UNIVERSITY OF CALIFORNIA
RIVERSIDE

Unraveling Fire Adaptation Traits in Pyrophilous Bacteria and Fungi

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Microbiology

by

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December 2024

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Acknowledgements

The research contained within this dissertation represents a significant portion of my life and could not have been completed alone. There are many individuals without which this research would not have been possible, and I would be remiss not to take time and acknowledge the support of the people who kept me going even when the going was hard.

It should go without saying that this dissertation and all of the research within it would not have been possible without the guidance and mentorship of my advisor Dr. Sydney Glassman. Over slightly more than 6 years Sydney has guided my path as I dove ever deeper into the mycological world. She has steered me back from countless rabbit holes I would have become lost down and has encouraged me at every turn to reach further than I thought I could. I could not have done this without her and though this chapter of our time together is ending, I know I have both a guiding presence and dear friend I can count on for throughout the rest of my career.

As much as a ship is nothing without her captain, she is also does not function without her crew and the crew of the Glassman lab are not only treasured colleagues but also friends that have both celebrated my victories with me and consoled me in my failures. From the very beginning when it was just Dr. Fabi Pulido and I as Sydney's students, the people within this lab have made it a second home. Fabi helped keep me sane with her constant mantra of "it is what it is". And even though our journeys take us

wherever they may, I know there will always be a lifelong friend waiting at every conference for us to get the chance to reconnect and I will be eagerly waiting as well.

Of Arik, Basubi, Esbeiry, Marcos, Ehsan, Maria, James, and Judy you were all instrumental in both the work that took place in this dissertation and in all the important moments along the way. From the guidance and encouragement of the post-docs Ehsan and Marcos to the support and shared adventures with my fellow grad students Arik, Basubi, and Esbeiry I will forever be grateful for time we spent together and I cannot wait to see what the future has in store for you all as well. For lab managers Judy, James, and Maria your constant readiness and willingness to step in and help was invaluable. I hope you remember our time together as fondly as I will.

The research undertaken in my last two chapters were projects that quickly became grand in scale and if it were up solely to my own two hands the work might have taken another 6 years. As such, I have to thank and acknowledge the small army of undergraduates and trainees that worked with me along the way. Jorge, Marely, Audrey, Wine-Jie, Priscilla, Ryan, Anna, Jenna, Aishwarya, Jesus, Sienna and Justin were all phenomenal assistants and I was privileged to mentor these bright upcoming scientists. And while I only succeeded in keeping Ryan and Aishwarya in research careers, all of these individuals have become fantastic scientists in their careers and I could not be prouder or more grateful of the work you did by my side.

I also have to thank the other mentors who have helped to shape this research and my path along my career. Before I even knew research was where my end goal lay, Robin Ikeda and Sam Gaddie were present to encourage me in any way possible as I continued

my studies. Without Sam taking a chance on a young undergraduate looking for his first job in science I would not have had the career opportunities which have helped me reach the Ph.D. in the first place. Without Robin and her contagious enthusiasm for science and teaching, I may not have ever considered a Ph.D. as a path I might follow. After joining California State University Dr. Paul Orwin took this budding would-be scientist and showed me a love for microbes. Without Paul I might be studying mammals right now and what a tragedy that might have been for all involved. And then there are the professors at UCR that helped to shape the scientist I am today. Dr. Kathy Borkovich first helped me in brainstorming ideas for my NSF GRFP, then served on my guidance and qualifying exam committees and I am grateful for her guidance and am proud of the collaborative *Neurospora* work we have engaged in together. Dr. Marko Spasojevic first managed the minor miracle of getting some statistics to stick in my brain (a feat for which I will eternally be grateful), then served on both my qualifying and dissertation committees and his guidance and patience has been of enormous help and impact to my Ph.D. Last but certainly not least there is Dr. Jason Stajich. I truly believe that from my first meeting with Dr. Jason Stajich that I gained an ally in the pursuit of my microbiological dreams. Jason was my first contact at UCR, he helped me set up my first rotation in a lab, he served on my guidance committee, chaired my qualifying exam committee, and then stayed on to advise in my dissertation committee. His guidance and advice have been constant companions throughout my Ph.D. and I have been truly lucky to have gotten the chance to work with him as I have.

A Ph.D. is an undertaking of endurance as much as it is about the science and research and mine would not have been possible without the support of the many friends and family who were by my side throughout my journey. I have been immensely fortunate to have been a part of the same core group of friends for the last 13 years and they have been with me through some of the best and worst times of my life. Alex, Flynn, Katy, Oscar, Yvonne, DJ, Kelsey, Mev, and Adam you have all been integral in the completion of this journey. Without your love and friendship this undertaking would have been impossible. You are family to me in every way that matters. As for the increasingly large family I am surrounded with, I love and thank you all for your support and for the assistance and celebration of every milestone along this road. To my sisters and brothers (both born and in-laws) I could not have done this without you. A very special thank you and acknowledgment must be reserved though for my parents both those I was born with and the ones I have gained along the way. Andy and Cindy, you both have treated me as if I was your own son from the very beginning and I genuinely could not have made it this far without your love and support. Jason, you joined our family when I was already almost an adult, but in the years since you have been a father to me in every way that has mattered and I cherish all the memories we have made together along the way. Lastly, to my mom who stood by me and has fiercely supported me my entire life I love you so much. Thank you for all the unwavering love, support, and encouragement you have shown me.

Last, but most important of all, I thank my wife Annie and my daughter Emily. Em, you were only 6 months old when I started this journey. Now you are 6 (almost 7 at

the time of writing) and have grown into person I could not be prouder of. I hope you know how much every little drawing, every note you wrote, every squeal of delight when I walked through the door kept me going when I wasn't sure I could anymore. I love you so much. Annie, you are my best-friend, my soul mate, my partner in all things. I am grateful every day that you are my wife and that I get to spend every moment of this journey with you. You carried me through this Ph.D. more than anyone and I truly could not have done this without you. Every late-night dinner, every little love note or surprise, every sign you made for when I returned home from a conference, every whispered word of encouragement when I thought it was time to give up kept me going more than you will ever know. I love you so incredibly much. Thank you.

ABSTRACT OF THE DISSERTATION

Unraveling Fire Adaptation Traits in Pyrophilous Bacteria and Fungi

by

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Doctor of Philosophy, Graduate Program in Microbiology
University of California, Riverside, December 2024
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Wildfires are an increasingly serious consequence of climate change and human development. As soil microbes are integral drivers of many ecosystem processes it is imperative to understand how fire will affect soil microbial communities. There is evidence of some pyrophilous, or “fire-loving” microbes, but this information is lacking in ecological functional understanding and much of the data regarding pyrophilous microbial trends comes from Pinaceae-dominated ecosystems. To address these gaps, we first took advantage of the Soberanes mega-fire of 2016, a serendipitous wildfire in a redwood-tanoak forest in which we had pre- and post-fire sampling from the exact same plots along with true unburned controls. We then characterized the post-fire soil bacterial and fungal communities and found similar increased dominance of pyrophilous taxa as described from other ecosystems and that response to fire was phylogenetically conserved at the class level for fungi and the phylum level for bacteria. By assessing the

patterns in pyrophilous microbes across multiple ecosystems we were able to construct a hypothetical framework of what traits drive fire-adaptation in pyrophilous microbes and related it to Grime's famous 1977 competitive-stress tolerator-ruderal (C-S-R) trait framework. We then made predictions about where some described pyrophilous taxa might be placed within the framework. Over the next four years, we created a large and diverse culture collection of pyrophilous microbial isolates which we could use to investigate pyrophilous microbial traits in a laboratory setting. In the course of this culture collection creation we tested the effect of various soil storage methods and culture medias, including the creation of a new pyrogenic organic matter (PyOM) media, on the culturing of pyrophilous microbes and found that freezing soil at -80ºC preserved pyrophilous microbes the best and that a combination of the PyOM media and an oligotrophic media capture the greatest diversity of pyrophilous microbes. Finally, we selected 20 pyrophilous fungi and profiled an array of pyrophilous traits using phenotypic assays including growth, enzymatic, thermotolerance, and N_2O emission assays. We used this trait data to plot these traits in functional trait space to statistically determine the trait-syndromes which explain pyrophilous fungal life strategies.

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Chapter I

Introduction to the Dissertation

1.1 Wildfires are Becoming Increasingly Severe

Changes in wildfires and wildfire regimes have increasingly become a major concern for people around the globe. Wildfire regimes are chiefly characterized by the frequency of fire events (also referred to as the "fire return interval"), the intensity of the fire (ie. how hot the fire burns), the severity of the wildfire (Gill and Allan 2008). Wildfire severity typically is quantified either by vegetation mortality metrics (tree crown loss, percent vegetation mortality) or soil burn severity quantified using a combination of vegetation mortality in conjunction with soil surface measurements such as ash depth (Keeley 2009). In many ecosystems, wildfires have historically been low severity, high frequency events that support the ecological functions of their ecosystems (Archibald et al. 2013). The low severity fires serve to clear away dead and decomposing vegetation opening space for new growth all while depositing the nutrients released from the dead vegetation back into the soil in the form of nitrogen rich ash (Wan, Hui, and Luo 2001; Bond and Keeley 2005). Many ecosystems such as Mediterranean and certain coniferous

forest systems depend on fire to allow the propagation of several key species within those ecosystems (Keeley et al. 2011; He and Lamont 2018). However, global climate change factors such as prolonged droughts (Mukherjee, Mishra, and Trenberth 2018), increasing average global temperatures (Westerling et al. 2006), and shifting seasonal precipitation patterns (Hajek and Knapp 2022), coupled with continual development along the wildland-urban interface (Hammer et al. 2007), have made ecosystems more susceptible to larger, more frequent, and more severe wildfire events than historical norms (Hantson, Pueyo, and Chuvieco 2015; de Groot, Flannigan, and Cantin 2013; Liu, L. Goodrick, and A. Stanturf 2013).

Thus, there is an increasing global concern over the ecological impacts of wildfire. It is estimated that in 2023 alone, over 93,000km^2 of tree cover was lost due to wildfire (MacCarthy et al. 2024). Additionally, the global tree cover lost to wildfire has increased by 6% every year since 2001 (Tyukavina et al. 2022). These increasingly severe wildfire events have even given rise to the phenomenon coined the "mega-fire" which is characterized by extraordinary size and severity that have outsized impacts on socio-economics (Adams 2013; Attiwill and Binkley 2013; Stephens et al. 2014). As these contributing factors to wildfire intensity continue to escalate, it is vital that we understand how these fires will affect our terrestrial ecosystems.

1.2 Soil Microbes Drive Key Ecosystem Functions, which are altered by wildfire

Soil microbes are essential drivers for a wide host of essential ecosystem functions (Heijden, Bardgett, and Straalen 2008). Arbuscular and Ectomycorrhizal fungi

are obligate symbionts of 80-90% of terrestrial plants (Tedersoo et al. 2014; Brundrett and Tedersoo 2018). Soil microbes are the primary drivers of plant litter decomposition (Purahong et al. 2016; Moorhead et al. 2013), and are an essential aspect of biogeochemical cycling (Crowther et al. 2019) including both carbon (Schimel and Schaeffer 2012) and nitrogen cycling (Kuypers, Marchant, and Kartal 2018). Wildfire dramatically alters soil chemistry, causing large depositions of nitrogen (Wan, Hui, and Luo 2001), raising the pH of soil (Certini 2005), and converting microbially accessible carbon sources into pyrogenic carbon forms (Pierson et al. 2019; Michelotti and Miesel 2015), which are largely composed of recalcitrant polycyclic aromatic hydrocarbons (Knicker 2011; Zeba et al. 2024). This is all in addition to fire's direct heating of the soil which can massively impact soil communities (Pingree and Kobziar 2019). As the soil microbiome is such an integral part of ecosystem function, it is imperative that we understand how these increasingly severe wildfires will affect soil microbes.

1.3 Fire Affects Soil Microbes Differentially

Fire has a dramatic effect on soil microbial communities (Pressler, Moore, and Cotrufo 2019). Wildfire reduces microbial species richness (Pressler, Moore, and Cotrufo 2019; Day et al. 2019; Certini et al. 2021), even by as much as 80% (Pulido-Chavez et al. 2023). Fire also reduces microbial diversity and community evenness (Pressler, Moore, and Cotrufo 2019), and can cause massive reductions of whole guilds of soil microbes including mycorrhizal fungi (Day et al. 2019; Dove and Hart 2017; Caiafa et al. 2023; Pulido-Chavez et al. 2023). Fire also causes large community composition shifts,

reducing bacterial phyla like Proteobacteria, Verrumicrobia and Cyanobacteria while increasing Firmicutes and Actinobacteria (Acea and Carballas 1996; Pressler, Moore, and Cotrufo 2019). Fire also shifts fungal communities from Basidiomycota to Ascomycota dominated communities (Pressler, Moore, and Cotrufo 2019; Pulido-Chavez et al. 2023; Day et al. 2019; Fox et al. 2022). It has also been documented that these changes to soil microbial communities can be long-lasting post-fire, taking upwards of 20 years to recover to pre-fire levels (Bárcenas-Moreno et al. 2011; Isobe et al. 2009; Pérez-Valera et al. 2018).

However, not all soil microbes are negatively affected by wildfire. Since 1909, evidence of pyrophilous or "fire-loving" microbes has existed (Seaver 1909; Seaver and Clark 1910). These pyrophilous microbes are taxa which consistently increase in abundance following fire, with the first pyrophilous microbes being fungi observed to fruit repeatedly after fire (Seaver and Clark 1910; Petersen 1970; Warcup and Baker 1963; Fox et al. 2022). Even as recently as 2020, detection of pyrophilous fungi through fruiting body surveys has still been employed (Hughes et al. 2020). More recent soil community DNA analyses have helped identify non-fruiting pyrophilous fungal species (Fox et al. 2022; Brown et al. 2019) which has also opened the field to include pyrophilous bacterial taxa (Brown et al. 2019; Whitman et al. 2019; Woolet and Whitman 2020; Johnson et al. 2023). As with pre-fire soil communities, these post-fire communities are integral in ecosystem function and recovery (Pérez-Valera et al. 2020), requiring a deeper understanding of pyrophilous microbial function and traits.

1.4 Trait-Based Frameworks as Powerful Tools for Understanding Ecological Functions

Trait-based approaches have gained popularity as a powerful way to understand and predict ecological functions of taxa or groups based on the observed traits of individual species. These traits are quantified measurements of how a given species grows (e.g. leaf size, seed weight, cell division rates, etc.) or interacts with its environment (e.g. carbon use efficiency, nitrogen cycling ability, production of defensive molecules, etc.). Use of trait-based assays and trait frameworks have enabled plant researchers to gain deeper insights into ecological functions and interactions (Laughlin 2014; Moretti and Legg 2009; Reich 2014; J. P. Grime 1977). Trait-based approaches to microbial functions have likewise given researchers new and deeper insights into the effect microbes have on their ecosystems (Barberán et al. 2017; Goberna and Verdú 2016; Krause et al. 2014; Martiny et al. 2015; Lustenhouwer et al. 2020). These trait assays have in turn led to frameworks which categorize trade-offs and lifestyles of microorganisms using defined trait suites (Malik et al. 2020; Camenzind et al. 2024; Allison and Goulden 2017; Maynard et al. 2019). The trade-offs I refer to stem from the hypothesis that it is incredibly rare for a given species to be good at all ecological functions and roles and therefore must specialize in certain functions to secure an ecological niche for itself (Kneitel and Chase 2004; J. P. Grime 1977; J. Philip Grime 1988). This specialization comes at the cost of being able to engage in an opposing life strategy (ex. growing fast vs. growing slowly) or causes specialization for one type of ecological function (such as improved carbon acquisition) at the expense of specializing

in another ecological function (ability to cycle nitrogen) (Kneitel and Chase 2004). Understanding of pyrophilous traits and experimentally derived trait-based frameworks exist for fire-adapted plants (Keeley et al. 2011; Bond and Wilgen 2012; Pausas et al. 2004), but our understanding of pyrophilous microbial traits is far more limited.

1.5 Limited Knowledge on Pyrophilous Microbial Functions and Traits

Most research on pyrophilous bacteria and fungi has been observational documenting which taxa increase in abundance and dominance post-fire (Pressler, Moore, and Cotrufo 2019; Fox et al. 2022; Petersen 1970; Bárcenas-Moreno et al. 2011). Here I refer both to the increasing abundance of certain phylogenetic groups discussed previously, and the work that exists characterizing the post-fire abundance of individual taxa like the fungal genus *Pyronema domesticum* (Seaver 1909; Bruns et al. 2020; Fischer et al. 2021; Fox et al. 2022) and the bacterial genus *Massilia* (Whitman et al. 2019; Woolet and Whitman 2020; Johnson et al. 2023). However, our understanding of the ecological functions of pyrophilous microbes and the traits that enable their adaptation to fire is in its infancy.

Our understanding of pyrophilous microbial functions is limited by knowledge of pyrophilous microbial traits. One hypothetical trait framework for pyrophilous microbes was published prior to the work in this dissertation (Whitman et al. 2019), but testing hypothetical trait frameworks requires quantitative measurements of phenotypic traits of individual pyrophilous microbial taxa (Krause et al. 2014), which requires cultures. Some limited data exists regarding a handful of pyrophilous fungal taxa, such as one study

suggesting that pyrophilous fungi may contribute to post-fire soil aggregation based on growth patterns and enzyme exudates of 3 pyrophilous isolates (Filialuna and Cripps 2021). A small amount of studies showed evidence that some pyrophilous fungi might be capable of metabolizing pyrogenic organic matter (PyOM) based on genomic and transcriptomic analysis (Steindorff et al. 2020; 2022; Fischer et al. 2021; Day et al. 2019), but did not do any direct experimentation to prove PyOM degradation or incorporation. The rest of the proposed traits of pyrophilous fungi are based on field observations without direct laboratory evidence to support the claims (Fox et al. 2022). The amount of accumulated knowledge regarding pyrophilous bacteria is even more scant. One study has hypothesized some pyrophilous traits of bacteria based on co-occurrence and genomic analysis (Whitman et al. 2019), but these traits remain hypothetical without laboratory testing. Additionally, one study has characterized some pyrophilous bacterial traits that drive post-fire recovery (Johnson et al. 2023) but this was done using whole-community analyses which can make asserting that a specific taxa is responsible for any given function difficult at best. Uncovering pyrophilous microbial traits will better inform researchers about pyrophilous microbial functions and help us predict how microbial communities and their functions respond to wildfire.

1.6 Overview and Objectives of the Dissertation

While several studies have been conducted profiling microbial response to fire (Pressler, Moore, and Cotrufo 2019; Dove and Hart 2017), there are still large gaps in our knowledge of post-fire microbes that need to be remedied. First, most studies of fires use

space-for-time substitutions to identify impacts of fire on soil microbiomes, but very few have the opportunity to sample the same locations pre and post-fire (Glassman et al ISME 2016, Bruns 1998). Both studies that sampled plots before and after a stand-replacing wildfire were in Pinaceae systems, where the vast majority of research on pyrophilous microbiomes exists (Pressler, Moore, and Cotrufo 2019). This begs the question, do microbes respond to fire similarly in non-pine ecosystems? Finding patterns of microbial fire response specific to pyrophilous taxa that transcend ecosystem type will greatly improve our predictions of pyrophilous microbial traits. Second, to test microbial traits it is necessary to have a culture. Trait profiling on cultures will help to fill gaps in our understanding of pyrophilous microbial function (Fox et al. 2022; Whitman et al. 2019), which would ideally be performed on a culture collection of the diverse bacterial and fungal species repeatedly identified post-fire (Pulido-Chavez et al. 2023; Fox et al. 2022; Pressler, Moore, and Cotrufo 2019). As wildfires are unplanned phenomena, there are certain logistical issues to test regarding the treatment of post-fire soils for pyrophilous microbial culturing (Černohlávková et al. 2009; Cui et al. 2014; Rubin et al. 2013). Additionally, choice of culturing media can play a crucial role in the ability to capture pyrophilous microbes (Meletiadis et al. 2001; Vieira and Nahas 2005) and thus testing of various medias needs to occur. Finally, once pyrophilous microbes are in hand to work with in the lab, a variety of assays would need to be conducted to experimentally determine the traits governing adaptation to fire and the post-fire environment (Fox et al. 2022; Krause et al. 2014). With that trait data collected, it may then be possible to visualize the trait syndromes that govern microbial adaptations to fire in such a way as to

begin to bring parity with the work done on plant traits (Reich 2014; Laughlin 2014; Keeley et al. 2011), and thus validate or refute hypothetical pyrophilous frameworks (Whitman et al. 2019).

1.7 Specific Aims

The goal of this dissertation is to elucidate pyrophilous microbial traits. In pursuit of this, the following specific aims are to be achieved:

1) Characterize microbial response to mega-fire in a redwood-tanoak (non-Pinaceae) forest, test for phylogenetic conservatism of microbial response to fire, and identify patterns of pyrophilous microbial activity post-fire which will help inform the potential traits which govern microbial adaptation to fire.
2) Test for the optimal soil storage method and laboratory media(s) to capture the greatest diversity of pyrophilous microbes in culture. This aim will supply pyrophilous microbial cultures for laboratory trait-based testing.
3) Test pyrophilous fungal cultures in a variety of phenotypic assays in order to profile their traits and identify the traits governing their adaptation to wildfire and to the post-fire environment. Additionally, once trait data is collected for a diverse group of pyrophilous fungi then trait syndromes will be identified through statistical testing in trait space ordinations to validate or refute hypothetical placements of pyrophilous taxa in trait frameworks.

