (2020). Fire as a fundamental ecological process: Research advances and frontiers. *Journal of Ecology*, 108(5), 2047-2069. https://doi.org/10.1111/1365-2745.13403

Mohagheghi, A., Grohmann, K. M. M. H., Himmel, M., Leighton, L., & Updegraff, D. M. (1986). Isolation and characterization of Acidothermus cellulolyticus gen. nov., sp. nov., a new genus of thermophilic, acidophilic, cellulolytic bacteria. *International Journal of Systematic and Evolutionary Microbiology*, 36(3), 435-443. https://doi.org/10.1099/00207713-36-3-435

Neary, D. G., Klopatek, C. C., DeBano, L. F., & Ffolliott, P. F. (1999). Fire effects on belowground sustainability: a review and synthesis. *Forest Ecology and Management*, 122(1-2), 51-71. https://doi.org/10.1016/S0378-1127(99)00032-8

Normand, P., Berry, A., & Benson, D. R. (2015). Acidothermus. *Bergey's Manual of Systematics of Archaea and Bacteria*, 1-3. https://doi.org/10.1002/9781118960608.gbm00039

Oliver, A. K., Callaham Jr, M. A., & Jumpponen, A. (2015). Soil fungal communities respond compositionally to recurring frequent prescribed burning in a managed southeastern US forest ecosystem. *Forest Ecology and Management*, 345, 1-9. https://doi.org/10.1016/j.foreco.2015.02.020

Orumaa, A., Agan, A., Anslan, S., Drenkhan, T., Drenkhan, R., Kauer, K., ... & Metslaid, M. (2022). Long-term effects of forest fires on fungal community and soil properties along a hemiboreal Scots pine forest fire chronosequence. *Science of The Total Environment*, 851, 158173. https://doi.org/10.1016/j.scitotenv.2022.158173

Palmer, B., Pietrasiak, N., Cobb, P., & Lipson, D. (2023). Using simulated wildland fire to assess microbial survival at multiple depths from biocrust and bare soils. Frontiers in Microbiology, 14, 1123790. https://doi.org/10.3389/fmicb.2023.1123790

Pausas, J. G., & Keeley, J. E. (2009). A burning story: the role of fire in the history of life. *BioScience*, 59(7), 593-601. https://doi.org/10.1525/bio.2009.59.7.10

Peay, K. G., Garbelotto, M., & Bruns, T. D. (2009). Spore heat resistance plays an important role in disturbance-mediated assemblage shift of ectomycorrhizal fungi colonizing Pinus muricata seedlings. *Journal of Ecology*, 97(3), 537-547. https://doi.org/10.1111/j.1365-2745.2009.01489.x

Pellegrini, A. F., & Jackson, R. B. (2020). The long and short of it: a review of the timescales of how fire affects soils using the pulse-press framework. *Advances in Ecological Research*, 62, 147-171. https://doi.org/10.1016/bs.aecr.2020.01.010

Philpott, T. J., Danyagri, G., Wallace, B., & Frank, M. (2025). Low-severity wildfire prevents catastrophic impacts on fungal communities and soil carbon stability in a fire-affected Douglas-fir ecosystem. *Geoderma*, 454, 117189. https://doi.org/10.1016/j.geoderma.2025.117189

Põlme, S., Abarenkov, K., Henrik Nilsson, R., Lindahl, B. D., Clemmensen, K. E., Kauserud, H., ... & Tedersoo, L. (2020). FungalTraits: a user-friendly traits database of fungi and fungus-like stramenopiles. *Fungal Diversity*, 105(1), 1-16. https://doi.org/10.1007/s13225-020-00466-2

Pulido-Chavez, M. F., Randolph, J. W., Zalman, C., Larios, L., Homyak, P. M., & Glassman, S. I. (2021). Bacterial and fungal communities experience rapid secondary succession during the first year following wildfire in California chaparral. *bioRxiv*, 2021-12. https://doi.org/10.1101/2021.12.07.471678

Pulido-Chavez, M. F., Randolph, J. W., Zalman, C., Larios, L., Homyak, P. M., & Glassman, S. I. (2023). Rapid bacterial and fungal successional dynamics in first year after chaparral wildfire. *Molecular Ecology*, 32(7), 1685-1707. https://doi.org/10.1111/mec.16835

Pressler, Y., Moore, J. C., & Cotrufo, M. F. (2019). Belowground community responses to fire: meta-analysis reveals contrasting responses of soil microorganisms and mesofauna. *Oikos*, 128(3), 309-327. https://doi.org/10.1111/oik.05738

R Core Team, R. (2020). R: A language and environment for statistical computing.

Rai, D., Silveira, M. L., Strauss, S. L., Meyer, J. L., Castellano-Hinojosa, A., Kohmann, M. M., ... & Gerber, S. (2023). Short-term prescribed fire-induced changes in soil microbial communities and nutrients in native rangelands of Florida. *Applied Soil Ecology*, 189, 104914. https://doi.org/10.1016/j.apsoil.2023.104914

Revillini, D., David, A. S., Menges, E. S., Main, K. N., Afkhami, M. E., & Searcy, C. A. (2022). Microbiome-mediated response to pulse fire disturbance outweighs the effects of fire legacy on plant performance. *New Phytologist*, 233(5), 2071-2082. https://doi.org/10.1111/nph.17689

Revillini, D., Searcy, C. A., & Afkhami, M. E. (2025). 50-year fire legacy regulates soil microbial carbon and nutrient cycling responses to new fire. *Soil Biology and Biochemistry*, 109868. https://doi.org/10.1016/j.soilbio.2025.109868

Robertson, K. M., Platt, W. J., & Faires, C. E. (2019). Patchy fires promote regeneration of longleaf pine (Pinus palustris Mill.) in pine savannas. *Forests*, 10(5), 367. https://doi.org/10.3390/f10050367

Salo, K., & Kouki, J. (2018). Severity of forest wildfire had a major influence on early successional ectomycorrhizal macrofungi assemblages, including edible mushrooms. *Forest Ecology and Management*, 415, 70-84. https://doi.org/10.1016/j.foreco.2017.12.044

Sharma, G.D. Effect of fire on soil microorganisms in a meghalaya pine forest. Folia Microbiol 26, 321–327 (1981). https://doi.org/10.1007/BF02927260

Semenova-Nelsen, T. A., Platt, W. J., Patterson, T. R., Huffman, J., & Sikes, B. A. (2019). Frequent fire reorganizes fungal communities and slows decomposition across a heterogeneous pine savanna landscape. *New Phytologist*, 224(2), 916-927. https://doi.org/10.1111/nph.16096

Stambaugh, M. C., Guyette, R. P., & Marschall, J. M. (2011). Longleaf pine (Pinus palustris Mill.) fire scars reveal new details of a frequent fire regime. *Journal of Vegetation Science*, 22(6), 1094-1104. https://doi.org/10.1111/j.1654-1103.2011.01322.x

Taylor, D. L., & Bruns, T. D. (1999). Community structure of ectomycorrhizal fungi in a Pinus muricata forest: minimal overlap between the mature forest and resistant propagule communities. *Molecular Ecology*, 8(11), 1837-1850. https://doi.org/10.1046/j.1365-294x.1999.00773.x

Wasserman, T. N., & Mueller, S. E. (2023). Climate influences on future fire severity: a synthesis of climate-fire interactions and impacts on fire regimes, high-severity fire, and forests in the western United States. *Fire Ecology*, 19(1), 1-22. https://doi.org/10.1186/s42408-023-00200-8

Weakley, A. S. (2020). Flora of the southern and mid-Atlantic states. University of North Carolina Herbarium, North Carolina Botanical Garden, Chapel Hill, NC, USA. https://ncbg.unc.edu/research/unc-herbarium/flora-apps/ [accessed 03 May 2023].