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Chapter II

Mega-fire in Redwood Tanoak Forest Reduces Bacterial and Fungal Richness and Selects for Pyrophilous Taxa that are Phylogenetically Conserved

2.1 Abstract

Mega-fires of unprecedented size, intensity, and socio-economic impacts have surged globally due to climate change, fire suppression, and development. Soil microbiomes are critical for post-fire plant regeneration and nutrient cycling, yet how mega-fires impact the soil microbiome remains unclear. We had a serendipitous opportunity to obtain pre- and post-fire soils from the same sampling locations after the 2016 Soberanes mega-fire burned with high severity throughout several of our established redwood-tanoak plots. This makes our study the first to examine microbial fire response in redwood-tanoak forests. We re-sampled soils immediately post-fire from

two burned plots and one unburned plot to elucidate the effect of mega-fire on soil microbiomes. We used Illumina MiSeq sequencing of 16S and ITS1 to determine that bacterial and fungal richness were reduced by 38-70% in burned plots, with richness unchanged in the unburned plot. Fire altered composition by 27% for bacteria and 24% for fungi, whereas the unburned plots experienced no change in fungal and negligible change in bacterial composition. We observed pyrophilous taxa that positively responded to fire were phylogenetically conserved, suggesting shared evolutionary traits. For bacteria, fire selected for increased Firmicutes and Actinobacteria. For fungi, fire selected for the Ascomycota classes Pezizomycetes and Eurotiomycetes and for a Basidiomycota class of heat-resistant Geminibasidiomycete yeasts. We build from Grime's Competitor-Stress tolerator-Ruderal (C-S-R) framework and its recent microbial applications to show how our results might fit into a trait-based conceptual model to help predict generalizable microbial responses to fire.

2.2 Introduction

The rise of the mega-fire is an anthropogenic phenomenon with unknown consequences for soil microbes and ecosystem processes. In the past, many wildfires were low severity, high frequency events (Archibald et al., 2013). These low-intensity naturally occurring wildfires helped clear away dead brush, revitalize the soil with nutrient deposition from pyrolyzed material, and assist some plants with their reproductive cycles (He & Lamont, 2018; Keeley et al., 2011). However, rising global

temperatures (Westerling et al., 2006), elongated droughts (Mukherjee et al., 2018), and development at the wildland-urban interface (Spyratos et al., 2007), have led to the rise of catastrophic mega-fires of unprecedented size, intensity, and socio-economic impacts that exceed what would be predicted from a historical fire regime (Stephens et al., 2014). Whereas many ecosystems (such as Mediterranean and Australian ecosystems) and plants are adapted to fire and may indeed require fire to reproduce (Keeley et al., 2011), whether plants or their associated soil microbiomes will survive mega-fires remains unknown.

The soil microbiome is an important driver of plant diversity and productivity (Van der Heijden et al., 2008) and soil biogeochemical cycling (Crowther et al., 2019). If soil microbiomes do not survive mega-fires, then associated plants, especially those reliant on symbiotic mycorrhizal fungi, may not regenerate (Collier & Bidartondo, 2009). Ectomycorrhizal fungi (EMF) and arbuscular mycorrhizal fungi (AMF) are key partners with plant roots that increase access to soil nutrients in exchange for photosynthetically derived carbon (Brundrett & Tedersoo, 2018). Moreover, soil bacteria and fungi are primarily responsible for litter decomposition (Glassman et al., 2018), nutrient cycling (Crowther et al., 2019), and soil aggregation (Lehmann et al., 2017), which are essential for ecosystem regeneration. As climate change continues to influence fire regimes with unknown consequences for ecosystem processes (Rogers et al., 2011), it is essential to determine how soil microbes that drive these processes are affected by mega-fires.

Previous studies have shown that while fire significantly decreases bacterial and fungal biomass (Dooley & Treseder, 2012) and richness (Pressler et al., 2019), fire is not totally sterilizing, and similar to plants, some taxa respond positively and are adapted to

fire (Keeley et al., 2011). Fires are known to shift fungal dominance from Basidiomycota to Ascomycota (Cairney & Bastias, 2007; Pérez-Valera et al., 2018; Semenova-Nelsen et al., 2019), and many Ascomycetes are known to fruit in abundance after fire. Indeed, century-old studies describe “pyrophilous", or fire loving, fungi from mushroom surveys (Seaver, 1909). Many of these pyrophilous fungi that fruit after fires are in the Ascomycete family Pyronemataceae (El-Abyad & Webster, 1968; McMullan-Fisher et al., 2011; Petersen, 1970) and heat-treated soil has recovered Ascomycetes in the genera *Aspergillus* and *Penicillium* (McGee et al., 2006; Warcup & Baker, 1963). Further, the Ascomycete *Neurospora crassa* is known to have heat activated spores (Emerson, 1948). While less common than Ascomycetes, Basidiomycetes can also respond positively to fire. For example, the Basidiomycete mushroom *Pholiota highlandensis* commonly fruits after wildfires (Raudabaugh et al., 2020), and the EMF species *Rhizopogon olivaceotinctus* significantly increased in frequency after a pine forest mega-fire (Glassman et al., 2016). However, less is known about the mycelial response of fungi to fire, and even less is known about bacteria, with less than 3% of studies examining microbial response to fire addressing their composition (Pressler et al., 2019). Yet, recent evidence suggests that pyrophilous bacteria may also exist (Whitman et al., 2019; Woolet & Whitman, 2020). For example, several taxa of bacteria significantly increased in frequency after a boreal wildfire, in particular the Actinobacteria *Arthrobacter* and the Proteobacteria *Massilia* (Whitman et al., 2019). Currently, most research on pyrophilous microbes is observational, and as such while we know that these taxa respond positively to fire, we do not yet know why.

While their small size and immense diversity has limited our understanding of fire effects on microbes, trait-based approaches provide promising avenues for categorizing microbial taxa according to suites of traits under which selective trade-offs might occur (Lustenhouwer et al., 2020; Malik et al., 2020). Trait strategies and trait syndromes are well established in the plant world including traits for wildfire response (e.g. thick bark, serotinous cones, vegetative re-sprouting) (Keeley et al., 2011; Pausas et al., 2004). Functional traits synthesize the wide diversity of species into trait groups (e.g., seed size and specific leaf area) each of which is associated with specific strategies such as longevity or drought tolerance. Due to energetic costs or evolutionary constraints associated with different functions, it is unlikely that one organism will succeed at all strategies and, therefore, will *trade off* the ability to perform one function for a less costly alternative (Reich, 2014). Identifying traits of pyrophilous microbes and trade-offs among them can improve our ability to model biogeochemical consequences such as carbon cycling under anthropogenically induced changes to fire regimes (Malik et al., 2020). A recent study hypothesized what some of the broad microbial fire response traits could be, including fast growers, thermotolerant structure producers, and resource acquisition of post-fire nutrients (Whitman et al., 2019). Expanding on this framework and identifying microbes that fit into these trait syndromes in a variety of biomes will provide key insights into microbial fire ecology and improve prediction of fire biogeochemical impacts. A first step in this expansion of the pyrophilous trait framework is to test the phylogenetic conservation of microbial fire response, meaning testing if more closely related organisms tend to response to fire in the same way (Martiny et al., 2013). By

comparing a clade's fire response to observations made in other fire events and in multiple ecosystems we can better predict how individual taxa will respond even if they are observed post-fire for the first time. This is because traits governing microbial phenotypic responses are often conserved within clades (Martiny et al., 2013). Thus, as the discovery of traits driving pyrophilous microbes' fire response are uncovered, the ecological role of related microbes will be better predicted in future post-fire sampling. For example, bacterial response to both nitrogen addition (Isobe et al., 2019) and simulated global climate change factors was phylogenetically conserved across all perturbations (Isobe et al., 2020), which improves prediction of disturbance response of closely related taxa. In this way we might be able to start better explaining observed shifts in fungal decomposition rates post-fire (Holden et al., 2013; Semenova-Nelsen et al., 2019) based on which fungi are present/absent after fire though additional study into the ecological roles of post-fire microbes will be needed to further this predictive modeling.

Most fire studies use burned areas compared to an unburned control as a substitute for not having pre-fire data from the burned region (Brown et al., 2019; Buscardo et al., 2010; Whitman et al., 2019), laboratory heating experiments (Bruns et al., 2020; Riah-Anglet et al., 2015), or prescribed fire to examine impacts on soil microbes (Brown et al., 2013; Fujimura et al., 2005). While these all provide useful information on microbial response to fire, none of these conditions replicate the size and severity of a mega-fire. Additionally, even though the severity of mega-fires can be patchy, the size of high severity patches tend to be much larger than many historical fires

(Collins & Roller, 2013; Stephens et al., 2014). Since it is unlikely to have microbial documentation before fires occur, the study of mega-fire impacts must be opportunistic. Two such studies exist, in which pre- and post-fire soil samples from the exact same sampling locations after a stand-replacing fire were available, and both were in Pine forests, and both focused solely on EMF (Baar et al., 1999; Glassman et al., 2016). Existing research on pyrophilous taxa also focuses largely on pine forests (Dove & Hart, 2017; Pressler et al., 2019), so it is unclear whether the same pyrophilous taxa will respond in other forest types or dryland ecosystems. It is worth noting that studies that examine fire and microbes exist in other biomes such as Australian Eucalyptus forests (McMullan-Fisher et al., 2011), Indonesian rainforests (Isobe et al., 2009), Mediterranean grasslands (Glassman et al., 2021) and shrublands (Bárcenas-Moreno et al., 2011; Pérez-Valera et al., 2018). However, two recent meta-analyses identifying post-fire microbial responses found that 66% to 93% of studies occurred in forests with the vast majority taking place in Pinaceae forests (Dove & Hart, 2017; Pressler et al., 2019). Expanding our knowledge to non-Pinaceae forests will allow us to determine if pyrophilous taxa and their traits are generalizable or ecosystem specific.

The California redwood (*Sequoia sempervirens*) and tanoak (*Notholithocarpus densiflorus*) forest is a charismatic forest of coastal California and southern Oregon that is adapted to high fire frequency (median fire return interval of 10 years; (Stephens et al., 2007)) with these trees possessing fire response traits including thick bark and vegetative re-sprouting (Paul Zinke, 1988; Stephens et al., 2007). These forests are now highly threatened by both the invasive pathogen *Phytopthora ramorum*, causative agent of

Sudden Oak Death (SOD), and changing fire regimes (Metz et al., 2013; Simler et al., 2018). It is possible that their associated soil microbiomes, including the AMF associated with redwoods (Afek et al., 1994) and EMF associated with tanoaks (Bergemann & Garbelotto, 2006) may help them survive these unprecedented disturbances. Yet, their wildfire response remains completely uncharacterized.

Here, we take advantage of the 2016 Soberanes Fire, a mega-fire burning >500 Km^2 , which burned through our study plots in a previously established plot network (Metz et al., 2011) (Figure 2.1). We were able to sample soils from two burned and one unburned plot immediately post-fire, so we could assess what microbes survived the mega-fire before rain could disperse in new microbes. Because we had an unburned plot, we were able to capitalize on a before-after control impact (BACI) (Conquest, 2000) experimental design for increased inference. We thus tested the following hypotheses: H1) the mega-fire would significantly decrease bacterial, total fungal, and mycorrhizal richness in comparison to the control plot, H2) fungal composition would shift from Basidiomycete to Ascomycete dominated, and H3) pyrophilous bacterial and fungal taxa would emerge and might have similarities to previously described pyrophilous taxa from pine-forests and would fit into a trait-based conceptual model based on post-fire resource acquisition, thermotolerance, or fast growth or colonization (Whitman et al 2019). Finally, we hypothesized that H4) adaptation to fire might be a phylogenetically conserved trait, as has been indicated for microbial response to nitrogen disturbance (Isobe et al., 2019).

2.3 Materials and Methods

2.3.1 Plot Description and soil sampling scheme

In 2006 and 2007, one hundred fourteen 500 m^2 circular plots were established in redwood forests across the California Big Sur region to study the effects of the emerging SOD outbreak (Metz et al., 2011). Big Sur has an average rainfall of 911mm/year, with most rain falling from October through April, and an average temperature range of 10.1°C to 16.1°C (Potter, 2016). On January 14th and February 4th 2013, soil was collected to determine soil microbial composition from a subset of those plots selected for dominance of tanoak as the only EMF host, accessibility from the road so as to keep soils on ice immediately after sampling, and similarity in slope, aspect, and elevation. We collected 12 soil cores at 2, 6, and 10m from the plot center in 4 cardinal directions in an attempt to adequately sample the soil microbial communities across the entire plot (Figure 2.1B). The top 10cm of soil was collected using field cleaned (with 70% EtOH) Bond 8050 Releasable Bulb Planters (~250 mL of soil). We acknowledge that cleaning corers between samples with EtOH may allow for some potential for cross-contamination of low-abundance taxa. However, given the size of the core (250ml) relative to the amount of soil from which DNA is extracted (0.25g), we think this possibility is low, and indeed, our effect sizes give us no indication that this cross-contamination could be statistically or ecologically significant. From July 22-October 12, 2016, the Soberanes Fire burned 534 km^2 in Big Sur, burning with high severity throughout a significant portion of the fire including two of our plots, as measured using differenced Normalized Burn Ratio (dNBR) calculated using Landsat imagery (Potter, 2016)(Figure 2.1A). On

the day that the fire was officially declared over (October 12, 2016), we were able access and collect soil samples from the exact same sampling locations (using GIS, meter tape, and notes) in one unburned (plot 058) and two burned plots (plots 601 and 603). All plots were in UTM zone 10 and slopes ranged from 19-34°, elevations from 491-744 m, distances from the road were 0.4 - 0.84 km, distances to the coast from 4.1-6.4 km, and distance between unburned and burned plots ranged from 42 – 43 km (Table A.2.1).

2.3.2 DNA Extraction and Storage

Soils were stored on ice in the field and homogenized the next day using a 2mm sieve, cleaning with water and 70% EtOH between samples. For DNA extraction, 0.25g of soil was weighed and placed into tubes from the MoBio Power Soil DNA Extraction Kit and stored at 4˚C. For both collection timepoints, DNA was extracted within the week of collection following manufacturer's protocols and stored at -20˚C until analyzed. DNA extractions from both timepoints were PCR amplified and sequenced together using the same protocols."

2.3.3 PCR Amplification of rDNA

We used the 515F-806R primer pair to amplify the V4 region of the bacterial 16S rRNA gene (Caporaso et al., 2011) and the ITS1F-2 primer pair (White et al., 1990) adapted for Illumina MiSeq (Smith & Peay, 2014) to characterize the ITS1 region of the fungal internal transcribed spacer region (Schoch et al., 2012). PCR recipe included 1μL of template DNA (in some cases diluted 1:10 to overcome inhibitors), 2μL of each primer

at 10μM concentration, 12.5μL of Accustart Toughmix (Quantabio, Beverly, MA, USA), and 7.5μL of PCR grade water for a total reaction volume of 25μL. Thermocycler conditions began with denaturation at 94°C for 3 min; followed by 29 amplification cycles for 16S of 45 s at 94°C, 1 min at 50°C, 90 sec at 72°C, followed by a 10-min final extension at 72°C; and for ITS: 30 s at 94°C, 30 sec at 52°C, 30 sec at 68°C, with a 10-min final extension at 68°C. A mock community (ZymoBiomics, Zymo, Irvine, CA) and negative DNA extractions and PCRs were also amplified and sequenced.

2.3.4 Illumina library preparation and sequencing

Bar-coded 16S and ITS PCR products from all 72 samples (12 samples per plot x 3 plots x 2 time-points) were combined at approximately equimolar concentration and cleaned as previously established (Glassman et al., 2018). We combined PCR products for either 16S or ITS based on band strength from gel electrophoresis, using 3μL for the weakest and 1μL for the strongest bands, then cleaned with AMPure magnetic beads (Beckman Coulter Inc., Brea, California, USA). We quality checked the 16S and ITS libraries for concentration and amplicon size using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) then combined at 60:40 ITS to 16S ratio prior to sequencing with an Illumina MiSeq 250bp paired-end run at the Institute for Integrative Genome Biology (IIGB) at University of California, Riverside (UCR).

2.3.5 Bioinformatics and OTU Table Construction

Demultiplexed sequences were received from the UCR IIGB and raw reads were analyzed for quality with FastQC, and primers and barcodes were trimmed with Cutadapt v. 1.16 (Martin, 2011). We then used UPARSE v11 (Edgar, 2013) to merge forward and reverse reads with fastq_mergepairs, quality filtered with fastq_filter with fastq_maxee of 0.5, dereplicated sequences, removed singletons, and clustered 97% OTUs following established protocols (Glassman et al., 2018). For 16S, 5.2 million (M) reads were analyzed, 4.6M (88.6%) merged under the constraints for quality control, and 4.3M reads (94.4%) passed the filtering step at an expected error rate of 0.5. OTUs were then assigned taxonomic information using a RDP classifier with 80% bootstrapping and the GreenGenes database (DeSantis et al., 2006) (accessed 4/25/2019) in QIIME 1.9.1 (Caporaso et al., 2010). Samples identified as mitochondria, chloroplasts, or unidentified were removed, leaving a remaining 14,282 OTUs. For ITS1, 9.2M reads were analyzed, 7.5M (81%) merged, and 5.6M (75.6%) passed the filtering step. Fungal taxonomy was assigned to 97% OTUs using the QIIME 1.9.1 BLAST protocol with 80% bootstrapping and the UNITE database (Kõljalg et al., 2005) (accessed 5/13/2019). Samples not identified as Kingdom Fungi were removed, resulting in 3,328 OTUs. Sequences were submitted to the National Center for Biotechnology Information Sequence Read Archive under BioProject accession number PRJNA659056 (Enright et al., 2020).

2.3.6 Data Analysis

All statistical analyses and figures were produced in R 4.0.2 (R Core Team, 2020) and all scripts are available at: https://github.com/sydneyg/SoberanesFire. In order to accurately compare richness across samples with uneven sequencing depth, samples were rarefied to an even sequencing depth per sample (10,367 for bacteria and 12,089 for fungi) using the "rrarefy" function in the Vegan R package (Oksanen, 2007). In this process negative DNA extractions and negative PCRs were removed due to low sequencing depth. Mock communities were examined and removed prior to analysis. Alpha diversity metrics were calculated with the "estimate" function (observed species, ACE and Chao1) in the BioDiversityR package (Kindt, 2019) and with the "diversity" and "exp" functions (Shannon, Simpson) in Vegan (Oksanen, 2007). ACE, Chao1 and observed Species were highly correlated for both bacteria (Figure A.2.4 A& B) and fungi (Figure A.2.4 B&C) so all analyses and figures are based on observed species number after rarefaction. Shannon metrics were used to detect changes in evenness and Simpson was used to detect changes in dominance. Normality was tested with a Shapiro test, then ANOVA was used to test the effect of fire versus time on bacterial and fungal richness, followed by a post-hoc Tukey HSD test. Percent reduction in species richness was computed using the average species richness in each plot before and after the fire. Species accumulation curves were also constructed using "speccacum" function in Vegan (Oksanen, 2007). Richness figures were created in ggplot2 (Wickham, Chang, et al., 2019).

We used FunGuild (Nguyen et al., 2016) to analyze the effects of fire on EMF, AMF and saprobic fungi. We applied FunGuild to the unrarefied fungal OTU table, then only included "Highly Probable" guild assignments. These OTUs were then selected from the rarefied full fungal dataset using a "semi_join" function from the tidyverse R package (Wickham, Averick, et al., 2019), resulting in a total of 463 OTUs, including 222 EMF, 25 AMF, and 76 saprobic fungi. Changes in per guild species richness were then calculated.

For bacterial and fungal community composition, we calculated Bray-Curtis dissimilarity matrices using the "vegdist" function in Vegan (Oksanen, 2007) on the rarefied and filtered OTU tables. We then tested the effect of time and fire on bacterial and fungal community composition with a two-way Permutational multivariate analysis of variance (PERMANOVA) (Anderson et al., 2008) as implemented with the Vegan "adonis" function. We visualized community compositional differences with nonmetric multidimensional scaling (NMDS) with the Vegan "metaMDS" function and used the Vegan "envfit" function to determine which taxa correlated well with ordination space, correcting for multiple tests with a Benjamini-Hochberg correction.

We identified pyrophilous taxa with four approaches. First, we identified taxa that correlated well with ordination space with "envfit" as described above. Second, we calculated percent changes in sequence abundance of dominant taxa (over 1% sequence abundant) before and after fire in the burned plots and visualized the most abundant taxa summarized by genera with rank abundance curves. Third, we used indicator species analysis (ISA) to identify microbial indicators in the burned plots before and after the fire

using the “multipatt” function in the Indicspecies package (Cáceres & Jansen, 2019). Finally, we ran the raw OTU tables through DESeq2 (Love et al., 2019) to identify log-fold changes in abundance of each OTU in the burned plots before and after fire. We then used the DESeq2 output to determine if the taxa that positively or negatively responded to fire were phylogenetically conserved using established protocols (Isobe et al., 2019). We created circular phylogenies using maximum likelihood trees using the RAxML pipeline (Stamatakis, 2014) for 16S and ITS1and then assigned each OTU a positive or negative response to fire based on the DESeq2 analysis. Then the tree was examined for the deepest node at which >90% of the OTUs shared the same response (positive or negative). These groups were then binned into consensus clades and the mean depth of the consensus clades was calculated with consenTRAIT (Martiny et al., 2013) as implemented with the Castor “get_trait_depth” function using 1,000 permutations. The consensus clades were then mapped onto the phylogeny and colored to visualize evolutionary relationships of taxa exhibiting positive or negative responses to fire using the Interactive Tree of Life (ITOL) (Letunic & Bork, 2007). The taxonomy of clades whose response was significantly more positive or negative than expected by chance was identified with a two-tailed exact test (Mc. Donald, 2015) against the equal distribution of positive and negative responses within each taxonomic group. Because of some of the known issues with using ITS for phylogenetic analyses an attempt to strengthen our phylogeny using GhostTree (Fouquier et al., 2016) was made. However, due to the inherent bias towards EMF in the GhostTree backbone phylogenies, aligning our OTUs to GhostTree resulted in the loss of 61.4% of OTUs rendering the GhostTree derived

phylogenies not suitable for our analyses. Therefore, we decided to move forward with ITS based analysis under the guiding principle that the trends and clustering observed in our analysis, supported by literature and our other analyses, are still meaningful despite the potential weakening of phylogenetic conclusions due to the limitations of ITS.