White, T. J., Bruns, T., Lee, S. J. W. T., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: A guide to methods and applications*, 18(1), 315-322. http://dx.doi.org/10.1016/B978-0-12-372180-8.50042-1

Whitman, T., Whitman, E., Woolet, J., Flannigan, M. D., Thompson, D. K., & Parisien, M. A. (2019). Soil bacterial and fungal response to wildfires in the Canadian boreal forest across a burn severity gradient. *Soil Biology and Biochemistry*, 138, 107571. https://doi.org/10.1016/j.soilbio.2019.107571

Yang, T., Tedersoo, L., Lin, X., Fitzpatrick, M. C., Jia, Y., Liu, X., ... & Chu, H. (2020). Distinct fungal successional trajectories following wildfire between soil horizons in a cold-temperate forest. *New Phytologist*, 227(2), 572-587. https://doi.org/10.1111/nph.16531

Yilmaz, N., Visagie, C. M., Houbraken, J., Frisvad, J. C., & Samson, R. A. (2014). Polyphasic taxonomy of the genus Talaromyces. *Studies in Mycology*, 78, 175-341. https://doi.org/10.1016/j.simyco.2014.08.001

CHAPTER 5

CONCLUSIONS

This dissertation demonstrates that fire regime legacy is a dominant force structuring plant and soil microbial communities in a fire-dependent longleaf pine ecosystem, with significant implications for community composition, species interactions, and ecosystem resilience. By integrating both above and belowground perspectives across multiple temporal and experimental contexts, this work reveals that variation in frequent fire regimes impose selective pressures that differentially affect plants and microbes, ultimately shaping recovery trajectories, ecosystem function, and stability.

In Chapter 2, I show that annual fires homogenize fungal communities, reduce plant diversity, and lower soil phosphorus, suggesting that highly frequent fire can diminish resilience by narrowing functional and taxonomic diversity. Contrary to expectations, annual fire favored ectomycorrhizal and saprotrophic fungal guilds, while maintenance fire supported more functionally diverse assemblages, including endophytes, pathogens, and parasitic fungi. These shifts in fungal functional guilds likely reinforce vegetation dynamics sustaining frequent fire regimes while influencing plant health and successional trajectories under the less frequent disturbance. These results demonstrate that fire regimes not only determine species richness but also restructure microbial diversity and functional guilds in ways that may feedback to aboveground community dynamics.

In Chapter 3, a plant-soil feedback (PSF) experiment revealed that soil microbial communities exert strong, species-specific effects on plant performance, with implications for coexistence and vegetation dynamics. Sensitive-briar performed better in live soils, whereas wiregrass experienced reduced survival and biomass, indicating that soil microbes can drive host-specific growth patterns. Annual fire soils produced strong negative PSFs for wiregrass, suggesting that highly frequent burning fosters antagonistic microbial communities that suppress a foundational graminoid. In contrast, PSFs were largely neutral under the maintenance fire regime, indicating that the less frequent recurrent fire regime may maintain more balanced plant-microbe interactions. These findings complicate the assumption that fire universally reinforces graminoid dominance and highlight that fire regimes shape not only aboveground community composition but also belowground processes that influence plant establishment. By showing that fire regime can disrupt beneficial microbial associations, this work underscores the importance of integrating PSFs into fire management and restoration strategies to enhance biodiversity, stability, and long-term ecosystem resilience.

Finally, in Chapter 4, I show that although fire-adapted ecosystems are typically resilient to frequent burning, annual fire regimes can lead to reductions in plant diversity, shifts in microbial composition, and changes in fungal functional guilds relative to maintenance fire regimes. In addition, plant and microbial communities exhibited divergent recovery trajectories, with bacteria largely unaffected but fungi declining sharply post-fire fire and recovering slowly, especially in subsurface soils. These findings reveal that fire regime and soil depth jointly structure microbial community responses, and that frequent fire may selectively filter for disturbance-tolerant taxa while excluding slower-growing or dispersal-limited organisms. Collectively, these results suggest that highly frequent burning can alter belowground biodiversity and function, challenging assumptions about the ecological neutrality of annual fire in a highly fire-dependent system and underscoring the critical role of fire regimes in sustaining resilience in pyrogenic ecosystems.

APPENDIX A

SUPPLEMENTARY INFORMATION: CHAPTER 2

Table A1. Effects of time since fire (TSF) on bacterial and fungal diversity metrics. TSF was included as a categorical predictor in linear models for each response variable (richness, diversity, evenness) to test whether time since fire confounded observed fire regime effects. TSF was not a significant predictor for any mode, except for a significant interaction between fire regime and TSF in the bacterial richness model ($p < 0.05$).

		Fungi			**Bacteria**		
Response	**Predictor**	**F-value**	**df**	**p-value**	**F-value**	**df**	**p-value**
Richness	TSF	0.928	1	0.339	1.570	1	0.542
	Fire Regime x TSF	0.002	1	0.962	5.290	1	**0.025**
Evenness	TSF	0.207	1	0.650	0.349	1	0.556
	Fire Regime x TSF	0.080	1	0.777	0.375	1	0.542
Diversity	TSF	0.427	1	0.515	0.269	1	0.605
	Fire Regime x TSF	0.137	1	0.712	1.952	1	0.167

Table A2. Top ten bacterial indicator taxa associated with each fire regime treatment. Taxonomic classifications are shown from phylum to species (where available). The corresponding indicator statistic and p-value are reported. Only taxa with significant associations ($p < 0.05$) are included.

Fire Regime	Phylum	Class	Order	Family	Genus	Species	Stat	P-value
Annual	Firmicutes	Bacilli	Paenibacillales	Paenibacillaceae	Ammoniphilus	n/a	0.681	0.0001
Annual	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	Bacillus deserti	0.591	0.0001
Annual	Firmicutes	Bacilli	Paenibacillales	Paenibacillaceae	Ammoniphilus	n/a	0.574	0.0001
Annual	Actinobacteriota	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	n/a	0.490	0.0001
Annual	Chloroflexi	Ktedonobacteria	Ktedonobacterales	Ktedonobacteraceae	uncultured	n/a	0.484	0.0001
Annual	Chloroflexi	TK10	TK10	TK10	TK10	Ellin6519	0.483	0.0001
Annual	Proteobacteria	Gammaproteobacteria	Burkholderiales	Nitrosomonadaceae	MND1	n/a	0.483	0.0002
Annual	Firmicutes	Bacilli	Alicyclobacillales	Alicyclobacillaceae	Tumebacillus	Tumebacillus sp.	0.483	0.0001
Annual	Chloroflexi	AD3	AD3	AD3	AD3	uncultured	0.473	0.0002
Annual	Chloroflexi	TK10	TK10	TK10	TK10	Ellin6543	0.460	0.0002
Maintenance	Proteobacteria	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	n/a	0.597	0.0001
Maintenance	Planctomycetota	Planctomycetes	Gemmatales	Gemmataceae	uncultured	uncultured	0.582	0.0001
Maintenance	Planctomycetota	Planctomycetes	Gemmatales	Gemmataceae	Gemmata	n/a	0.514	0.0001
Maintenance	Verrucomicrobiota	Verrucomicrobiae	Chthoniobacterales	Xiphinematobacteraceae	Candidatus	n/a	0.513	0.0001
Maintenance	Proteobacteria	Alphaproteobacteria	Elsterales	uncultured	uncultured	Alphaproteobacteria	0.506	0.0003
Maintenance	Actinobacteriota	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium	n/a	0.504	0.0001
Maintenance	Planctomycetota	Planctomycetes	Isosphaerales	Isosphaeraceae	Tundrisphaera	n/a	0.491	0.0001
Maintenance	Proteobacteria	Alphaproteobacteria	Elsterales	uncultured	uncultured	uncultured soil	0.485	0.0001
Maintenance	Acidobacteriota	Vicinamibacteria	Vicinamibacterales	uncultured	uncultured	n/a	0.482	0.0002
Maintenance	Proteobacteria	Alphaproteobacteria	Elsterales	uncultured	uncultured	uncultured Stella	0.477	0.0001

Table A3. Top ten fungal indicator taxa associated with each fire regime treatment. Taxonomic classifications are shown from phylum to species (where available). The corresponding indicator statistic and p-value are reported. Only taxa with significant associations (p < 0.05) are included.