2.4 Results

2.4.1 Change in Microbial Richness After Fire

After rarefaction, we found a total of 12,322 bacterial and 2,878 fungal OTUs. In the burned plots, we found 9,116 bacterial OTUs pre-fire and 6,172 post-fire. For fungi, we found 1,869 fungal OTUs pre-fire and 987 post-fire within the burned plots. Fire significantly reduced both bacterial and fungal observed species richness in the burned plots, whereas richness remained unchanged in the unburned plot (Figure 2.2). Results did not differ if we treated samples independently ($F_{1,44} = 23.75$, $p < 0.001$) versus if we counted them as nested within plots ($F_{1,1} = 65.73$, $p < 0.001$). The average number of OTUs per sample decreased by 70% for fungi and 52% for bacteria in plot 603 and by 38% for fungi and 40% for bacteria in plot 601. This is in contrast to the unburned plot (058), which had equivalent per sample mean richness to the burned plots pre-fire but experienced no change in either bacterial or fungal per sample richness during the three years (Figure 2.2; Table A.1.1). Fire also resulted in large and significant reductions in average per sample evenness (Shannon diversity index decreased by 82-83% for fungi and 65-78% for bacteria) and large increases in average per sample dominance in the burned plots (measured as the inverse of Simpson's index (Whittaker, 1965), which

increased by an average of 71-78% per sample for fungi and by an average 76-82% per sample for bacteria) with no significant change in either index for the unburned plot (Table A.2.2). Average EMF richness per sample decreased by 68% in one burned plot (plot 603), was unchanged in the other burned plot, but increased by 26% over the 3-year time span in the unburned plot (Figure A.2.2). Average AMF and saprobic richness per sample both decreased in burned plots (saprobes: 69% in plot 601 and 76% in plot 603; AMF: 60% in plot 601 and 80% in plot 603) and both increased in the unburned plot over time (saprobes: 24%; AMF: 83%; Figure A.1.2).

2.4.2 Change in Microbial Composition After Fire

Fire resulted in a large and significant change in bacterial (Adonis $R^2 = 0.27$, $p < 0.001$) and fungal (Adonis $R^2 = 0.24$, $p < 0.001$) community composition, while the unburned plot experienced no change in fungal composition and a relatively smaller change in bacterial composition ($R^2 = 0.09$, $p < 0.01$) (Figure 2.3; Table A.2.3). Compositional changes in bacteria were largely driven by increases in the Actinobacteria and Firmicutes phyla post-fire and decreases in the Proteobacteria, Gemmatimonadetes, Verrumicrobia, Chloroflexi, Elusimicrobia, Planctomycetes, Acidobacteria, Bacteroidetes, and Saccharibacteria phyla (Figure 2.3D). Compositional changes in fungi were driven by large increases in the Basidiomycete genus *Basidioascus* and the Ascomycete genus *Penicillium* and decreases in the Mucoromycota genus *Mortierella*, and the Ascomycete genera *Ilyonectria*, *Metarhizium*, *Cladophialphora*, *Pectenia*, *Humicola*, and *Exophiala* (Figure 2.3F).

2.4.3 Change in Relative Sequence Abundance of Bacterial Taxa After Fire

Pre-fire, bacterial OTUs dominating (≥1% of sequence abundance) the burned plots were primarily Proteobacteria (84.4%) and Acidobacteria (15.6%) with the most dominant taxa, Proteobacteria species in *Bradyrhizobium* and *Rhodoplane*s, occupying around 2.3% sequences each (Figure 2.4A; Table A.2.4). Post-fire, there was a shift in the dominant phyla (≥1% total sequences), with a loss of Proteobacteria and a dominance of Firmicutes (82%) and Actinobacteria (18%). The most abundant taxon also had an increase in dominance, a Firmicute in the genus *Sporosarcina*, dominating 6.9% of the sequences, followed by Firmicutes genera *Fictibacillus, Thermoflavimicrobium, Bacillus, Solibacillus, Cohnella,* and Actinobacteria genera *Micromonospora, Pseudonocardia,* and the family Micromonosporaceae (Figure 2.4A).

2.4.4 Change in Relative Sequence Abundance of Fungal Taxa After Fire

Pre-fire, dominant fungal phyla (≥1% total sequences) belonged to the Basidiomycota (62%), followed by 25% Mucoromycota, 10% to Ascomycota, and 3% unidentifiable to phylum (Figure 2.4B; Table A.2.5). Pre-fire, the most dominant fungal species were the Basidiomycete *Hygrocybe acutoconica* (5.94%) and the Mucoromycete *Morteriella baineri* (4.62%). Post-fire, dominant fungal phyla taxa transitioned to Ascomycota (65%), with Basidiomycota falling to 35%, and a complete loss of the Mucoromycota. There was also a large increase in dominance with the most abundant taxon post-fire, a Basidiomycete Geminibasidiomycete yeast *Basidioascus undulatus*,

accounting for 28% of the sequences (Figure 2.4B). The other top taxa were dominated by genera in the Ascomycota *Penicillium* 13.1% and *Fusarium* 1.2% and the family Pyronemataceae (*Tricharina* 13.6%, *Peziza* 10.2%, *Anthracobia* 7.6%, *Scutellina* 7.2%, *Pyronema* 4.2%).

2.4.5 Bacterial indicator species

Bacterial indicator species analysis (ISA) revealed 86 indicator OTUs shared between the burned plots pre-fire, and 21 OTUs indicative of the post-fire community (Table A.2.6). The top ten taxa identified for the post-fire grouping were two OTUs from the Firmicutes family Thermoactinomycetaceae, one OTU from the Actinobacteria family Thermomonosporaceae, OTUs from the Firmicutes genera *Thermoflavimicrobium, Fictibiacillus, Cohnella, Paenibacillus, Bacillus, Tepidibacterium,* and the Actinobacteria genus *Streptomyces*.

2.4.6 Fungal indicators species

Fungal ISA identified 13 indicator taxa for the burned plots pre-fire and 5 indicator taxa post-fire (Table A.2.7). The 13 pre-fire taxa identified were 4 species from the Mucoromycota genus *Mortierella* and the rest Ascomycetes belonging to the genera *Polyphilus, Pycnopeziza, Thelonectria, Pectenia, Cladophialophora,* and *Phomopsis*. The five fungal indicators of the post-fire group were all Ascomycetes, one in the Aspergillaceae, *Penicillium decumbens*, and the rest in the Pyronemataceae: *Anthracobia* sp., *Geopyxis alpina, Peziza vacinii*, and *Tricharina praecox.*

2.4.7 Phylogenetic conservation of pyrophilous taxa

Lineages of both bacteria and fungi that negatively or positively respond to fire appear to be phylogenetically conserved at the class level for fungi and the phylum level for bacteria (Figures 2.5 & 2.6). There is strong clustering of bacteria positively responding to fire in the Firmicutes and Actinobacteria and a few positive responders in the Acidobacteria (Figure 2.5; Table A.2.8). All other phyla belonged to consensus clades that either did not show the patterned response to fire or responded negatively. For fungi, the lineages that positively respond to fire include the Ascomycete class Pezizomycetes (containing Pyronemataceae) and the Basidiomycete class Geminibasidiomycetes (containing *Basidioascus*) (Figure 2.6; Table A.2.9). There are also positive interactions within some of the other classes, which appear to be conserved at the order level rather than class level, namely the Eurotiomycetes order Eurotiales (contains Aspergillaceae) and the Agaricomycete order Russulales. All other groups belonged to consensus clades that responded negatively or did not show the phylogenetic patterned response.

2.5 Discussion

Here, we take advantage of the 2016 Soberanes Fire burning through our plots and show for the first time the effects of a mega-fire on bacterial and fungal communities with a rare pre- and post-fire dataset in a redwood tanoak forest. In accordance with our hypotheses, the mega-fire significantly decreased bacterial and fungal richness (H1) (Figure 2.2); fungal composition shifted from Basidiomycete to Ascomycete dominated

(H2) (Figure 2.4); many pyrophilous taxa previously identified from other biomes appeared in a redwood tanoak forest and are likely generalizable and may fall into trait categories analogous to plants (H3); and adaptation to fire is likely a phylogenetically conserved trait across bacteria (Figure 2.5) and fungi (Figure 2.6) (H4).

We found that the mega-fire led to large and significant reductions in bacterial and fungal richness. Reduction in microbial richness is a typical result after fire (Brown et al., 2019; Day et al., 2019; Pérez-Valera et al., 2018; Whitman et al., 2019), however the degree to which fire affects richness varies. In our study, fire reduced the average species richness per sample by up to 52% for bacteria and up to 70% for fungi (Figure 2). In contrast, a meta-analysis of fungal response to fire found an average richness reduction of 28% (Dove & Hart, 2017). However, within the literature the range for both fungal (12-80%) (Brown et al., 2019; Pulido-Chavez et al., 2021) and bacterial (20-58%) (Brown et al., 2013; Pérez-Valera et al., 2018) richness reduction can be quite large. This is likely due to differences in fire severity, which is often not measured or consistently reported in post-fire microbial surveys, despite that more severe fires have been shown to have greater impacts on the soil community (Whitman et al., 2019). Moreover, when reported, it is often based on plant mortality measured at coarse levels (30 km^2 in the case of the Soberanes Fire) (Potter, 2016) that do not correspond well to the scale of the soil core and can lead to wide disparities in the presence or absence of live plants, duff, or ash in soil cores all taken in high severity defined fire zones. A few studies have taken the effort to assess fire severity metrics in the field as opposed to relying solely on satellite data (Brown et al., 2019; Day et al., 2019; Glassman et al., 2021; Whitman et al., 2019).

There is also the possibility that discrepancies in the literature about the effect of fire on microbial richness could be due to the evolutionary effect of fire on the ecosystem. Much like how frequently burned plant communities have evolved traits that make them more resilient or resistant to fire (Keeley et al., 2011), it is possible that the soil microbial communities could also have adapted to fire. This might be due to higher prevalence of thermotolerant taxa, such as those discussed in our trait framework (Figure 2.7), and could confer resistance to the effect of fire felt in more sensitive communities. We also found that EMF richness declined following fire, which makes sense given large host mortality, and is in accordance with other high severity fires in pine forests (Glassman et al., 2016; Pulido-Chavez et al., 2021; Reazin et al., 2016). We feel confident in attributing the observed changes in richness to the effect of fire and not other confounding variables like time or seasonality given that our control plot exhibited no change in either bacterial or fungal richness despite a 3-year time span and seasonal difference between sampling efforts. We acknowledge that this confidence could have been further strengthened with the inclusion of additional control plots but unfortunately due to logistical and funding constraints wrought by the Soberanes fire we were only able to sample one unburned plot. This large-scale reduction in the microbial richness can have far reaching impacts on the surrounding ecosystem from radically altered nutrient cycling (Crowther et al., 2019) to the inability to reestablish critical plant species (Van der Heijden et al., 2008).

The mega-fire led to large compositional shifts in both bacterial and fungal communities, explaining 27% of the community shift for bacteria and 24% for fungi

(Figure 2.3). Studies often report significant changes in fungal compositional turnover post-fire but variable change in bacterial composition (Pérez-Valera et al., 2018; Pressler et al., 2019). However, sampling immediately post-fire does reveal significant reductions in bacterial richness and evenness (Brown et al., 2019; Ferrenberg et al., 2013) and one study showed that the reduction in richness and evenness becomes more drastic with increasing fire severity (Brown et al., 2019). Even within the same biome, changes in microbial composition can be as short lived as 21 days after simulated soil heating (Bárcenas-Moreno & Bååth, 2009) or as long as 19 years post-fire (Pérez-Valera et al., 2018), both studies taking place in Spanish Mediterranean shrublands though it is difficult to compare results from lab heating studies and field studies. A study in a Chinese pine forest speculated that bacterial composition would recover within one growing season based on data from 6 months post-fire (Li et al., 2019). This disparity in resilience is likely due to differences in fire severity and timing of post-fire sampling. Fires that are not as severe often produce smaller shifts in bacterial and fungal composition (Buscardo et al., 2010). The effect of fire on microbial richness and composition is also likely more transient in some biomes and longer lasting in others, which once again could be due to evolutionary adaptation within the microbial communities. As such, studies that sample 1-2 years post-fire may miss changes in bacterial and fungal communities depending on the biome. In the case of our data, these observed changes stemmed from a severe mega-fire where the changes to the landscape can be quite drastic and took place in a well-established forest and therefore recovery could take much longer.

Changes in dominance can be important indicators of how post-fire microbial communities assemble, as has been documented in plant communities (Moser & Wohlgemuth, 2006). Fires often lead to shifts in plant community evenness to increased dominance by specific fire-adapted species such as *Pinus palustris* in Southeastern United States, (Glitzenstein et al., 1995) or *Ceanothus* (Lawson et al., 2010) or *Arctostaphylos* shrubs (Vogl & Schorr, 1972) in California chaparral. These effects can be more pronounced when fire severity increases (Kuenzi et al., 2008). Fire similarly alters microbial community assembly processes (Ferrenberg et al., 2013). Here, we find patterns of fire-induced dominance in microbial communities, with a single fungal taxon (*Basidioascus undulates*) dominating 28% and a single bacterial taxon (*Sporosarcina spp.*) dominating 7% of the sequences post-fire. While changes in dominance have been documented in microbial communities post-fire (Pérez-Valera et al., 2018; Whitman et al., 2019), they are at lower levels (2-4% for most abundant taxon) likely because they sampled later after the fire and after the first rains post-fire thus obscuring initial compositional shifts. An experimental fire in laboratory "pyrocosm" (a small-scale highly controlled experimental fire to test the effects of fire on soil samples) similarly found huge increases in dominance with the most abundant taxon *Pyronema domesticum* occupying 57% of the sequences within 2 weeks of the fire (Bruns et al., 2020). We interpret this level of dominance to indicate the opening of niche space due to massive microbial death of the pre-fire dominants that allows the few microbial taxa that are thermotolerant or capable of capitalizing on post-fire resources or eating microbial necromass to take over. This interpretation of results links well with the hypothesized

placements of *Basidioascus* and *Sporosarcina* as potentially thermotolerant taxa dominating post-fire in our study.

After fire, we found a complete turnover in the abundant bacterial taxa (>1% sequence abundant) from Proteobacteria and Acidobacteria domination to Firmicutes and a few Actinobacteria (Figure 2.4). These changes are likely driven by thermotolerance of endospore forming Firmicutes, which have also been found to increase in abundance following laboratory heat treatments of soil samples (Filippidou et al., 2016; Jurburg et al., 2017) and soon after Mediterranean fires (Pérez-Valera et al., 2020). Post-fire environments are often characterized by increases in pH (Neary et al., 1999), which may favor both thermotolerant and alkaline tolerant Actinobacteria (Shivlata & Tulasi, 2015). Indeed, while the effect of fire mediated pH changes on soil microbial communities have not been directly studied, results of indirect analysis seems to suggest that post-fire pH shifts may favor bacteria over fungi (Brown et al., 2019; Dooley & Treseder, 2012). More research is needed to fully assess the effect of fire related pH shift and which specific taxa might be benefiting from the high post-fire pH. Of the specific genera dominating our communities post-fire, only *Thermoflavimicrobium* (Yoon et al., 2005) and *Sporosarcina* (Jurburg et al., 2017) have previously been shown to increase after laboratory heating, supporting that they may possess thermotolerant traits hypothesized within our framework (Figure 2.7). Other studies have shown an increase in Actinobacteria and Firmicutes post-fire, though only the *Bacillus spp.* in our study is a potentially shared taxon with those other studies (Ferrenberg et al., 2013; Li et al., 2019; Whitman et al., 2019). In contrast, most other groups of bacteria negatively responded to

fire, or only responded positively sporadically within a clade (Figure 2.5). Lineages can have differing responses within a clade when lineages challenged with the same evolutionary pressures do not respond the same way (Isobe et al., 2019). Furthermore, the degree to which a trait is conserved within lineages may reflect the genetic complexity of the response or trait, with more complex responses being less conserved due to needing many pathways to be conserved across all taxa of a lineage (Isobe et al., 2019). It may be then that the genetic drivers of Firmicutes' response to fire are either less genetically complex or more genetically integral to that phylum while Actinobacteria's response may be genetically varied or require the conservation of a greater number of genetic pathways in order to successfully adapt to fire. Finally, as this study is among the first to comprehensively characterize the soil microbiome of the charismatic redwood-tanoak forests, we note a few similarities in our pre-fire soils with a recent bacterial study of coastal redwood roots which also showed the most abundant taxa as *Bradyrhizobium* and *Rhodoplanes* (Willing et al., 2020). Additionally, a study of the microbial composition of the coastal redwood's sister genus, the giant sequoia (*Sequoiadendron giganteum),* also showed domination by *Bradyrhizobium* and Sinobacteraceae sp. of the Proteobacteria (Carey et al., 2019). The similarities with the giant sequoia soil communities also extend to fungi, with *Hygrocybe* dominating both our redwood forests pre-fire and in the giant sequoia forest.

After the fire, the fungal communities were dominated (28%) by the yeast *Basidioascus undulates,* which was rare (0.05%) pre-fire. *Basidioascus* and *Geminibasidium* are newly described heat-resistant and xerotolerant Basidiomycete

yeasts isolated from laboratory heat-treatments to soil (Nguyen et al., 2013). While not previously described as pyrophilous, it is possible that they should be given their exhibited thermotolerance. Only one other wildfire study has found *Basidioascus* to increase post-fire (Pulido-Chavez et al., 2021) though not with the level of dominance in our study. A couple recent studies have found an increase in dominance of the sister genus *Geminibasidium*, including a study of a *Pinus ponderosa* forest in the American Pacific Northwest (Pulido-Chavez et al., 2021) and a study of *Larix* and *Betula* dominated forests in northeastern China (Yang et al., 2020). *Geminibasidium* was also present at 0.05% sequence abundance, the same amount as our pre-fire soil, in an unburned giant sequoia forest (Carey et al., 2019). While Geminibasidiomycetes are positively selected for by fire (Figure 2.6), likely due to their thermotolerance and xerotolerance (Nguyen et al., 2013), they have been missed by existing descriptions of pyrophilous fungi that are mainly based on fruiting body surveys (McMullan-Fisher et al., 2011).

Despite the most dominant post-fire fungus being a Basidiomycete, overall the community shifted from Basidiomycete to Ascomycete dominated post-fire, with particular increases in the Pezizomycete and Eurotiomycete lineages (Figure 2.6). The shift from Basidiomycete to Ascomycete dominance is well established in the post-fire fungal literature (Cairney & Bastias, 2007) with similar findings across diverse biomes ranging from Mediterranean shrublands of Spain (Pérez-Valera et al., 2018) to pine savannas of the American southeast (Semenova-Nelsen et al., 2019). Four of the five fire indicator taxa (Table A.2.7) belong to the Pyronemataceae, which have long been known

to fruit extensively after fires (Seaver, 1909). In addition to evidence from fruiting body surveys, the Pyronemataceae genus *Pyronema* can also dominate belowground mycelium by as much as 60% after experimental laboratory fires (Bruns et al., 2020) and increased 100 fold in frequency after prescribed fires in a *P. ponderosa* forest (Reazin et al., 2016). *Geopyxis alpina* was also an indicator of fire, and at least two species of *Geopyxis* species are known to fruit exclusively on burned soil (Wang et al., 2016). The Pyronemataceae produce sporocarps that are small orange cup fungi that appear on pyrolyzed material after burning (El-Abyad & Webster, 1968). This repeated observation of fruiting on burned material may suggest some capacity for using unique compounds produced through combustion, a post-fire nutrient acquisition trait as outlined in figure 2.7, and was recently explored in a study on *Pyronema domesticum* charcoal metabolysis (Fischer et al., 2021). It is also possible that their color or morphology makes them better adapted to post-fire scenarios, for example many of them are orange and contain carotenoids (Carlile & Friend, 1956) which may add UV protection (Luque et al., 2012). However, it is also likely that they survive fires due to their ability to form resistant propagules called sclerotia (Smith et al., 2015) which may be thermotolerant (Richter & Barnard, 2002). While Eurotiomycetes are widespread fungi, it is possible that their ability to grow fast and produce ubiquitous spores help them colonize open niche space after fires, as they were similarly common after wildfires in a Canadian boreal forest (Whitman et al., 2019) and in northeastern China (Yang et al., 2020). This would be an example of the fast-growth pyrophilous trait (Figure 2.7) analogous to fire adapted plants sprouting in the newly opened niche of burn zones (Keeley et al., 2011). Moreover, several species of

Aspergillus and *Penicillium* have evidence of heat activated spores (Warcup & Baker, 1963). Much like our reasoning for why some bacterial clades responded better to fire, the Pezizomycetes may all share a similar, conserved response to fire that is less genetically complex, more basal, or more deeply conserved than the response exhibited by the Eurotiales, which may explain why all lineages within the Pezizomycetes responded positively to fire while not all lineages of the Eurotiomycetes did (Figure 2.6). When discussing the phylogenetic signal obtained in our analyses, we are aware of the limitations of ITS based phylogenies compared with phylogenies constructed involving 18S or 28S data and therefore the statistical significance portrayed in our consenTRAIT analysis (Figure 2.6) may not be as reliable as the analysis done with the 16S phylogeny (Figure 2.5). However, we feel confident in continuing to draw meaningful conclusions from our phylogenetic analysis as the observed signal is tightly clustered within clades, and the positively responding taxa from which we form additional hypotheses (eg. Pyronemataceae, Eurotiales, Geminibasidiomycetes) have strong support in the literature behaving as observed (McMullan-Fisher et al., 2011; H.T. Nguyen et al., 2013; Petersen, 1970; Seaver & Clark, 1910). Future phylogenetic studies on pyrophilous fungi would benefit from sequencing which includes 18S or 28S information.

Critically analyzing similarities between certain groups of pyrophilous microbes has brought us to the hypothesis that there may be traits that characterize a microbe's ability to thrive post-fire, which could be placed into a conceptual framework (H3). These traits may be analogous to the traits exhibited by fire-tolerant plants, which themselves fall into fairly well defined suites (vegetative re-sprouters, structural

resistance to fire, and rapid colonizers post-fire) (Keeley et al., 2011). We thus propose that microbes survive fire by analogous traits to plants and build from Grime's Competitive-Stress-Ruderal (C-S-R) model (Grime, 1977), its adaptation to mycorrhizal fungi (Chagnon et al., 2013) and to microbial decomposers (Malik et al., 2020), and a recent study from a Canadian boreal forest fire (Whitman et al., 2019). Pyrophilous microbes appear to survive fire via trade-offs among microbial ability to acquire post-fire resources (analogous to Grime's C), tolerate heat or desiccation (analogous to Grime's S), or early colonize or grow fast (analogous to Grime's R) (Figure 2.7). Furthermore, as trade-offs among traits can be used to predict decomposition rates among wood decomposer fungi (Lustenhouwer et al., 2020), we predict that trade-offs among fire response traits might enable prediction of post-fire biogeochemical cycling. For example, the DEMENT model uses traits to improve forecasting of soil carbon by considering trade-offs between a microorganism's ability to decompose litter versus tolerate drought (Allison & Goulden, 2017). Similarly, trade-offs between a microbe's ability to survive fire by thermotolerance versus decomposing pyrogenic organic matter might predict changes in carbon cycling post-fire. This could be further highlighted by a trade-off of post-fire nutrient acquisition strategy microbes growing slowly as they work to breakdown recalcitrant carbon forms present in PyOM, slowly releasing this carbon for cycling, as opposed to fast-growing microbes taking advantage of easily accessible labile carbon. We hypothesize that several known pyrophilous taxa observed in this study and in others would fall into these suites of traits. For post-fire resource acquisition, basidiomycetes such as *Pholiota highlandensis* or *Lyophyllum atratum* might be more

likely to degrade pyrolyzed organic matter due to their enzymatic capabilities, as supported by recent sequencing of their genomes (Steindorff et al., 2020), and *Fusarium* may be capable of denitrifying highly abundant nitrogen (Maeda et al., 2015). For thermotolerance, *Neurospora crassa* has heat-activated spores and *Rhizopogon olivaceotinctus* has spores that increase after heat treatment (Bruns et al., 2019; Emerson, 1948) and *Pyronema* may produce thermotolerant sclerotia (Moore, 1962). *Aspergillus* and *Penicillium* are likely fast-colonizers due to highly abundant spore production. However, it is possible that certain species fall along a continuum of traits and that trade-offs may not be so strict. For example, *Aspergillus* and *Penicillium* species are also likely thermotolerant (Warcup & Baker, 1963), and some *Penicillium* species have been found to degrade polycyclic aromatic hydrocarbons (PAHs), such as those produced through combustion (Leitão, 2009). Another fungal example could be *Pyronema*, which in addition to thermotolerance may be of the resource acquisition strategy given the recent study on its ability to metabolize charcoal (Fischer et al., 2021) and thus fall along a gradient of thermotolerance to post-fire resource acquisitive. Similarly, the yeast *Basidioascus* tolerates stress with its xero- and thermo-tolerance but as a yeast may also be a fast-colonizer since it is unicellular. As our hypothesis regarding post-fire microbial trait suites expands, it may evolve into a more multi-faceted niche space as is currently used in plant trait models (Díaz et al., 2016).

We propose that this trait based conceptual model works for pyrophilous bacteria as well as fungi (Figure 2.7). For example, for post-fire resource acquisition, there are a few studies claiming *Arthrobacter* may be capable of degrading pyrogenic organic

matter (Fernández-González et al., 2017; Woolet & Whitman, 2020) and so may *Nocardiodes* (Woolet & Whitman, 2020). For thermotolerance, members of the Firmicutes are good candidates since they are almost all spore-forming bacteria (Barberán et al., 2017; Filippidou et al., 2016). For fast growth or colonization, the Proteobacteria genus *Massilia* has been hypothesized as a fast responder due to a predicted high 16S copy number (Nemergut et al., 2016; Whitman et al., 2019). We believe that categorizing pyrophilous microbes into traits will improve prediction of biogeochemical cycling post-fire. Just as redwoods survive fire with thick bark and tanoaks are vegetative re-sprouters, it is likely that microbes have similar strategies and this diversity of fire-responsive traits both above and belowground will enhance forest resilience to the unprecedented rise of mega-fires.

2.6 Conclusions

In conclusion, we present the first study examining the immediate effects of a megafire on both bacterial and fungal communities with a rare data set of pre- and post-fire samples and also the first study to comprehensively characterize the soil microbial communities of a redwood tanoak forest. We know of only two other instances where pre- and post-fire samples from the same locations exist (Baar et al., 1999; Glassman et al., 2016), but both were in pine forests, focused only on EMF communities, and both lacked an unburned control. We identified a massive increase in the Basidiomycete yeast *Basidioascus* and the bacterial Firmicutes post-fire, and we showed that pyrophilous bacteria and fungi are phylogenetically conserved at the class level. By comparing our

work to other recent molecular characterizations of post-fire microbes in Pinaceae forests (Bruns et al., 2020; Glassman et al., 2016; Whitman et al., 2019), we can now begin to generalize traits of post-fire microbes to other forest types and compare them to analogous traits in plants (Figure 2.7). For example, we hypothesize certain bacteria (Firmicutes) and fungi (Pyronemataceae) appear to be able to survive fire with thermotolerant structures, and other fungi (*Penicillium*) or bacteria (*Massillia)* are fast-responders, and trade-offs might exist among these traits. Future studies of post-fire systems in a broad variety of ecosystems, and experimental determinations of microbial traits, will allow us to further characterize and generalize traits of post-fire microbes so that we can refine our conceptual model and reach the level of knowledge of post-fire traits of plants.

2.7 Acknowledgements

We would like to acknowledge Monterey Peninsula Regional Park District (Plots 601, 603) and the University of California's Landels-Hill Big Creek Reserve (Plot 058). We thank Michael Ernandes and Tom Bruns for their assistance in collecting pre-fire soils, Judy Chung for assistance with molecular work, and James Randolph for assistance with conceptual figure artwork. DJE is supported by the National Science Foundation Graduate Research Fellowship and the Oregon Mycological Society Scholarship.

2.8 Competing Interests

The authors declare no conflicts of interest or competing financial interests in relation to the work described.

2.9 Data Accessibility and Benefit-Sharing:

Sequences are accessioned under BioProject number PRJNA659056 at the National Center for Biotechnology Information Sequence Read Archive. All scripts used to analyze the data are available at: https://github.com/sydneyg/SoberanesFire.

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2.11 Figures

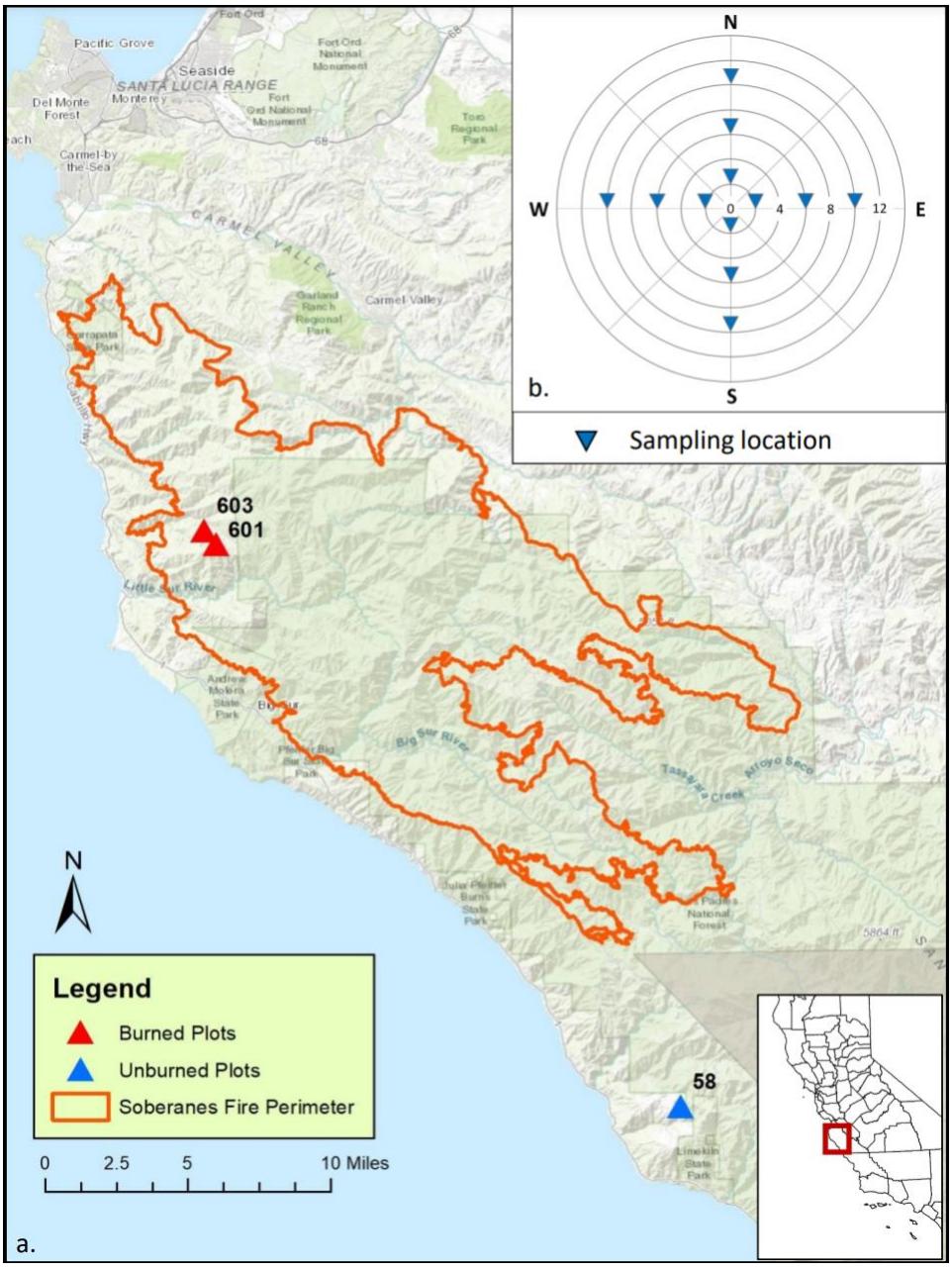

Figure 2.1: A) Map of the Soberanes Fire of 2016 with fire perimeter in orange. Triangles represent locations of individual plots in the plot network that were sampled in 2013 with burned plots in red and unburned plot in blue. B) Sampling scheme used in all plots with soils collected at 2, 6, and 10 meters from the plot center in each cardinal direction (indicated by inverted blue triangles on sampling scheme).

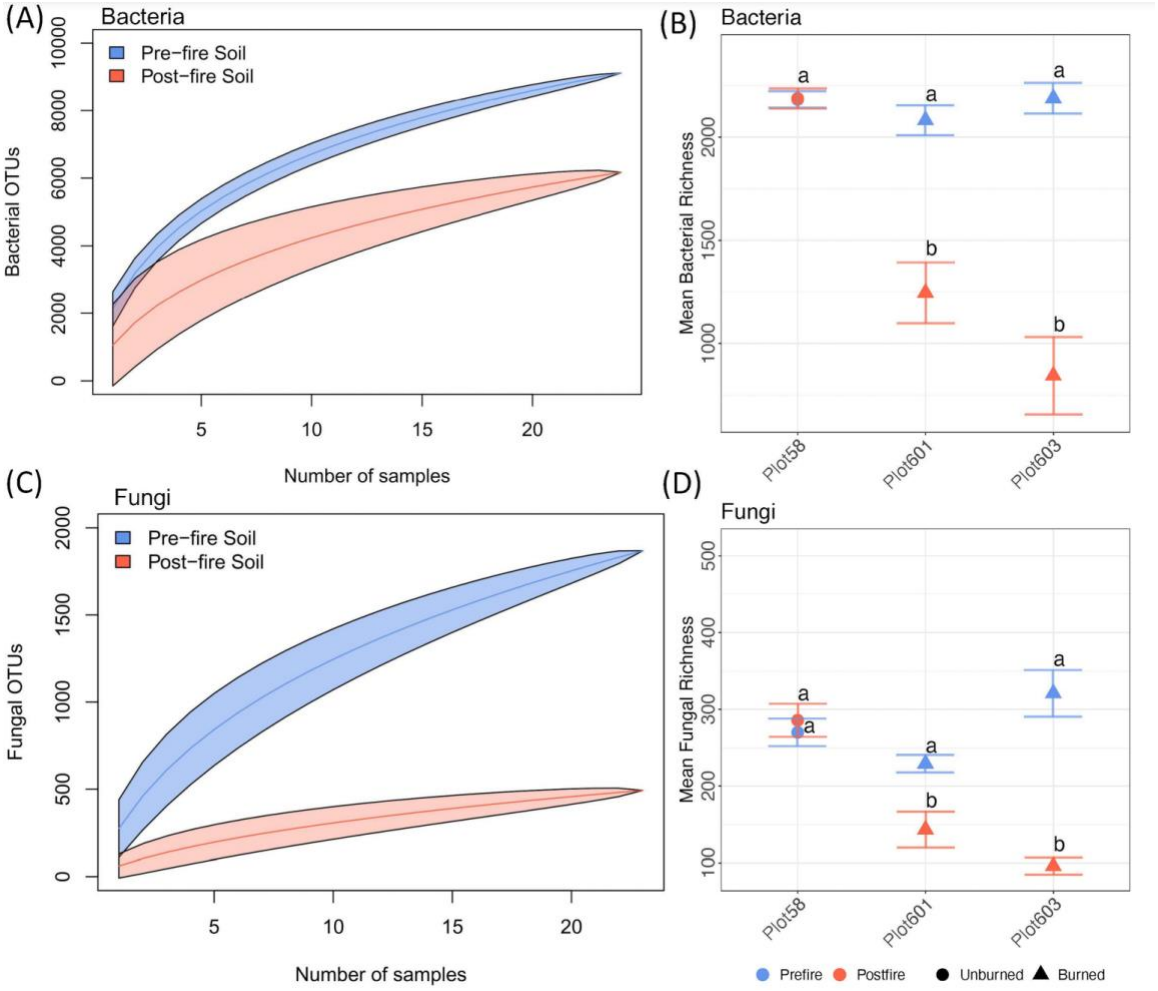

Figure 2.2: Fire reduced bacterial (A) total richness and (B) mean per sample richness in burned plots and fungal (C) total richness and (D) mean per sample richness in burned plots. Species accumulation curves represent total pre- and post-fire bacterial and fungal OTUs within the burned plots with transparency around the line representing the standard deviation. Mean per sample richness shown plus and minus the standard error. Colors differentiate sampling in 2013 pre-fire and in 2016 immediately post-fire. Shapes differentiate burned (plots 601 and 603) and unburned plots (plot 58). Statically significant difference in richness was tested using ANOVA (for burned plots, $F_{1,1} = 65.73$, $p < 0.001$, for unburned $F_{1,1} = 0.005$, $p = 0.943$). Letters represent Tukey HSD differences.

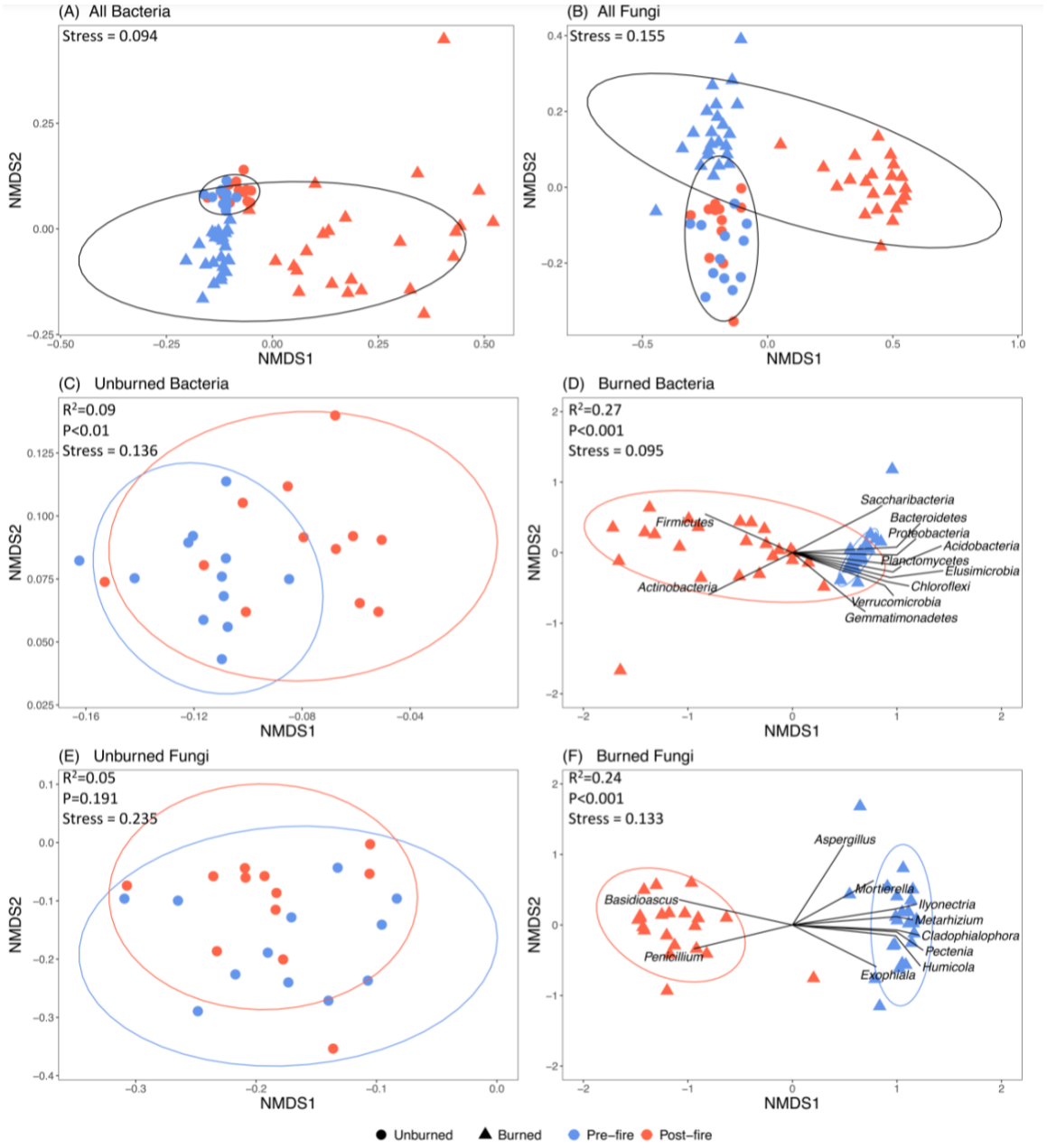
(A) All Bacteria
Stress = 0.094
(B) All Fungi
Stress = 0.155
(C) Unburned Bacteria
R²=0.09
P<0.01
Stress = 0.136
(D) Burned Bacteria
R²=0.27
P<0.001
Stress = 0.095
Saccharibacteria
Bacteroidetes
Proteobacteria
Acidobacteria
Planctomycetes
Elusimicrobia
Chloroflexi
Verrucomicrobia
Gemmatimonadetes
Firmicutes
Actinobacteria
(E) Unburned Fungi
R²=0.05
P=0.191
Stress = 0.235
(F) Burned Fungi
R²=0.24
P<0.001
Stress = 0.133
Aspergillus
Mortierella
Ilyonectria
Metarhizium
Cladophialophora
Pectenia
Humicola
Exophiala
Basidioascus
Penicillium
NMDS1
NMDS2
Unburned
Burned
Pre−fire
Post−fire

Figure 2.3: NMDS of Bray-Curtis Dissimilarity ordinations comparing bacterial composition A) in all plots, C) unburned plot, and E) burned plots and fungal composition in B) all plots, D) unburned plot, and F) burned plots with colors indicating the 2013 pre-fire and 2016 post-fire samplings and shapes differentiating burned and unburned plots. All ordinations were done in 2 dimensions and stress is indicated for each NMDS. Ellipses represent 95% confidence interval from the centroid for each group. Notice the difference in scales for all NMDS with much smaller scales for NMDS axes in unburned plots representing a much smaller degree of compositional turnover than for burned plots with much larger axes. Adonis R^2 and p-values represent the difference in composition for pre- versus post-fire. Envfit model depicts which taxa are driving the changes in community composition for D) bacterial or F) fungal communities. The top phyla contributing to change are shown. Identification of the top contributors was done by R^2 value after constraining p value at less than 0.001 and adjusted p -value to less than 0.01.