Fire Regime	Phylum	Class	Order	Family	Genus	Species	Stat	P-value
Annual	Ascomycota	Dothideomycetes	Pleosporales	unidentified	unidentified	unidentified	0.596	0.0001
Annual	Ascomycota	Dothideomycetes	Pleosporales	Didymosphaeriaceae	Paraphaeosphaeria	Paraphaeosphaeria verruculosa	0.500	0.0001
Annual	Ascomycota	Dothideomycetes	Pleosporales	Periconiaceae	Periconia	Periconia lateralis	0.447	0.0001
Annual	Ascomycota	Dothideomycetes	Pleosporales	Didymosphaeriaceae	Paraconiothyrium	Paraconiothyrium brasiliense	0.446	0.0001
Annual	Ascomycota	Dothideomycetes	Venturiales	unidentified	unidentified	unidentified	0.399	0.002
Annual	Mucoromycota	Mucoromycotina	Mucoromycotina	Mucoromycotina	Bifiguratus	unidentified	0.366	0.006
Annual	Ascomycota	Sordariomycetes	Hypocreales	Stachybotryaceae	Striaticonidium	n/a	0.354	0.0028
Annual	Ascomycota	Leotiomycetes	Helotiales	n/a	n/a	n/a	0.353	0.0006
Annual	Ascomycota	Dothideomycetes	Pleosporales	unidentified	unidentified	unidentified	0.350	0.0013
Annual	Ascomycota	Eurotiomycetes	Chaetothyriales	n/a	n/a	n/a	0.344	0.0058
Maintenance	Ascomycota	Dothideomycetes	Pleosporales	Didymellaceae	n/a	n/a	0.457	0.0001
Maintenance	Ascomycota	Dothideomycetes	Capnodiales	Teratosphaeriaceae	Devriesia	Devriesia acadiensis	0.436	0.0001
Maintenance	Ascomycota	Sareomycetes	Sareales	Sareaceae	Sarea	Sarea resinae	0.423	0.0006
Maintenance	Ascomycota	Dothideomycetes	Capnodiales	Teratosphaeriaceae	Devriesia	Devriesia acadiensis	0.391	0.0001
Maintenance	Ascomycota	Dothideomycetes	Mytilinidales	Gloniaceae	Cenococcum	n/a	0.384	0.0005
Maintenance	Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	Tremella	unidentified	0.374	0.0001
Maintenance	Basidiomycota	Geminibasidiomycetes	Geminibasidiales	unidentified	unidentified	unidentified	0.372	0.0017
Maintenance	Ascomycota	Dothideomycetes	Capnodiales	Teratosphaeriaceae	unidentified	unidentified	0.363	0.0001
Maintenance	Ascomycota	Sordariomycetes	Hypocreales	Hypocreales	Acremonium	Acremonium spinosum	0.360	0.0018
Maintenance	Ascomycota	Sordariomycetes	Coniochaetales	Coniochaetaceae	Coniochaeta	unidentified	0.354	0.0013