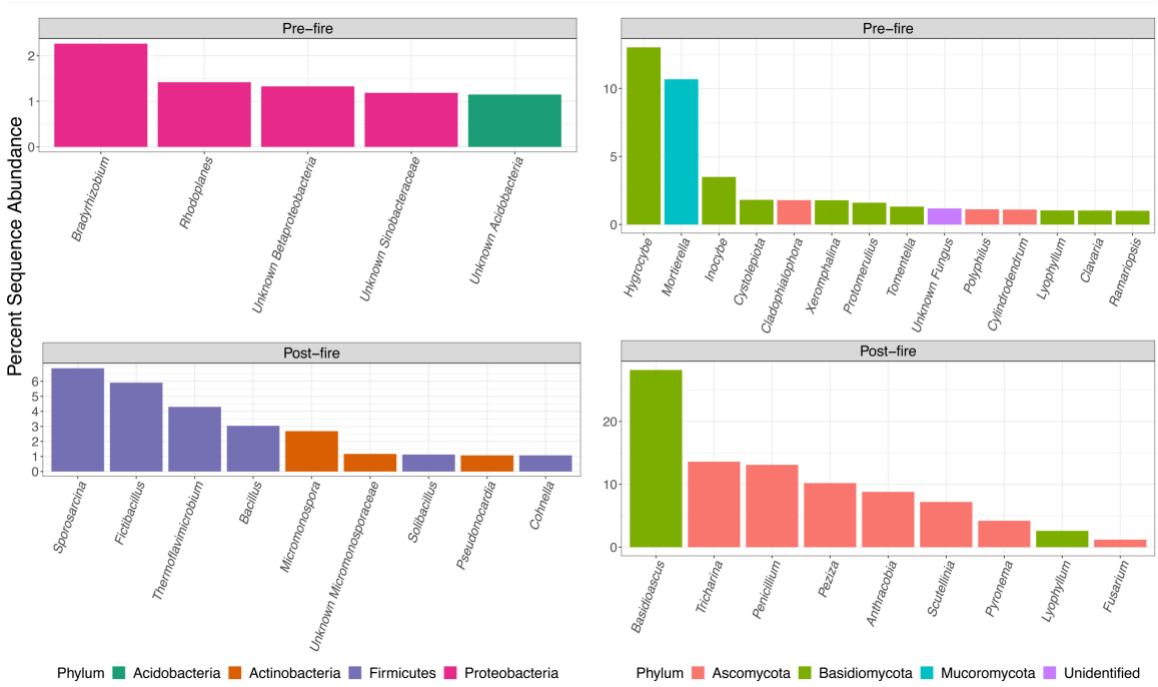

Figure 2.4: Rank abundance curves of taxa ≥1% sequence abundant for bacteria and fungi pre-fire (top) vs. post-fire (bottom) grouped by genus and colored by phyla. Where genus level identification could not be determined, a higher order of classification is given.

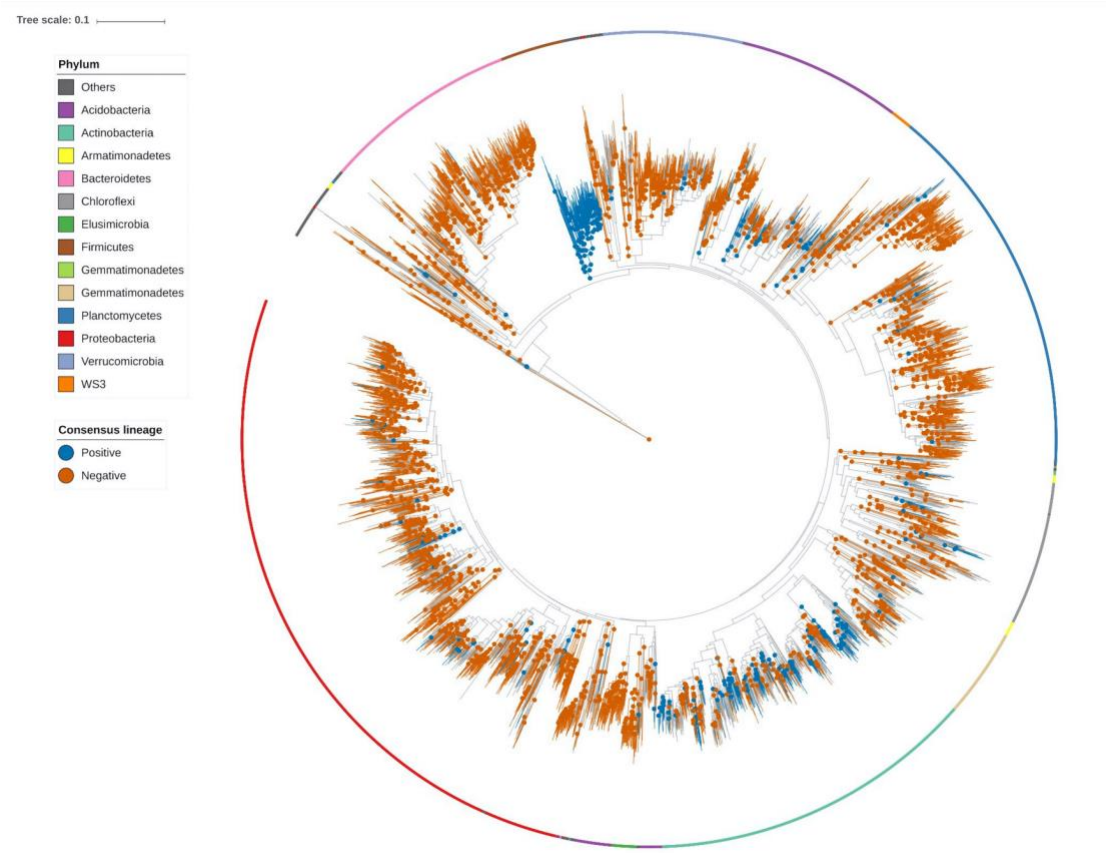

Figure 2.5: Circular phylogeny of all bacterial OTUs observed based on 16S rRNA. Consensus Lineage refers to the response to fire as measured using DeSEQ2 analysis. Lineages are colored based on positive (blue) or negative (orange) response to fire. The colored bars circling the outside of the phylogeny correspond to each OTUs respective phyla.

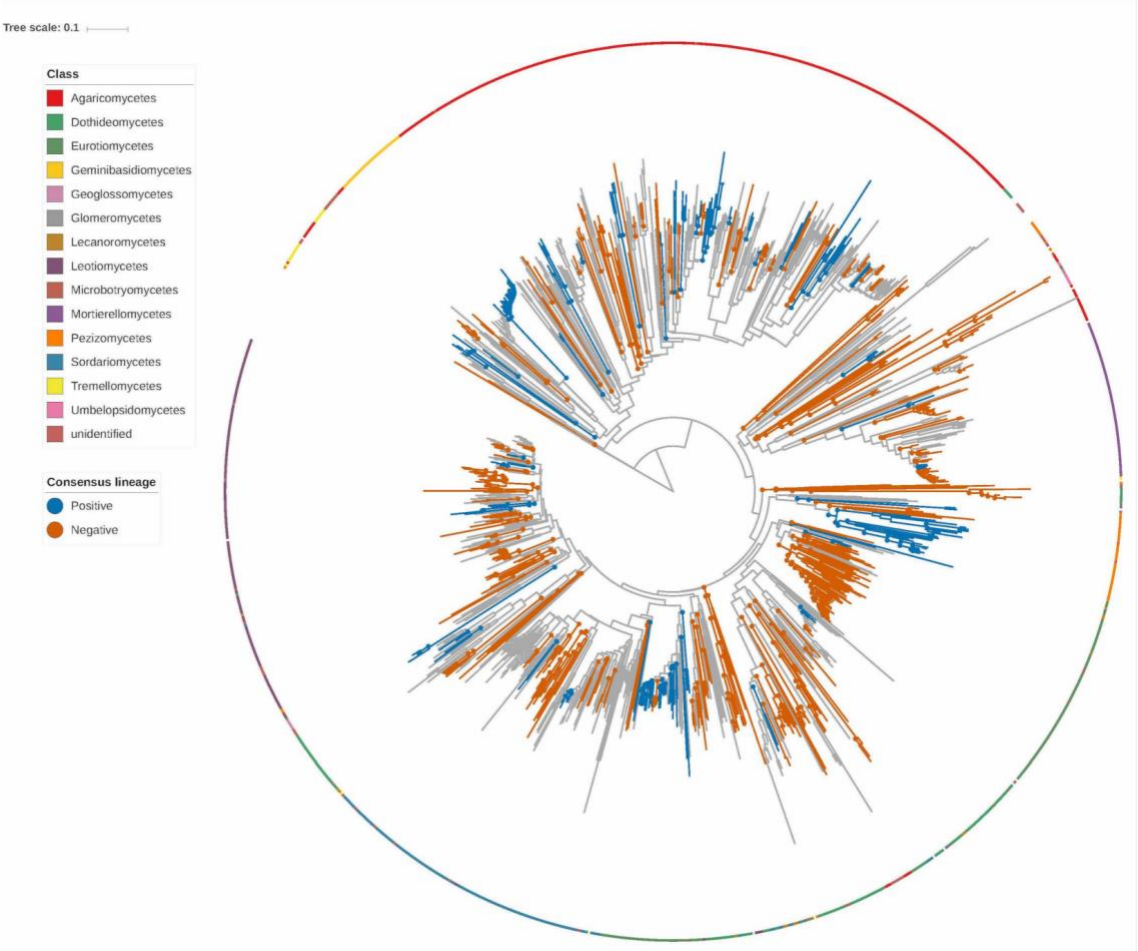

Figure 2.6: Circular phylogeny of all fungal OTUs observed based on ITS1. Consensus Lineage refers to the response to fire as measured using DeSEQ2 analysis. Lineages are colored based on positive (blue) or negative (orange) response to fire. The colored bars circling the outside of the phylogeny correspond to each OTUs respective class.

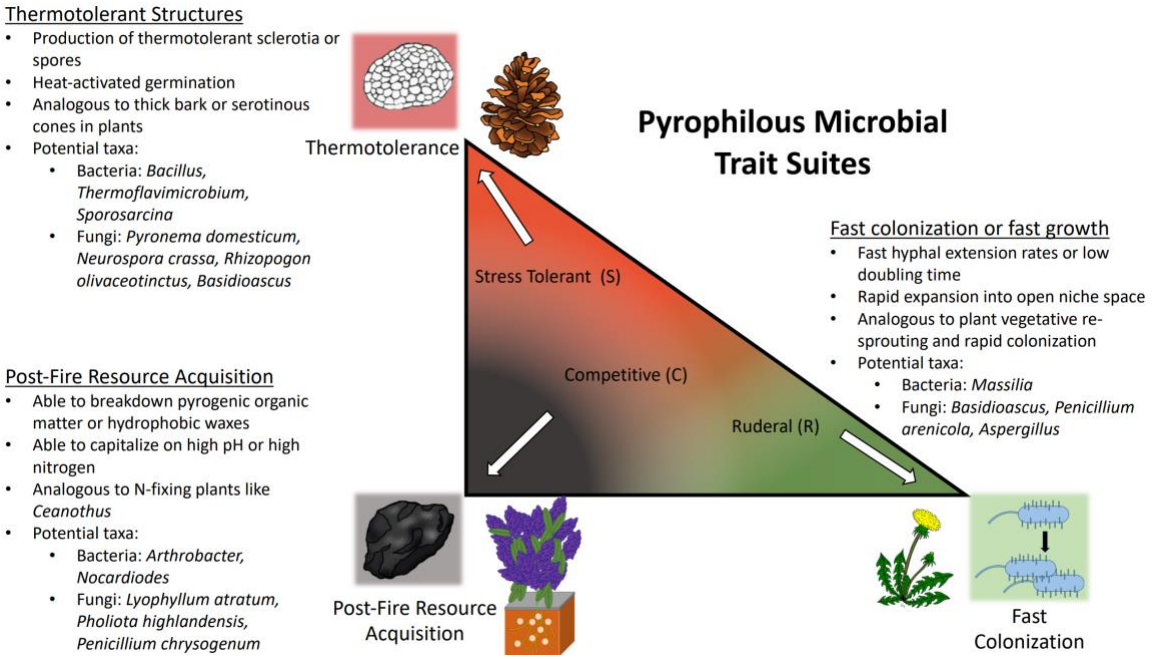

Figure 2.7: Conceptual model of hypothesized trait suites for post fire microbes, in comparison to analogous trait suits of fire adaptation in plants. Grimes CSR ecological trade-off directions are placed inside the triangle following where the corresponding pyrophilous microbial trait suite is hypothesized to relate. A color gradient between trait suites represents that these suites may not be exclusive but rather taxa may fall along a gradient between trait suites. A representation of the microbial trait is placed at each corner (Fast colonization = replicating bacteria, Thermotolerance = fungal sclerotium, Post-fire nutrient acquisition = colonized charcoal) along with an analogous plant trait representation (fast herbaceous growth, serotinous cone, root nodule forming post-fire colonizer, respectively). A brief description of the pyrophilous trait suites and hypothesized taxa placement is located on the right side of the figure.

Chapter III

Evaluating Best Practices for Isolating Pyrophilous Bacteria and Fungi from Burned Soil

3. 1 Abstract

A live microbial culture is invaluable to assess traits and functions via 'omics and biophysical assays. However, it is not always logistically feasible to culture immediately from freshly obtained soil, and selecting the proper media for culturing is not trivial. While building a culture collection of pyrophilous microbes obtained from burnt soil, we tested the best: 1) method of storing soil to retain culturable viability; and 2) media to garner the most microbial diversity. We tested four methods of soil storage (dried, stored at 4ºC, stored at -80ºC alone or in glycerol) and compared to fresh soil obtained 6 months after a severe California chapparal shrubland wildfire. For bacteria, soil frozen at -80ºC with glycerol preserved the greatest diversity (25 species, 13 genera) compared to fresh soil (26 species, 13 genera). For fungi, soil stored at -80ºC alone preserved the greatest

diversity (10 species, 3 genera) compared to fresh soil (13 species, 7 genera). We also tested 3 media types: rich media (Lysogeny Broth (LB) for bacteria; Malt Yeast Agar (MYA) for fungi), oligotrophic media (Reasoner's 2 Agar (R2A) and media made from pyrogenic organic matter (PyOM). For bacteria, culturing on LB and R2A garnered the greatest diversity (LB = 26 species, 13 genera, R2A = 27 species, 15 genera). For fungi a combination of R2A and PyOM captured the greatest diversity (R2A = 15 species, 8 genera, PyOM = 12 species, 6 genera). For both bacteria and fungi, some species of interest were only captured using the PyOM media. Using a combination of these methods from 2018-2022, we cultured >500 isolates (286 bacteria; 258 fungi) from burned soils of 7 Southern California wildfires. These cultures are an invaluable resource for pyrophilous microbial laboratory experiments.

3.2 Introduction

In microbiological research, few laboratory resources are as valuable as live microbial cultures. Though advances in the fields of molecular biology and DNA sequencing have freed microbiologists from the need to work with culturable species exclusively (Franzosa et al. 2015), microbial culture collections remain an invaluable resource. Pure cultures are essential components for high quality whole-genome assembly (Hugenholtz 2002; Haridas et al. 2011), genetic engineering experiments (Malik and Claus 1987), and the phenotypic profiling of microorganisms (Kämpfer 2014; Alster et al. 2021). Additionally, isolated microorganisms greatly improve the interpretation of metabolomics data (Swenson et al. 2018) and possession of a diverse

culture collection can unlock a plethora of applied microbiological research directions (Malik and Claus 1987; Reyes-César et al. 2014; Carini 2019). Once isolated and properly stocked, microbial culture collections become a permanent resource for future microbiological research (Caktu and Turkoglu 2011; Stackebrandt et al. 2014; Arnold and Lutzoni 2007). This in turn creates a veritable breeding ground for collaborative research. However, there are many hurdles to creating microbial culture collections and each collection poses its own unique challenges (Caktu and Turkoglu 2011).

The various factors that can be modified when attempting to culture microorganisms are as varied as there are microbes to isolate. For example, media selection may require grappling with an endless number of variables including consideration of the proper carbon sources, nitrogen and phosphorus content, salt concentrations, pH, and a host of other micronutrient considerations (Carini 2019; Davis, Joseph, and Janssen 2005; Stevenson et al. 2004). One must also consider whether to make the media nutrient rich or poor (oligotrophic), incubation temperature, water and oxygen availability, whether to alternate light and dark cycles, and the length of time to incubate samples (Davis, Joseph, and Janssen 2005; Stevenson et al. 2004; Leadbetter 2003). All this complexity contributes to so many microorganisms remaining uncultured (Prakash et al. 2013). And though recent advances in our understanding of microbiology have begun to make the adage of "99% of microbes are unculturable" outdated (Carini 2019; Stevenson et al. 2004; Leadbetter 2003; Martiny 2019), the fact remains that many microorganisms currently still lie beyond our reach.

One group of understudied microorganisms that is of growing interest are the pyrophilous (or "fire-loving") microbes. These microbes are the first to increase in abundance following wildfire and they can persist in the environment for many years after the fire has ended (Seaver and Clark 1910; Pérez-Valera et al. 2018; Pressler, Moore, and Cotrufo 2019). While there are many studies identifying which microbes become more abundant post-fire, there are very few studies characterizing how pyrophilous microbes interact with their environment (Fox et al. 2022). Wildfires dramatically change the landscapes they burn and create legacy effects that can impact soil microorganisms and their overall ecosystems for many years following the fire (Wan, Hui, and Luo 2001; Pierson et al. 2019; Michelotti and Miesel 2015). With rising global frequency and severity of wildfires across many ecosystems (de Groot, Flannigan, and Cantin 2013; Hantson, Pueyo, and Chuvieco 2015), understanding how pyrophilous microbes modify and interact with their environments is of critical importance. An important step to characterizing the ecology of pyrophilous microbes will be laboratory testing of pyrophilous culture isolates which can provide data on how individual pyrophilous taxa compete for resources, cycle post-fire nutrients, and interact with the post-fire environment. However, there are numerous technological and logistical challenges to creating a culture collection of pyrophilous microbes.

In addition to the well-known hurdles of creating a microbial culture collection, attempting to culture microbes associated with wildfires presents its own set of challenges. The first set of challenges are the logistic challenges associated with working with post-fire environmental samples. Wildfires are unpredictable natural phenomena. As

such, it may not always be possible to have the infrastructure for large-scale culturing efforts (personnel available, media selected and created, broth tubes or petri dishes ordered, culturing conditions decided and established) in place when a wildfire burn scar is ready to be sampled. As part of this logistical navigation, often times post-fire soil samples may have to be stored for varying lengths of time before they can be used. This is a logistical problem shared among many environmental culturing efforts ranging from culturing of marine microbes (Joint, Mühling, and Querellou 2010) to culturing from Antarctic soils (Malosso et al. 2006). While many insights have been gained regarding these logistical hurdles such as culturing as early as possible (Caktu and Turkoglu 2011; Černohlávková et al. 2009) and methods for freezing samples to improve cultural viability, their efficacy is not always consistent and the viability of culturable microorganisms may be reduced depending on the soil storage method (Černohlávková et al. 2009; Rubin et al. 2013). In addition to the logistics, wildfires radically alter environmental nutrient landscapes (Caon et al. 2014; Pierson et al. 2019). Wildfire pyrolysis turns labile, easily accessible carbon sources into difficult to degrade pyrogenic organic matter (PyOM) (Michelotti and Miesel 2015; Preston and Schmidt 2006). This PyOM is often enriched in polycyclic aromatic hydrocarbons (PAHs) that may require specialized metabolic pathways to process (Reyes-César et al. 2014; Woolet and Whitman 2020; Aranda et al. 2017; Fischer et al. 2021). This can lead to accumulated char persisting in the environment for up to and exceeding 100 years (Knicker 2011). Additionally, wildfires can deposit an enormous amount of inorganic nitrogen into the environment as organic nitrogen previously locked in plant biomass becomes released

during burning (Caon et al. 2014; Pulido-Chavez et al. 2023; González-Pérez et al. 2004; Wan, Hui, and Luo 2001). This high-nitrogen environment can radically alter the nutrient strategies that microorganisms may employ post-fire and may limit the microbes capable of surviving. For example, many species of fungi are known to be nitrophobic (Lilleskov et al. 2019). These radical nutrient shifts may result in commonly used laboratory media not providing appropriate nutrient conditions to cultivate post-fire microbes.

Here, our goal was to create a large culture collection of pyrophilous microbes to use to test the traits that govern pyrophilous microbial adaptation to fire and the post-fire environment via biophysical assays and genomics. Keeping in mind the above described logistic and nutrient-related challenges, we used soil from a local high severity chaparral wildfire to test 1) the best methods to store post-fire soil to retain pyrophilous microbial culturable viability and 2) which laboratory media best captured the diversity of pyrophilous microbes. For soil storage we tested soils stored for 3 months in a variety of ways (air-dried, refrigerated at 4°C, and frozen at -80°C with and without glycerol) versus cultured immediately from the freshly obtained soil samples (Figure 3.1). For media type we tested rich media (lysogeny broth (LB) agar for bacteria, malt-yeast agar (MYA) for fungi), an oligotrophic media (Reasoner's 2 Agar (R2A)), or a media created in-house from PyOM to test the effect of media selection on the culturability of pyrophilous bacteria and fungi (Figure 3.1). We tested the impact of our treatments on abundance (CFUs: Colony Forming Units) and microbial richness and composition (assessed via Sanger sequencing of 16S for bacteria or ITS for fungi). We hypothesized (H1) that air-dried soil would retain the greatest diversity of pyrophilous bacteria and

fungi compared to fresh soil since post-fire soils already tend to be fairly dry in chaparral environments (Pulido-Chavez et al. 2023; Stoof, Wesseling, and Ritsema 2010) thus leaving the soils to air dry would be the least dramatic disturbance for the soil microorganisms contained within the samples. We further hypothesized (H2) that a combination of the rich and PyOM media would obtain the greatest diversity of pyrophilous microbes as the rich media would be able to grow a wide range of bacteria and fungi and the PyOM media would capture those pyrophilous species with unique metabolic requirements specific to post-fire burn scars. Although we specifically sought to create a pyrophilous culture collection, the insights gleaned from this study not only showcase potential strategies for culturing fastidious pyrophilous microorganisms but can also aid environmental microbiologists in decision making about storage and media selection for all types of environmental culturing. The culmination of this research was the creation of a large and diverse collection of pyrophilous microorganisms of >500 isolates (286 bacteria; 258 fungi) from burned soils, mushrooms, and smoke of 7 Southern California wildfires.

3.3 Methods

3.3.1 Site Description

To test the best soil storage method and culture media to cultivate pyrophilous microbes, we leveraged a local high-severity wildfire of fire-adapted chaparral shrublands for sampling. The El Dorado wildfire burned ~92km^2 Manzanita (*Arctostaphylos)* dominated chaparral shrublands in the area surrounding Yucaipa, CA

from September through November of 2020. We examined high-resolution successional data of bacterial and fungal communities after a similar nearby high-severity Manzanita dominated chaparral wildfire (Pulido-Chavez et al. 2023), and compared to other post-fire studies that had successional data (Bárcenas-Moreno et al. 2011; Caiafa et al. 2023), to identify 6 months post-fire the ideal time to attempt to isolate a wide range of pyrophilous bacteria and fungi. Therefore, we collected soil samples from high-severity zones 6 months after the El Dorado fire in June 2021. Sites of the fire were characterized as having 50 to 75 percent slopes with soil characteristics described as dry, sandy soils in the Springdale family-Lithic Xerorthents association.

3.3.2 Soil Collection and Storage

Approximately 250mL of soil from 0-10 cm below the ash layer of burned soil was collected from 9 plots with ethanol cleaned releasable bulb planters and placed into whirl-pak bags 6 months post-fire following the same methods as used in a similar ecosystem (Pulido-Chavez et al. 2023). After collection, soil was 2mm sieved and either used as fresh inoculum for cultivation or stored in four different ways: air dried, refrigerated (4ºC), frozen (-80ºC), or suspended 50:50 by volume soil with glycerol to create a soil slurry and then frozen (-80ºC) (Figure 3.1).

3.3.3 Culturing Medias Used

Fresh soil samples were cultured immediately onto rich media (lysogeny broth (LB) agar for bacteria, malt-yeast agar (MYA) for fungi), an oligotrophic media

(Reasoner's 2 Agar (R2A)), or a media created in-house from PyOM to test the effect of media selection on the culturability of pyrophilous bacteria and fungi. After 3 months of storage, stored soil samples were cultured onto rich media. This means that the cultures obtained from freshly obtained soil samples on rich media served as both the "rich media" condition for the media experiment and the "fresh" comparison for the soil storage experiment (Figure 3.1).

LB agar and broth were produced using powdered LB Broth, Miller (Fisher Scientific, CAS# 73049-73-7). When making solid LB agar, 15g per liter of bacteriological grade agar (Apex Chemicals, Cat # 20-273) was added to DI water. For each liter of Malt-Yeast Agar (MYA) and broth 5g of powdered Malt Extract (Research Products International, Genessee, CAS# 8002-48-0) and 5g of powdered Yeast Extract (Sigma-Aldrich, Cat # 70161) was used. For solid agar media, 15g per liter of bacteriological grade agar (Apex Chemicals, Cat # 20-273) was added. R2A agar was produced using powdered R2A agar (Criterion, Cat # 16721) following manufacturer directions.

Pyrogenic Organic Matter (PyOM) Media: We developed an in-house recipe of PyOM agar media using biochar harvested from the El Dorado Fire burn sites. Biochar was ground into a fine powder prior to use with a coffee grinder (Chefman, Model: RJ44-OPP-RED). In a 2L Erlenmeyer flask, 50g of ground biochar was added followed by 1200mL of DI water. The mouth of the flask was covered and left on a stirring hotplate for 24 hours at 40°C after which it was removed from heat. The solution was then

allowed to settle for 8 hours. Then, the liquid contents of the flask were carefully siphoned into a clean Erlenmeyer flask, being very careful to minimally disturb the sediment at the bottom **(**Figure B.2.1**)**. The liquid was then passed through cheesecloth, and then twice through a coffee filter to remove any remaining sediment before being transferred to clean containers for autoclaving. For each liter of PyOM media 13g of bacteriological grade agar (Apex Chemicals, Cat # 20-273) was added. All media types (LB, MYA, and R2A, after mixing with DI water per manufacturer directions, and home-made PyOM media) were autoclaved at 121ºC for 45 minutes.

Antibiotics: For media used to isolate fungi and intended to resist bacterial growth, filter sterilized gentamycin and ampicillin (Hardy Diagnostics, Santa Maria, Ca), were added to the molten agar after autoclaving, once it had cooled to 60ºC, for a final concentration of 10mg/mL each. For media used to isolate bacteria and intended to resist fungal growth, cycloheximide (Research Products International, CAS # 66-81-9) was added to the molten agar, once it had cooled to 60ºC for a final concentration of 20mg/L.

3.3.4 Microbial Isolation Method

Soil suspension inoculum was prepared by making a slurry of 1g of soil and 5mL of autoclaved deionized water. A dilution series was made from the soil slurry down to concentrations between 1:10 to 1:1000. This inoculum was spread onto LB, MYA, R2A, or PyOM media supplemented with cycloheximide if culturing bacteria or ampicillin and gentamicin if culturing fungi. These plates were then parafilmed and allowed to grow for

a week. Fungi and bacteria were then morphotyped using size, color, and growth rate. Bacteria were isolated by picking a well isolated colony using a sterile 10µL pipet tip and then streaking for isolation onto a fresh LB plate without antifungals. Fungi were isolated by using a flame-sterilized scalpel to cut a 1 cm^2 square of the growing edge of a fungus out of the agar plate. This square was then transferred to a fresh MYA plate without antibacterials and placed growing-side down into the center of the plate. In cases where microbes would not establish onto LB or MYA, they were maintained on the media they originated from (either R2A or PyOM). Plates were allowed to incubate at room temperature for 1 week before re-isolating following the same procedure. This was repeated until three clean isolations were obtained prior to DNA extraction and stocking.

3.3.5 Microbial Stocking Method

Bacteria: Once a thrice purified isolate was obtained and DNA was extracted, each bacterial isolate was stocked for future use. A colony was picked aseptically from the last isolation streak plate using a sterile 10µL pipet tip and placed into 3mL of LB broth in a sterile 15mL Falcon tube. This was then grown under shaking incubation at 180rpm for 24-72hrs until the liquid culture became turbid. From this liquid culture, 1mL of culture suspension was added to a 2mL cryovial and combined with 1mL of sterile 40% glycerol, resulting in a final cell suspension that was 20% glycerol. This was vortexed briefly and then frozen at -80ºC. For each isolate, 2 stock tubes were made.

Fungi: Once a thrice purified isolate was obtained and DNA was extracted, each fungal isolate was stocked for future use. On a 100mm agar plate, a 1cm^2 cube of fungus was allowed to grow until it came within approximately 1cm of the edge of the Petri dish. Then, using a sterile transfer tube, 5mm disks were punched from the growing edge of the fungus. These disks were then transferred to a 2mL screw-cap tube containing 1mL of autoclaved tap water. For each stock tube 8-10 disks were obtained, and for each isolate 2 stock tubes were made. These tubes were then stored at room temperature for future use.

3.3.6 Soil Storage Method Experimental Design

For each of the 9 soil samples collected, we divided soils from each subsample into either fresh vs stored for 3 months air-dried, frozen, or frozen in glycerol, then 1g was combined with 5mL of sterile DI water in 4 separate 50mL falcon tubes to create four initial solutions for each of the soil samples collected. The initial solutions were then used to create a dilution series ranging from 1:10 to 1:10,000 for each storage method. 100µL of each solution in the dilution series was then plated on MYA plates with ampicillin and gentamicin and LB plates with cycloheximide. The plates were then wrapped in parafilm and placed in a drawer to be kept at room temperature for approximately one week. Cell counts and unique morphology counts were then done on the plates. Plates containing between 30-500 colonies were considered “countable” plates and the initial CFU concentrations were calculated using the number of colonies obtained multiplied by the dilution factor used. This led to each treatment having between 6 and 9 countable replicate plates. Morphotypes were determined using size, color, and growth

rate. We then isolated, extracted DNA, and stocked each bacterial and fungal unique morphology (Figure 3.1).

3.3.7 Effect of Media Type Experimental Design

The fresh soil suspension inoculum was prepared by making a slurry of 1 gram of soil and 5mL of deionized water. The soil suspension was then diluted down to concentrations between 1:10 to 1:10,000. This inoculum was spread onto MYA media with ampicillin and gentamicin, LB media with cycloheximide, R2A media (with antibacterials or cycloheximide) and PyOM media (with antibacterials or cycloheximide). The plates were allowed to grow for a week after inoculation before being morphotyped and CFUs were counted as described in the previous section. Fungi and bacteria were morphotyped using size, color, and growth rate. Individual morphotypes were then isolated (Figure 3.1).

3.3.8 DNA Extraction & PCR

DNA Extraction: DNA was extracted from all unique isolates using 10µL of home-made Extract-N-Amp Extraction Solution (Tris stock, EDTA, KCl, NaOH, DI H_20; full recipe in B.1.1). For bacteria, one well-isolated colony was picked using a sterile 10µL pipet tip, and for fungi, a $1mm^2$ area of the growing edge of the fungus was scraped with a flame-sterilized probe. The tip or probe was then submerged and swirled in the Extraction solution. After the Extraction Solution was inoculated, the samples were heated at 65ºC for 10 minutes followed by 95ºC for 10 minutes. Once finished, 10µL of a Dilution

Solution (3% BSA) was added and the samples were left to chill overnight at 4ºC. Samples were either frozen at -20ºC or immediately amplified.

PCR Amplification of rRNA genes and Sanger sequencing: The bacterial 16S rRNA region was amplified using a PA forward and PH reverse primer (Edwards et al., 1989) and the fungal ITS region was amplified with the ITS1F forward (Gardes & Bruns, 1993) and ITS4 reverse primer (White et al., 1990). Thermocycler conditions were as follows: 94℃ for 2 minutes, then 35 cycles of 57℃ (for fungi) or 62℃ (for bacteria) for 1 minute, 72℃ for 1 minute, then 94℃ for 1 minute, ending with a final extension of 72℃ for 8 minutes. The DNA was cleaned up using 3μL of diluted Exosap mastermix (Applied Biosystems, Waltham MA) per 7μL PCR product before submission for diluting and preparing for Sanger sequencing. Samples were Sanger sequenced in the forward direction using either Genewiz (San Diego, CA), Eurofins Genomics (Louisville, KY), or the UCR Institute for Integrative Genome Biology Genomics Core services.

BLAST Identification: Raw sequences were returned as .ab1 chromatogram files. Sequences were then trimmed in either SnapGene or Geneious Prime to remove low quality regions and were saved as FASTA files. Taxa were identified using NCBI BLASTn to create hit tables of top 10 hits. IDs were assigned using a combination of query cover, error value, percent identity, and a consensus of hits which obtained the same quality scores. In general, an isolate was identified at species level if the query cover was 100%, max e score was < 0.01, and percent identity was >98%. If percent

identity fell to ~95% then isolates were identified to genus, at 90% isolates were left at family, at 85-90% isolates were identified to class, and at < 85% isolates were only identified to phylum level.

3.3.9 Data Analysis

All statistical analyses were conducted, and figures were produced, in R 4.0.2 (R Core Team 2020) and all scripts are available on GitHub. Normality was tested with a Shapiro test, then ANOVA was used to test the effect of treatment (whether soil storage method or media selection) on bacterial and fungal CFU counts, followed by a post-hoc Tukey HSD test. Heatmap-like visualization of genera and species level diversity were created using the "geom_tile" function in ggplot2 (Wickham et al. 2019). All sequences have been deposited to NCBI GenBank.

In addition to the test of the best storage methods and media selection for isolating pyrophilous microbes, here we present our culture collection of pyrophilous microbes. For a table of all isolates, the fires from which they were sampled, time of collection, location data, and substrate from which species were isolated please see Supplementary File 3.2.

3.4 Results

3.4.1 Microbes Obtained

From the 2020 El Dorado Fire 6-month timepoint soils we obtained across all experimental methods a total of 170 bacterial isolates representing 4 phyla, 10 orders, 31

genera and 83 species. We also obtained 160 fungal isolates representing 3 phyla, 9 orders, 15 genera and 35 species. These isolates displayed a wide variety of morphologies (Figure 3.2) and all isolates are listed in Table B.3.1. These were the isolates used for further analysis in this study. Of these isolates, 138/170 bacterial isolates composed of 17 genera and 71 species and 152/160 fungal isolates composed of 8 genera and 30 species had literature support for inclusion as potential or confirmed pyrophilous species (Enright et al. 2022; Pulido-Chavez et al. 2023; Woolet and Whitman 2020; Aranda et al. 2017; Caiafa et al. 2023; Seaver and Clark 1910; Petersen 1970; Whitman et al. 2019; Brown et al. 2019; Bárcenas-Moreno and Bååth 2009; Ferrenberg et al. 2013). Pyrophilous isolates are listed in Table B.3.2.

3.4.2 Effect of Storage Method Selection on CFUs

Soil storage method significantly impacted the number of CFUs obtained per gram soil for both bacteria (ANOVA: $F_{1,4} = 19.97$, $p < 0.001$; Figure 3.3A) and fungi (ANOVA: $F_{1,4} = 19.78$, $p < 0.001$; Figure 3.3B). For both bacteria and fungi, soil stored air-dried (Bacteria: 2.31×10^5 CFUs/gram, Fungi: 1.33×10^4 CFUs/gram) and stored at -80ºC with glycerol (Bacteria: 1.31×10^5 CFUs/gram, Fungi: 6.69×10^3 CFUs/gram) retained the fewest CFUs/gram soil. For bacteria, soils stored at 4ºC (6.98×10^5 CFUs/gram) or at -80ºC without glycerol (6.39×10^5 CFUs/gram) were not significantly different from fresh soils (5.73×10^5 CFUs). For fungi, fresh soils had the highest average CFUs/gram soil (3.07×10^4 CFUs/gram), followed by soil frozen at -80ºC without glycerol (2.66×10^4 CFUs/gram), and then stored at 4ºC (2.16×10^4 CFUs/gram).

3.4.3 Effect of Media Selection on CFUs

Choice of growth media significantly impacted the number of CFUs obtained per gram soil for both bacteria (ANOVA: $F_{1,2} = 39.43$, $p < 0.001$; Figure 3.3C) and fungi ($F_{1,2} = 7.77$, $p < 0.01$; Figure 3.3D). For both bacteria and fungi, PyOM media yielded the fewest CFUs per gram (Bacteria: 1.66×10^5; Fungi: 1.03×10^4), and rich media (LB for bacteria and MYA for fungi) had the highest average CFUs per gram (Bacteria: 5.73×10^5; Fungi: 3.07×10^4). Soil slurry plated on oligotrophic R2A media had the second highest average CFU counts per gram for bacteria (3.20×10^5). For fungi, CFU counts per gram on R2A (2.20×10^4) were statistically equivalent to that on MYA.

3.4.4 Effect of Storage Method Selection on Diversity

Bacteria: Method of soil storage strongly affected the diversity of bacteria with fresh soil retaining the highest diversity and dried soil retaining the lowest diversity (Figure 3.4A). In assessing diversity, diversity among genera was given greater consideration than diversity among species as more diverse genera would cover greater phylogenetic diversity than diversity within species alone. For this reason, figures are ordered by number of genera first, then number of species. For bacteria, in the soil storage experiment we obtained a total of 21 bacterial genera (Figure B.2.2A and B.2.3) and 64 bacterial species (Figure B.2.2A and B.2.4). Fresh soil was the most diverse, capturing 62% of these genera and 41% of these species. The second most diverse treatment was frozen soil without glycerol which captured 62% of the genera and 30% of species. This

was followed closely by the frozen with glycerol treatment at 57% of genera and 39% of the species. Next was soil stored at 4ºC with 48% of genera and 22% of the species. Lastly, the dried soil captured 38% of genera and 23% of the species. Only 3 genera (*Arthrobacter, Curtobacterium, Streptomyces*) were isolated across all 5 storage treatments whereas 8 genera (*Brevibacterium, Fontibacillus, Glutamicibacter, Massilia, Ornithinimicrobium, Paeniglutamicibacter, Pseudoarthobacter, and an unidentified Firmicute*) were isolated from a single treatment either fresh, refrigerated, frozen, or frozen with glycerol (Figure B.2.3). It is more difficult to discern additional patterns among the species level diversity that are not additionally genus level patterns, but this is probably largely due to the fact that most species (64%) were only observed once and only the species *Curtobacterium oceanosedimentum* was observed in all treatments (Figure B.2.4).

Considering only the isolates that have literature support for being pyrophilous, we isolated from our soil storage experiment 13 bacterial genera and 51 bacterial species (Figures 3.4A, 3.5A; B.2.5). In this subset, frozen soil with no glycerol actually captured the largest share of the taxa with 85% of the genera and 33% of the species. Second most diverse was the Fresh soil with 62% of genera and 35% of species. This was followed closely by soil stored frozen with glycerol (54% of genera, 39% of species) and soil that was refrigerated (54% of genera, 22% of species). The least diverse bacterial communities were obtained from the dried soil which accounted for 38% of genera and 24% of the species.

Fungi: For fungi, in total we obtained 6 genera (Figure B.2.2B and B.2.6) and 23 species (Figures B.2.2B and B.2.7) from the soil storage experiment, of which we found literature support as pyrophilous for 3 genera (Figures 3.4B and 3.5B) and 20 species (Figure B.2.8). Fresh soil was the most diverse, capturing 100% of all 6 genera, whereas frozen and refrigerated soil both captured 50% of the genera, followed by 33% in dried and soil frozen with glycerol performing the worst, capturing only *Penicillium* (17% of the total) (Figure B.2.6). For species, fresh soil captured 52%, followed by 43% in frozen, and 30% in refrigerated and 26% in frozen with glycerol (Figure B.2.7). For the pyrophilous taxa, fresh, frozen, and refrigerated soil all captured 100% of the 3 fungal genera whereas dried captured 2 (*Aspergillus, Penicillium*) and frozen with glycerol on captured 1 (*Pencillium*; Figure 3.5B). For pyrophilous species, frozen soil captured 50%, followed closely by 45% in fresh, 35% in refrigerated and 30% in air-dried (Figure B.2.8).

Overall, for pyrophilous isolates, soil frozen without glycerol contained the most diverse bacterial and fungal populations. The worst storage method for pyrophilous bacterial diversity was air drying soil while freezing with glycerol was the worst storage method for pyrophilous fungal diversity.

3.4.5 Effect of Media Selection on Diversity

Bacteria: Choice of growth media strongly affected bacterial isolate diversity with R2A and LB capturing the highest numbers of bacterial genera and species. For bacteria, we obtained a total of 24 genera and 61 species across all media types (Figures B.2.2C, B.2.9 and B.2.10). Both LB and R2A obtained equally diverse communities, each capturing

58% of the total genera and 44% of the total species. Isolates obtained from PyOM were the least diverse, capturing 33% of genera and 18% of species. However, when we subset for isolates with support for being pyrophilous, the outcome is slightly different. For pyrophilous bacteria we obtained a total of 16 genera and 49 species (Figure 3.4C, 3.6A; B.2.11). Among pyrophilous bacteria, R2A captured the greatest diversity accounting for 69% of total genera and 49% of total species. Second most diverse was LB with 56% of total genera and 39% of total species. Lastly, PyOM obtained the lowest diversity of pyrophilous bacterial isolates with 44% of total genera and 20% of total species. Notably, while the PyOM media captured the least diverse bacterial community, the PyOM media was the only media to capture certain taxa of interest such as *Noviherbaspirillum* (Figure 3.6A) (Whitman et al. 2019; Caiafa et al. 2023; Pulido-Chavez et al. 2023).