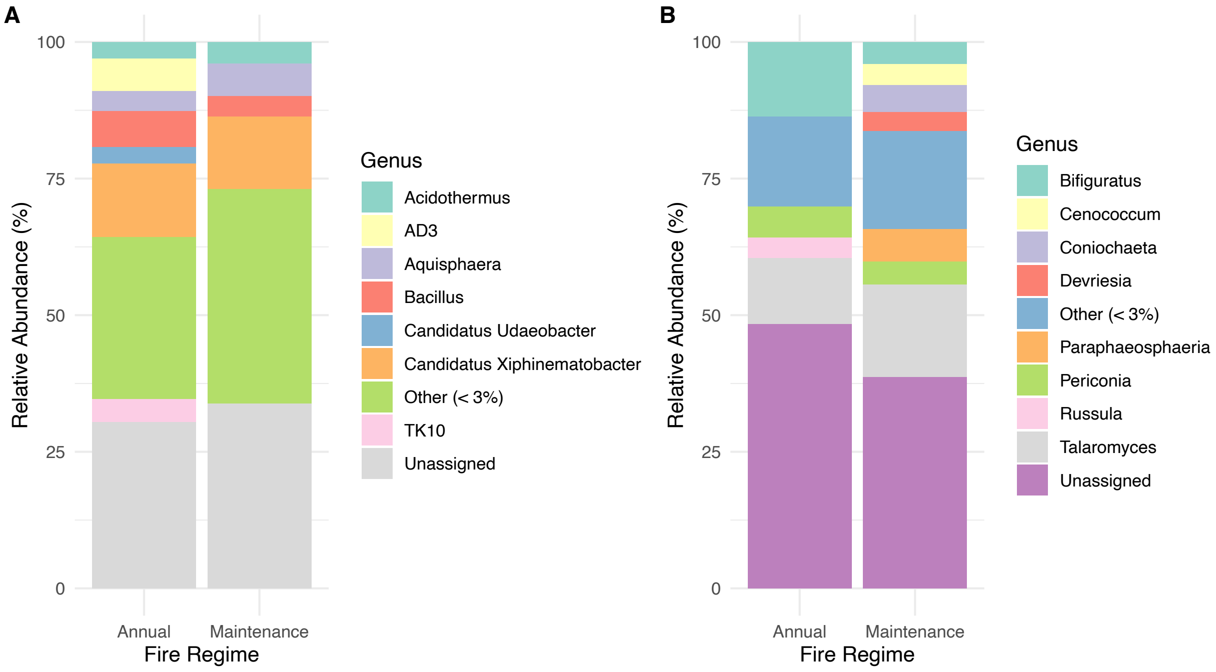

Figure A1. Mean relative abundance (%) of A) bacterial and B) fungal indicator genera from the indicator species analysis across fire regime treatments. All indicator taxa plotted were statistically significant ($p < 0.05$).

APPENDIX B

SUPPLEMENTARY INFORMATION: CHAPTER 3

Table B1. Results of ANOVAs examining the effects of fire regime, soil sterilization treatment, and their interaction on soil chemistry variables after the conditioning phase of the experiment.

Response	**Effect**	**Sum Sq**	**Df**	**F value**	**Pr(>F)**	**Sign.**
OM	Fire Regime	3.8002	1	87.9377	**< 0.0001**	***
	Sterility	0.0882	1	2.0402	0.1587	
	Fire Regime*Sterility	0.0202	1	0.4667	0.4973	
P1	Fire Regime	0.14951	1	15.11	**0.0002**	***
	Sterility	2.22219	1	224.587	**< 0.0001**	***
	Fire Regime*Sterility	0.15777	1	15.945	**< 0.0001**	***
P2	Fire Regime	0.0018	1	0.0288	0.8658	
	Sterility	5.3542	1	84.331	**< 0.0001**	***
	Fire Regime*Sterility	0.1187	1	1.8694	0.177	
K	Fire Regime	156.82	1	23.7858	**< 0.0001**	***
	Sterility	190.82	1	28.9429	**< 0.0001**	***
	Fire Regime*Sterility	25.35	1	3.8451	0.05487	
Mg	Fire Regime	3010.42	1	165.6676	**< 0.0001**	***
	Sterility	370.02	1	20.3626	**< 0.0001**	***
	Fire Regime*Sterility	36.82	1	2.0261	0.1602	
Ca	Fire Regime	70110	1	200.2149	**< 0.0001**	***
	Sterility	2470	1	7.0548	**0.01028**	*
	Fire Regime*Sterility	1760	1	5.0273	**0.02893**	*
pH	Fire Regime	0.04817	1	5.7966	**0.01938**	*
	Sterility	0.62017	1	74.6332	**< 0.0001**	***
	Fire Regime*Sterility	0.02817	1	3.3897	0.0709	
CEC	Fire Regime	5.046	1	131.4715	**< 0.0001**	***
	Sterility	0.024	1	0.6253	0.4324	
	Fire Regime*Sterility	0.0167	1	0.4342	0.5126	
NO_3	Fire Regime	52.27	1	3.7641	0.05741	
	Sterility	437.4	1	31.5	**< 0.0001**	***
	Fire Regime*Sterility	41.67	1	3.0007	0.08873	

Table B2. Results of ANOVA examining the effects of fire regime, soil sterilization treatment, and source soil (individual plant species grown in each species' source soil) on soil chemistry after the feedback phase.