Fungi: We obtained a total of 14 genera and 29 species across all media types (Figures B.2.2D, B.2.12, and B.2.13). R2A media yielded the most diverse fungi, capturing 57% of total genera and 48% of total species. Both MYA and PyOM equally yielded 43% of total genera and 41% of total species. Considering only pyrophilous fungi, we obtained 8 genera and 23 total species across all media types (Figure 3.4D, 3.6B; B.2.14). The most diverse isolate community was still isolated from R2A, with R2A capturing 89% of total pyrophilous genera and 57% of the species. Second most diverse among pyrophilous fungi was PyOM with 50% of total genera and 43% of total species. Among pyrophilous fungi MYA was the least diverse, capturing 38% of the total genera and 39% of the total species. Additionally, PyOM was the only media to capture *Neurospora*, a well-

documented post-fire fungus with relatively little knowledge about its ecological role (Figure 3.6B) (Kuo et al. 2014).

For both bacteria and fungi R2A captured the most diverse isolate population both among total isolates and pyrophilous isolates. Among pyrophilous isolates, PyOM media was the least diverse for bacteria but captured a very interesting and important pyrophilous organism and MYA was the least diverse for fungi.

3.5 Discussion

Throughout our experiments we managed to obtain a remarkable array of bacterial and fungal morphotypes (Figure 3.2). We found that contrary to our hypothesis, freezing soil was the best method of storing soil to retain the viability of culturable bacteria and fungi (Figures 3.4 & 3.5). Additionally, we found that the best media for culturing pyrophilous microbes was the oligotrophic R2A media (Figures 3.4 & 3.6). We did find that the novel solid agar media we created from field-harvested PyOM did contribute significantly to the diversity of the fungal isolates and was the only media to capture certain pyrophilous bacterial and fungal taxa of interest. The efforts in these experiments have informed our continual culturing efforts and resulted in a collection of over 500 post-fire microbial isolates (Supplementary File 3.1).

Here we conclude that from both a CFU and diversity-based perspective, freezing cells at -80°C is the most effective soil storage method for retaining 85% of the culturable pyrophilous bacterial genera (Figures 3.3A and 3.4A). It is possible that there could have been a slight amount of heterogeneity in the microbial communities between soil samples

(Jung and Kang 2014), however we believe this to be fairly unlikely since all experiments derived from a single, well-mixed, sieved soil subsample in order to reduce the effect of heterogeneity on the results. While CFU based analyses are useful in giving us an approximation of the total number of culturable cells present in the original sample and a broad overview of the effectiveness of certain culturing practices at cultivating cells, it does not capture the important distinction of diversity on the isolates obtained. This method captured the vast majority of the bacterial genera that we were interested in examining further based on post-fire sequencing studies that reported significant changes in post-fire bacterial diversity (Enright et al. 2022; Pulido-Chavez et al. 2023; Bárcenas-Moreno et al. 2011; Caiafa et al. 2023; Brown et al. 2019). We were surprised by this result as the post-fire soils were very dry to begin with, hence our hypothesis that drying would have the least impact on microbial viability. One previous study found that freezing at -20ºC was very detrimental to sandy, dry soils bacterial communities and instead found that refrigeration at 4ºC was better at retaining microbial biomass and respiration (Černohlávková et al. 2009). While we did not freeze soils at -20ºC and only froze samples at -80ºC, we did not observe the same detrimental effect of freezing on our soil bacterial communities, despite the El Dorado Fire soils being dry and sandy as well. Conversely, Rubin et al found that freezing at -20ºC preserved bacterial community structure (as assayed using 16S sequencing profiles) the best though they do note that their study only covered soils of one type from a single climatic region (Rubin et al. 2013). It is possible that the post-fire soils that we collected contain a high concentration of various solutes released from burning that could have buffered the microbial

communities from the worst effects of freezing. It is also worth noting that the bacterial genus *Massilia* was only obtained from soil that was preserved by freezing at -80°C with glycerol. This genus has been repeatedly observed post-fire with very little understanding about its ecology (Pulido-Chavez et al. 2023; Caiafa et al. 2023; Whitman et al. 2019; 2022), making it a prime candidate for further study. Therefore, it may be of value for researchers to freeze some soil with glycerol as well as without, to capture some of these more potentially vulnerable taxa.

Soil storage method seemed to matter less for pyrophilous fungi than bacterial genera. While there was one method that stood out as the worst (freezing at -80°C with glycerol), the rest of the methods captured most of the diversity observed (Figures 3.4B and 3.5B). This could be due to the resilience of fungal spores to a variety of disturbance effects (Falconer, Otten, and White 2015), but it is also very possible that MYA was not the ideal culture medium for pyrophilous fungi, given the success of R2A and PyOM media at cultivating pyrophilous fungi (Figures 3.4D and 3.6B). Future work repeating the storage experiments utilizing R2A and PyOM might improve pyrophilous fungal isolate retention.

Even with the media selection caveat to our storage experiments, there was some very surprising conclusions we were able to draw. We were both interested and surprised to find that our hypothesis that air-drying soil would be the most effective storage method was fully unsupported by our results. As previously noted, earlier studies on soil storage with the intent of preserving microbial communities can be contradictory and thus identifying the best soil storage method can be both difficult and potentially specific to

each soil type (Černohlávková et al. 2009; Rubin et al. 2013). In fact, both in terms of CFU data and diversity, air-dried soil commonly performed as one of the worst methods of storing soil for retaining culturable viability of both pyrophilous fungi and bacteria (Figures 3.3, 3.4, and 3.5). This finding is important as it is very common for soil samples to be dried before laboratory use (Rubin et al. 2013; Castaño et al. 2016; Cui et al. 2014) and if those experiments are to include culture work then researchers could be significantly reducing their obtainable species by not culturing from fresh soil or by storing soil samples frozen at -80°C. It is worth noting that we were unable to find studies exploring the impact of soil storage on fungal communities and as such may indicate a strong need for these methods to be tested across kingdoms.

In assessing the most effective culturing media for capturing pyrophilous bacteria and fungi, we were surprised at how well the oligotrophic media R2A performed. While the rich medias (LB and MYA) produced the most CFUs for both bacteria and fungi, R2A media was the second-highest producer of CFUs for both groups (Figure 3.3). Considering that R2A was developed to culture bacteria from potable drinking water (Reasoner and Geldreich 1985), this would suggest that a large number of post-fire microbes prefer a more oligotrophic environment. This was further supported by the diversity of pyrophilous genera obtained by these experiments, with R2A consistently producing the most diverse pyrophilous bacterial (Figure 3.4C) and fungal (Figure 3.4D) communities. While shifting life-strategies of microbial communities under stress can be extremely varied and nuanced (Ho, Di Lonardo, and Bodelier 2017), under an increasing stress gradient (such as carbon stress, soil moisture stress, or heat stress) oligotrophic

bacteria and fungi can be favored (Hernandez et al. 2021). It may be therefore that the low-nutrient environment of R2A media may actually be ideal for capturing a wide diversity of the heavily-stressed post-fire environment. This is a surprising result as post-fire environments tend to be high in nitrogen and phosphorus, even if the carbon landscape has radically changed (Wan, Hui, and Luo 2001; Michelotti and Miesel 2015). While previous studies exist examining the role of culture media conditions on microbial culturability (Vieira and Nahas 2005; Sánchez-Clemente et al. 2018; Malosso et al. 2006; Stevenson et al. 2004), these studies often take either a fine-scale approach to tuning a specific culture medium or focus on very broad-scale patterns of culturability such as relying on CFU-based metrics alone or utilizing only rich media. Continued exploration of media conditions is essential to increasing the number of cultured microbial species and insights gained from studies like this one will help open new paths in culture media development. It is worth noting that while our home-made PyOM media only captured 50% of pyrophilous fungi (Figure 3.4D) and 44% of bacteria (Figure 3.4C), it was also the only media to successfully isolate key pyrophilous taxa of interest both bacteria (i.e. *Noviherbaspirillum soli*) and fungi (*Neurospora crassa*) (Figure 3.6). These taxa are particularly interesting because like *Massilia*, *Noviherbaspirillum* has been repeated observed increasing in abundance post-fire (Enright et al. 2022; Pulido-Chavez et al. 2023; Caiafa et al. 2023) despite very little understanding of its ecology, and *Neurospora crassa* is famously found post-fire and is widely used as a genetics model organism despite very little understanding about its ecological role in the environment (Kuo et al. 2014). Therefore, we conclude that the best media to use to maximize pyrophilous

bacterial and fungal diversity if you can only select one is R2A, but best to also include a PyOM media if specifically interested in isolating the most finicky and perhaps interesting pyrophilous microbes.

While this study has made significant observations regarding effective culturing practices for pyrophilous bacteria and fungi, we would be remiss not to acknowledge both some of the limitations of this study and the additional work still before us. Except for the PyOM media, we limited ourselves to commonly available laboratory culturing media. We acknowledge there is a near endless range of possible medias to use (Vieira and Nahas 2005) and there may be more effective media available for the culturing of pyrophilous microorganisms. However, we assert that the broad trends (rich media vs. oligotrophic media vs. PyOM) hold valuable insight into the culturing of these microorganisms. As for the PyOM media itself, it is a complex undefined media and uses a water-extraction based method of incorporating nutrients from PyOM. Recent work has identified that there are some nutrients that are more water-soluble in PyOM than others (Zeba et al. 2024) so it is possible that our PyOM media does lack some nutrients that could be playing a role in post-fire metabolic requirements and us such capturing a more limited sampling of the microbial community. Additionally, the culturing effort in this study all took place aerobically at room temperature and as such we once again are capturing a narrower diversity of organisms than exist within our system. Further, future work could endeavor to test a wider variety of culturing and incubation factors including testing a range of growth temperatures (Bárcenas-Moreno and Bååth 2009; Vieira and

Nahas 2005) and media pH (Davis, Joseph, and Janssen 2005; Sánchez-Clemente et al. 2018).

Finally, while we acknowledge that separating bacterial and fungal cultures based on colony morphology is difficult at best (Ben-Jacob, Cohen, and Gutnick 1998; Sousa et al. 2013; Watrud et al. 2006), we decided that choosing which colonies to isolate based on morphotype (and when there was any shred of doubt between two cultures, isolating both) and then backing up identification with Sanger sequencing was an acceptable concession in the face of the technical and logistic demands of isolating, stocking, and identifying every single colony we were able to grow. This does mean that we could have missed some species-level diversity since some species can share extremely similar colony morphologies (Sousa et al. 2013; Watrud et al. 2006), but as we captured 28 bacterial genera and 14 fungal genera across our experiments we still assert that the isolates cultivated contribute substantially to the diversity of available culturable species and that insights derived from the diversity of our isolates across treatments are still meaningful. This is especially poignant as this is the first large-scale culturing effort of post-fire bacteria we were able to identify, and one of the largest non-mushroom fungal culturing efforts of pyrophilous fungi since Seaver began classifying pyrophilous species in 1910 (Seaver and Clark 1910). Future culturing efforts could leverage techniques like dilution to extinction culturing (Collado et al. 2007; Yang, Kang, and Cho 2016) to capture a broader species-level number of identified isolates.

3.6 Conclusions

Our experiments found that from high-severity burn scars in a chaparral ecosystem that storing soil samples at -80°C without glycerol preserved the greatest diversity of culturable pyrophilous bacteria and fungi. We found that certain taxa of interest were only captured from soil frozen with glycerol, indicating a potential value in storing some soil in that method as well. We found that the best laboratory media for culturing pyrophilous bacteria and fungi was the oligotrophic media R2A, and the newly created solid agar media created from field-harvested PyOM also captured a diverse community of isolates including some taxa of interest that were only captured on PyOM. The methods we employed captured many groups of interest from previous pyrophilous microbial studies and serve as an additional examination of prudent laboratory practices when considering future culturing efforts from soil.

Finally, the isolates discussed in this study are one portion of a much larger culturing effort of pyrophilous bacteria and fungi that leveraged 7 different wildfires, culturing from soil, smoke, and fungal fruiting bodies, and contains over 500 isolates of bacteria and fungi (Supplemental File 3.1). By testing and improving culture methods and undergoing large-scale culturing efforts, we can significantly increase the diversity of microbial cultures available for multi-omics, phenotypic, and applied microbiological research. For example, a culture collection is necessary to perform biophysical assays and genomics to assess traits that can yield insights into ecological processes and trade-offs (Maynard et al. 2019). Sequencing genomes from isolates with concurrent biophysical assays is a powerful yet under-used tool to gain insight into microbial traits and connect

genomic information to trait expression (Chase et al. 2017). Obtaining the cultures is the first step to characterizing their traits via ‘omics and phenotypic methods.

3.7 Acknowledgements

We thank the San Bernardino National Forest for allowing us to collect the soils needed for this study following the El Dorado wildfire. We thank many individuals who contributed to the Glassman lab pyrophilous microbial culture including lab managers Judy Chung, James Randolph, and Maria Ordonez, collaborators Tom Bruns and Monika Fischer, UCR PhD students Fabiola Pulido-Chavez, Arik Joukhajian, and Mark Yacoub, and UCR undergraduates Wine-Jie Lipardo, Audrey Reichard, Priscilla Shultz, Jorge Ponce, and Nathan Heger. This project was funded by the Department of Energy BER Award #DE-SC0023127 and United States Department of Agriculture-NIFA Award #2022-67014-36675 to SIG and the NSF GRFP to DJE. We also thank Dylan Enright’s Dissertation Committee members Jason Stajich and Marko Spasojevic and anonymous reviewers for their helpful feedback on the manuscript.

3.8 Competing Interests

The authors declare no conflicts of interest or competing financial interests in relation to the work described.

3.9 Data Accessibility and Benefit-Sharing:

Sequences will be accessioned and available at the National Center for Biotechnology at Information Sequence Read Archive at time of publication. All scripts used to analyze the data will likewise be available on GitHub.

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3.11 Figures:

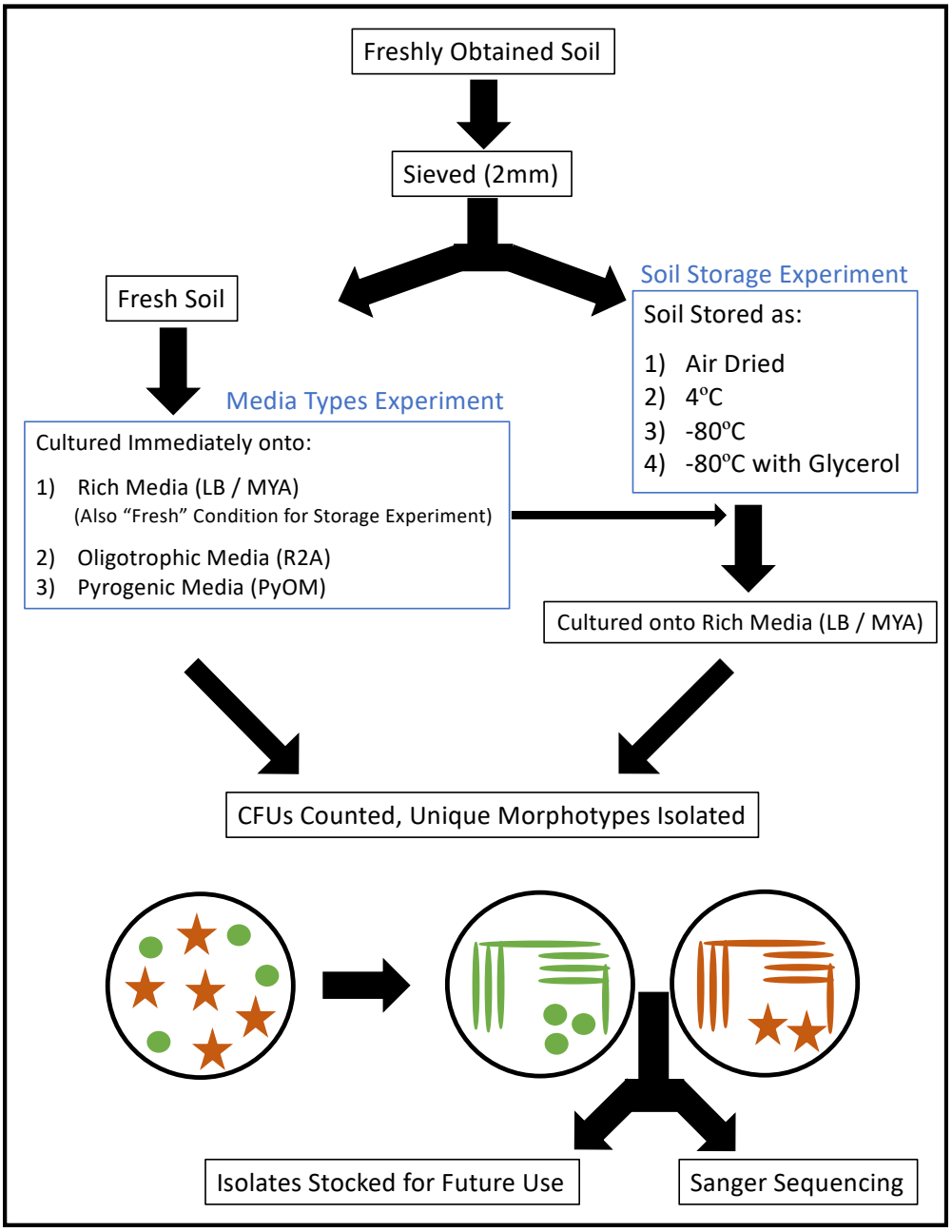

Figure 3.1: A conceptual diagram of the experimental design used in this experiment. "LB" is abbreviated for Lysogeny Broth, "MYA" is Malt Yeast Agar, "R2A" is Reasoner's 2 Agar, and "PyOM" is Pyrogenic Organic Matter. Note that the cultures obtained from fresh soil plated onto rich media also serve as the "Fresh" storage condition for the soil storage experiment. Green circles and orange stars symbolize two different morphotypes collected from the same initial plate.

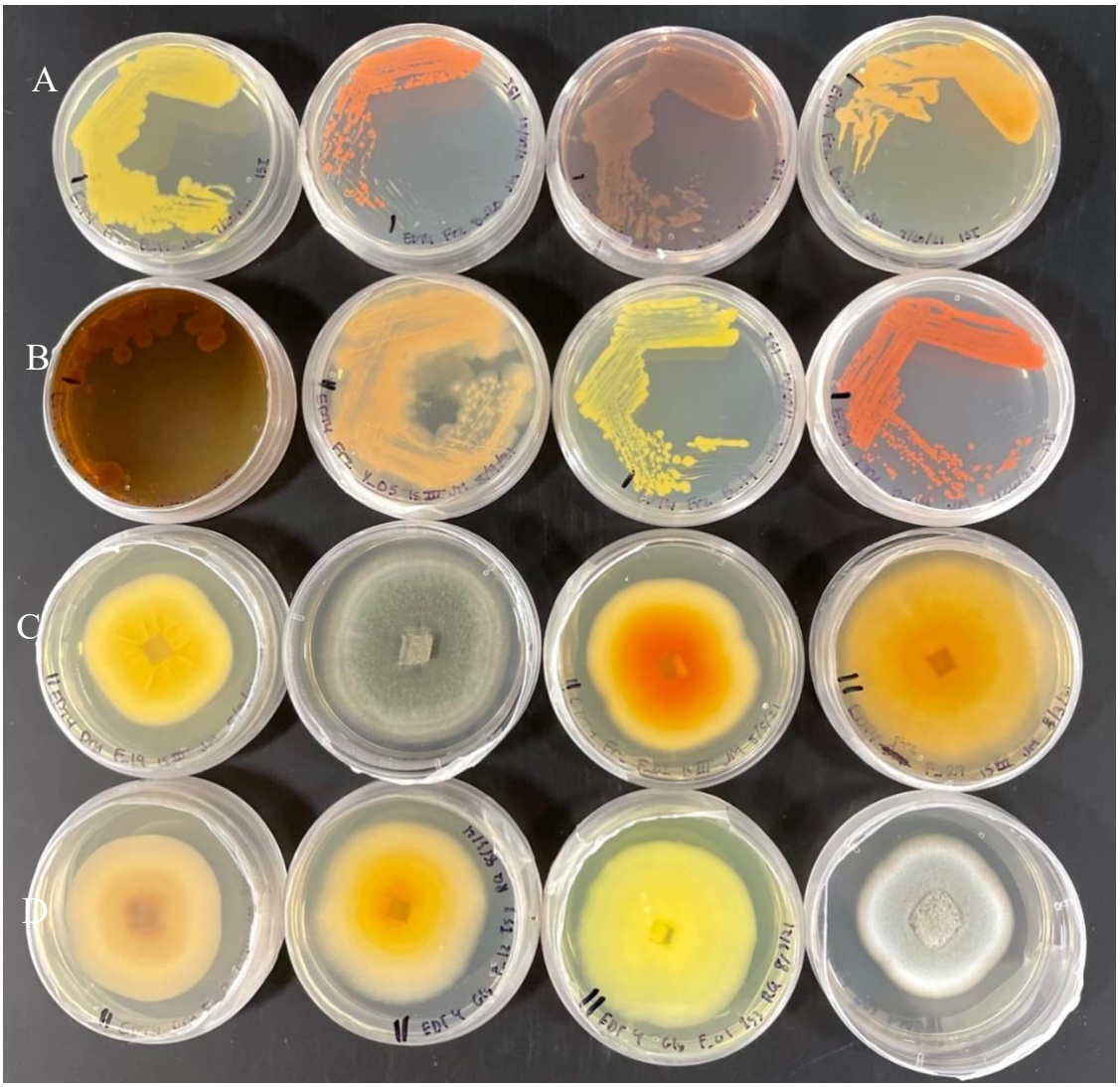

Figure 3.2: A snapshot of the morphological diversity of the cultures obtained from the El Dorado Fire across all soil storage and media type experiments. Identities were obtained via Sanger sequencing of the 16S or ITS regions for bacteria and fungi, respectively. Moving left to right across each row, the isolates shown are A)*Glutamicibacter bergerei, Arthrobacter agilis, Streptomyces mauvecolor, Kocuria rosea,* B) *Arthrobacter bussei, Holtermaniella festucosa, Pedobacter panaciterrae, Curtobacterium oceanosedimentum,* C) *Penicillium radiatolobatum, Penicillium fagi, Penicillium glabrum, Penicillium thomii,* D) *Penicillium chalabudae, Penicillium radiatolobatum, Penicillium murcianum, Penicillium adametzii.*