Response	Effect	Sum Sq	Df	F value	Pr(>F)	Sign.
P	Fire Regime	4.61	1	0.2342	0.629	
	Sterility	944.39	1	47.9823	< **0.0001**	***
	Soil Source	67.98	2	1.7269	0.182	
	Species	440.95	2	11.2	< **0.0001**	***
K	Fire Regime	23	1	0.054	0.815	
	Sterility	67039	1	95.974	< **0.0001**	***
	Soil Source	7953	2	5.6	**0.004**	**
	Species	95	2	0.07	0.931	
Mg	Fire Regime	7098	1	1.0985	0.296	
	Sterility	379439	1	62.989	< **0.0001**	***
	Soil Source	27087	2	2.186	0.115	
	Species	113425	2	9.93	< **0.0001**	***
Ca	Fire Regime	95324	1	1.233	0.268	
	Sterility	8414148	1	59.41	< **0.0001**	***
	Soil Source	591466	2	2.0337	0.134	
	Species	1201537	2	4.17	**0.017**	*
pH	Fire Regime	0.0127	1	0.6399	0.425	
	Sterility	0.0386	1	1.9454	0.165	
	Soil Source	0.0074	2	0.1861	0.830	
	Species	3.64	2	91.74	< **0.0001**	***
Mn	Fire Regime	5.5	1	0.033	0.854	
	Sterility	1311.4	1	15.59	**0.0001**	***
	Soil Source	159.3	2	0.948	0.390	
	Species	2028.4	2	13.4	< **0.0001**	***
Zn	Fire Regime	0.659	1	1.233	0.268	
	Sterility	37.006	1	72.309	**0.0001**	***
	Soil Source	5.942	2	5.738	**0.003**	**
	Species	2.73	2	2.73	0.06	
NO_3	Fire Regime	42.7	1	1.9013	0.170	
	Sterility	49.3	1	2.1915	0.141	
	Soil Source	18.4	2	0.4098	0.6644	
	Species	613.22	2	15.85	< **0.0001**	***

APPENDIX C

SUPPLEMENTARY INFORMATION: CHAPTER 4

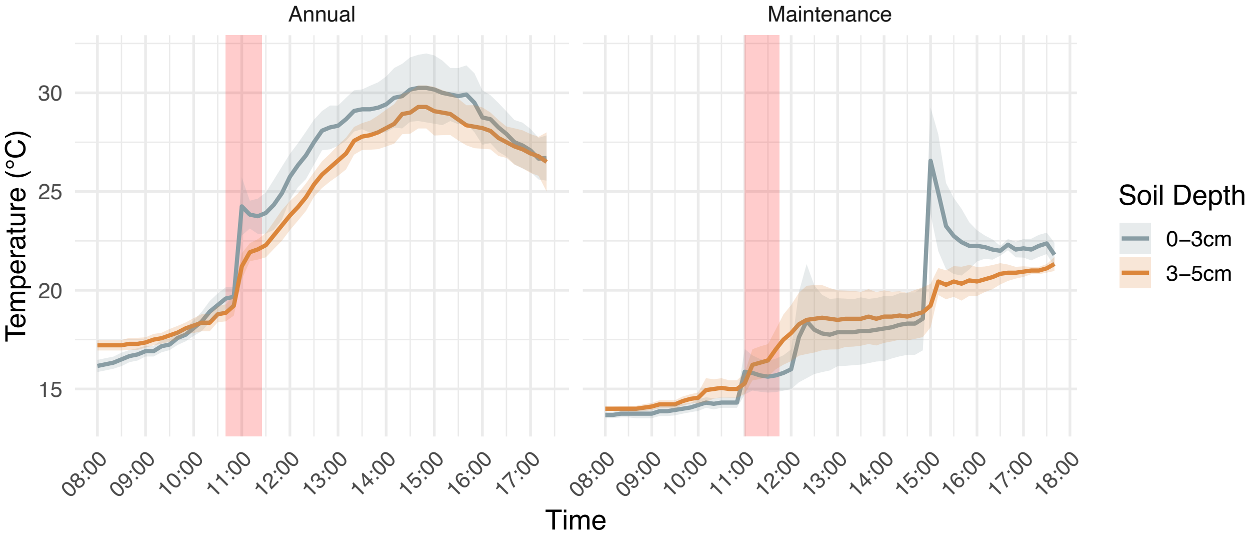

Figure C1. Temperature before, during, and after the prescribed fire events at the two fire regimes (annual, maintenance). Shaded red regions indicate the duration of active fire. Line represents temperature measurements over time, with different colors denoting soil depth (blue = 0-3 cm, orange = 3-5 cm).