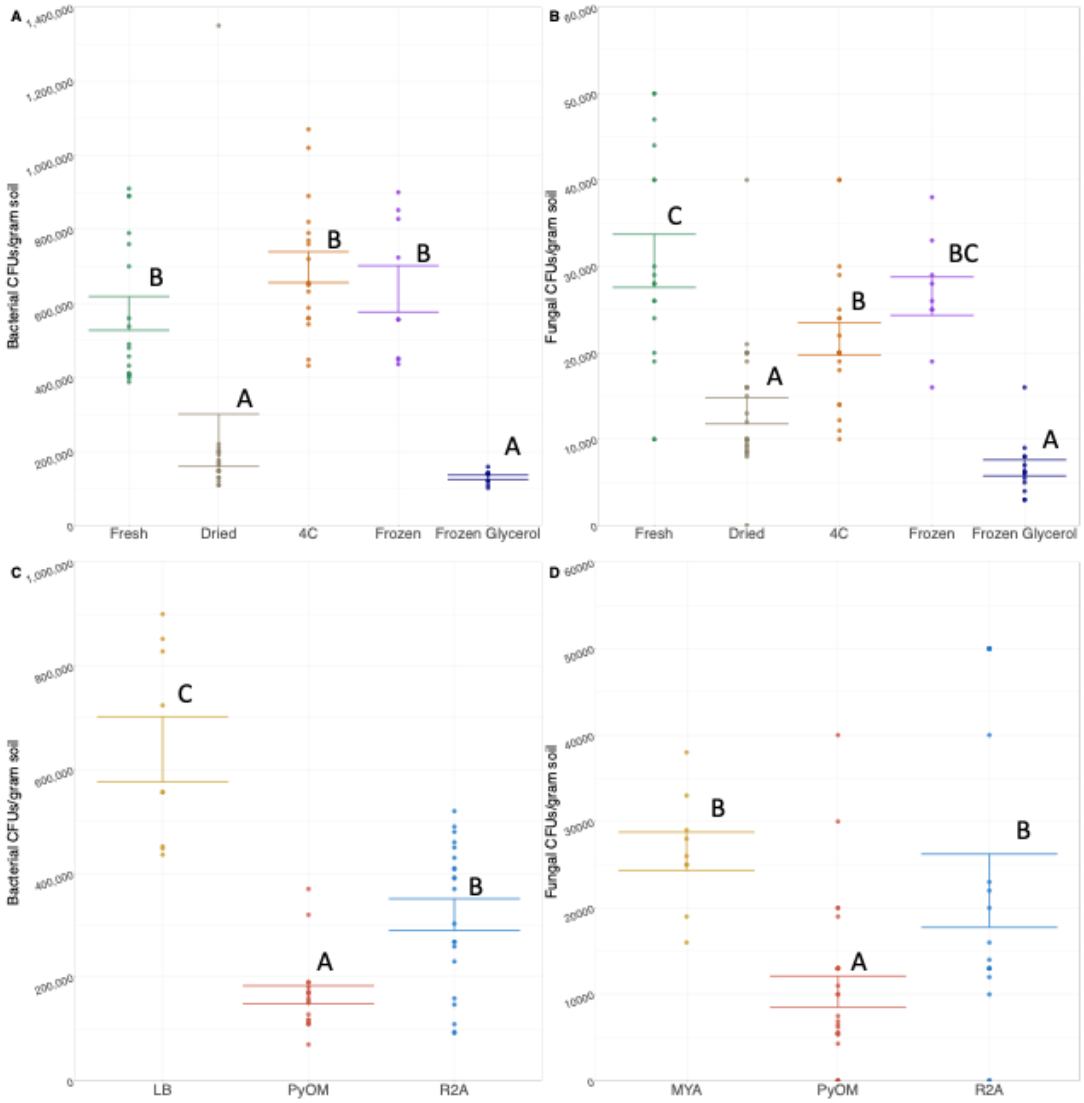

Figure 3.3: Calculated capturable CFUs per gram soil for bacteria among the soil storage experiment (A) and the media type experiment (C) and fungal CFUs per gram soil in the soil storage experiment (B) and the media type experiment (D). Significance was tested using ANOVA and Tukey Post-Hoc lettering has been applied. Points and bars are colored by treatment.

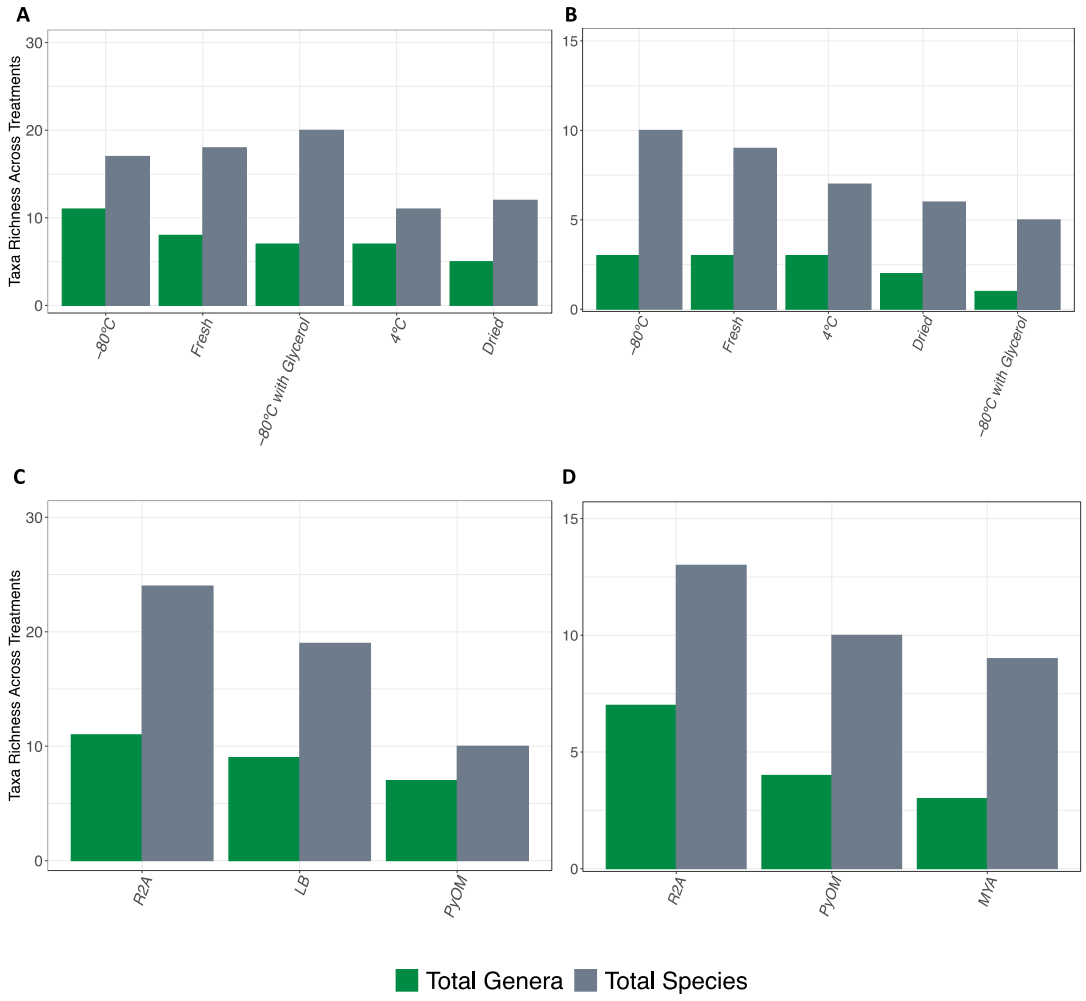

Figure 3.4: Summarized counts of pyrophilous bacterial and fungal genera (green) and species (grey) obtained from the soil storage experiment (A, Bacteria & B, Fungi) and from the media types experiments (C, Bacteria & D, Fungi). Treatments are ordered by highest amount of genera first, then species count second.

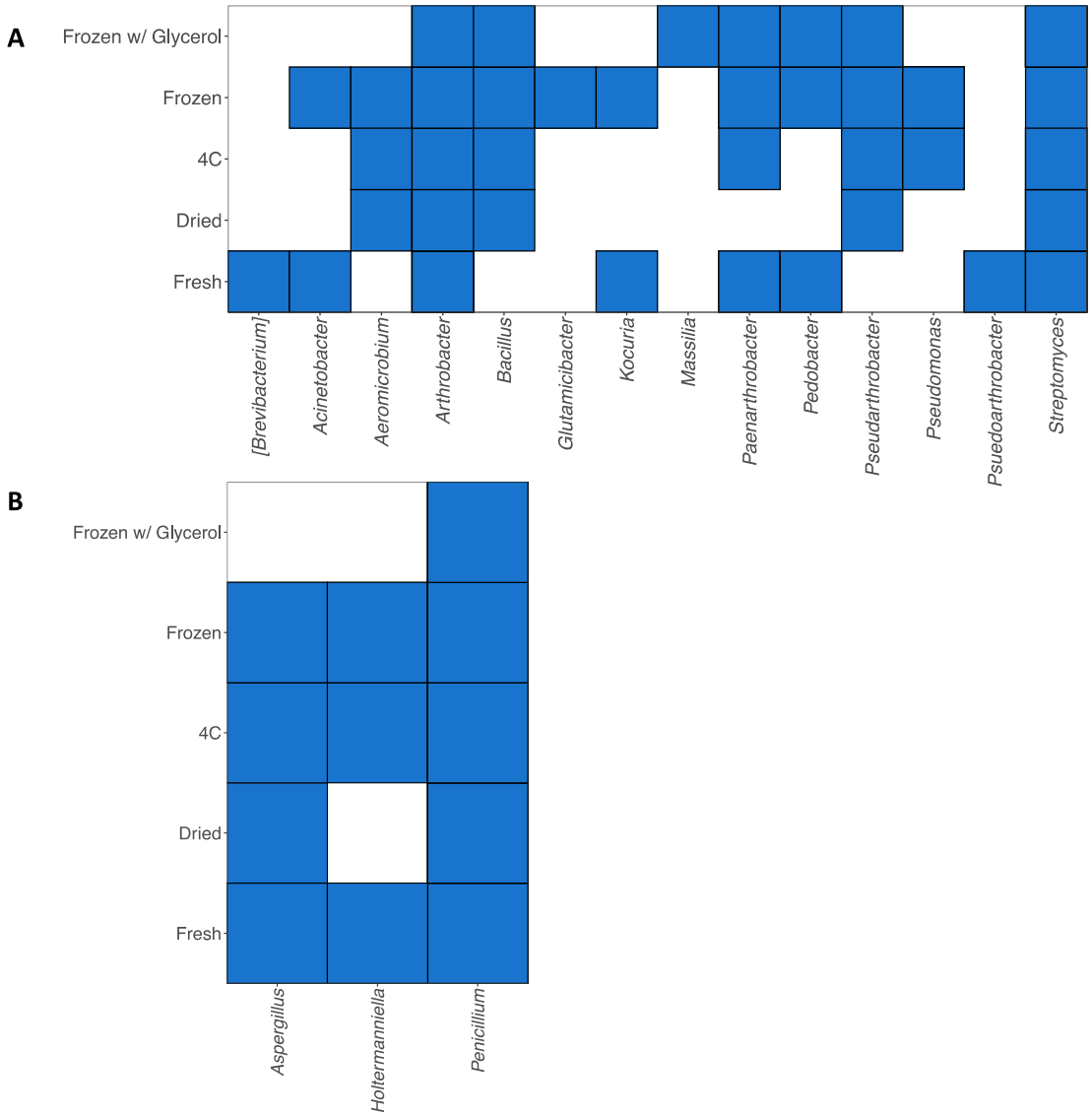

Figure 3.5: Heatmap-style visualization showing the diversity of pyrophilous bacterial (A) and fungal (B) genera across the soil storage experiment. A filled blue square indicates the presence of the genus on the x axis in the soil storage method indicated on the y axis.

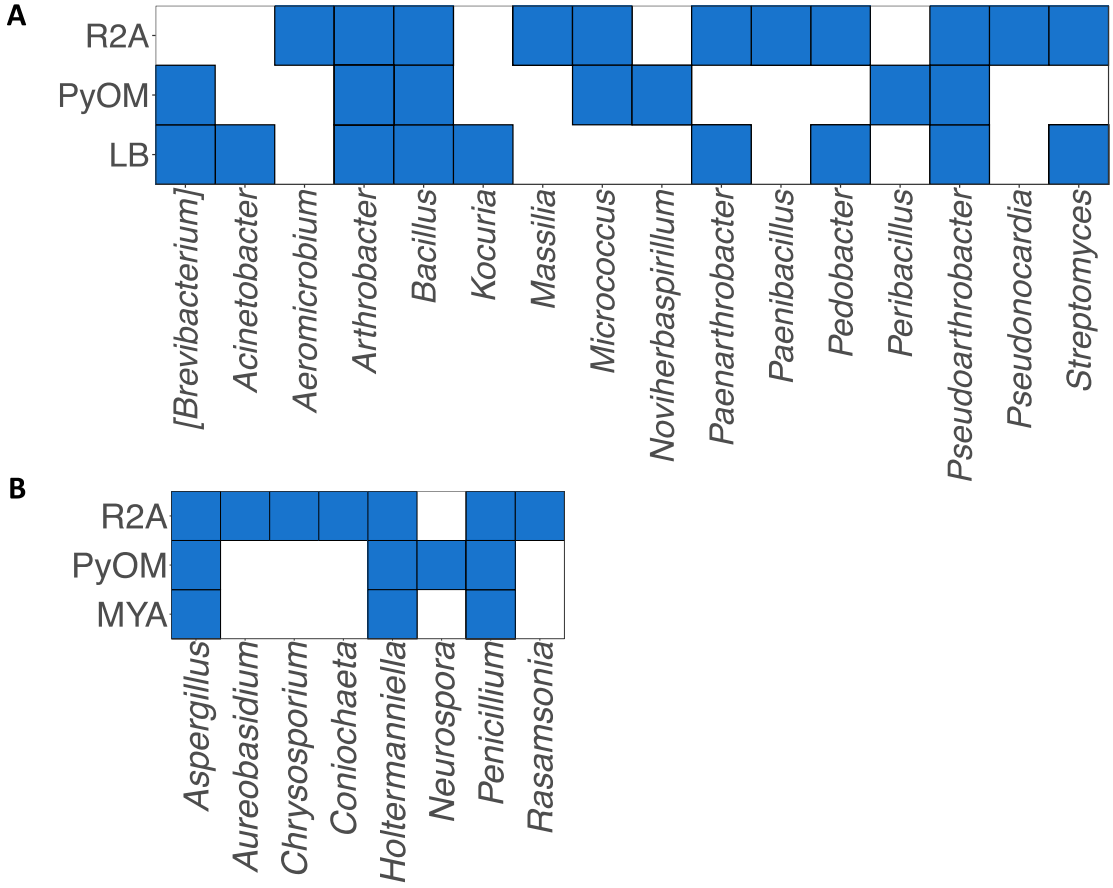

Figure 3.6: Heatmap-style visualization showing the diversity of pyrophilous bacterial (A) and fungal (B) genera across the media types experiment. A filled blue square indicates the presence of the genus on the x axis in the media indicated on the y axis.

Chapter IV

Unraveling the Traits that Allow Fungi to Survive Wildfires

4.1 Abstract

Fire is an important disturbance that helps maintain the biodiversity and stability of many natural ecosystems. However, due to a combination of development, wildfire suppression, and climate change, fires are increasing in frequency and severity across the globe. While severe wildfires can reduce fungal richness by up to 70%, several "fire-loving" or pyrophilous taxa greatly increase in abundance post-fire. However, the traits that enable pyrophilous taxa to thrive post-fire are largely unknown. We developed a framework based on plant ecological theory suggesting that pyrophilous microbes survive fires by either capitalizing on post-fire resources, thermotolerance, or fast colonization in absence of competitors. We selected 20 species of pyrophilous fungi that we had cultured from mushrooms and soils after local wildfires representing 6 classes, 8 orders, and 15

genera across the Ascomycota and Basidiomycota. We tested pyrophilous traits via biophysical assays including maximum survivable temperature, hyphal extension rate and hyphal density on malt-yeast agar (MYA) vs. pyrolyzed organic matter (PyOM) media, and nutrient cycling via greenhouse gas emissions and enzymatics. Overall, members of the Eurotiales favored stress-tolerant and nutrient acquisitive lifestyles possessing high thermotolerance (up to 50ºC), high hyphal density (up to 5.28mg/cm^2), and strong inorganic nitrogen cycling via fungal denitrification (nitrous oxide production up to 13.23ppm N_2O/day) but as a trade-off had slow hyphal extension rates (average less than 2.2mm/day). In contrast, members of the Pezizales favored a fast-growth lifestyle with fast hyphal extension rates (up to an average of 24.8mm/day) and utilized labile carbon and organic nitrogen sources at the expense of thermotolerance (highest 40ºC) or inorganic nitrogen cycling. Trait-space modeling supported these trends as distinct hotspots of trait accumulation probability.

4.2 Introduction

Fungi are key drivers of vegetation recovery, carbon, and nitrogen cycling (Crowther et al. 2019; Heijden, Bardgett, and Straalen 2008). Severe wildfires can dramatically reduce fungal biomass, species richness, and diversity (Dooley and Treseder 2012; Pressler, Moore, and Cotrufo 2019). Fires alter fungal community composition, in some cases removing whole guilds such as mycorrhizal fungi (Enright et al. 2022; Pulido-Chavez et al. 2021; Buscardo et al. 2010; Caiafa et al. 2023), which are critical to plant reestablishment (Brundrett and Tedersoo 2018).

Additionally, fire-driven changes in soil N chemistry caused both by burning and the addition of pyrogenic organic matter (PyOM), coupled with altered microbial activity, may substantially impact N loss via soil greenhouse gas (GHG) emissions (Yang et al. 2020), with critical implications for long-term ecosystem recovery, regional air quality, and Earth's climate (Smithwick et al. 2005).

Although fires can reduce fungal biomass and richness for decades (Bárcenas-Moreno et al. 2011; Pérez-Valera et al. 2020), pyrophilous (or "fire-loving") thrive post-fire (Fox et al 2022). Pyrophilous fungi are absent or low abundance pre-fire but dramatically increase in abundance post-fire as fruiting bodies or high sequence abundance (Seaver and Clark 1910; Petersen 1970; Glassman et al. 2016; El-Abyad and Webster 1968; Fox et al. 2022). Through observational studies we have discovered patterns such that species of the Ascomycota family Pyronemataceae within the Pezizales, genera *Aspergillus* and *Penicillium* within the Eurotiales, and some Basidiomycota yeasts *Holtermaniella* and *Basidioascus* and mushrooms such as *Lyophyllum* and *Pholiota* tend to dominate post-fire (Enright et al. 2022; Pulido-Chavez et al. 2021; Caiafa et al. 2023; Brown et al. 2019; Pérez-Valera et al. 2020; Bárcenas-Moreno et al. 2011). A recent study in a high severity chaparral wildfire demonstrated distinct trade-offs in sequence based abundance of pyrophilous fungal taxa (Pulido-Chavez et al. 2023) such that taxa that we expected might be capable of post-fire resource acquisition increased in abundance toward the end of the first-year post-fire as genes for degrading pyrogenic organic matter and cycling inorganic nitrogen increased (Pulido-

Chavez et al. 2024). Therefore, it is likely that changes in abundance of pyrophilous microbes and their traits drive important ecosystem functions post-fire.

Trait based approaches have already transformed community ecology into a more quantitative and predictive science (Lavorel and Garnier 2002; McGill et al. 2006), and are rapidly advancing microbial ecology (Malik et al. 2020; J. B. H. Martiny et al. 2015). Plant ecologists have long identified traits that determine plant responses to fire, including physiological and ecological adaptations like thermotolerant structures, thick bark, seed banking, and fast colonization (Keeley et al. 2011; Pausas et al. 2004). Our understanding of analogous traits in microbes is more limited, so we have adapted Grime's Competitor-Stress tolerator-Ruderal (C-S-R) plant trait-based framework (J. P. Grime 1977) or soil microbiomes. Grime's C-S-R posits that competitors (C) maximize resource capture, stress tolerators (S) prevail under stressful conditions, and ruderals (R) are fast growers. Building upon previous microbial adaptations of Grime's C-S-R (Malik et al. 2020; Chagnon et al. 2013; Whitman et al. 2019), we hypothesize that pyrophilous microbes possess analogous traits including 1) exploitation of post-fire resources (Grime's C), 2) resistance to heat stress (Grime's S), and 3) fast growth or colonization (Grime's R) (Enright et al. 2022). Importantly, due to energetic costs or evolutionary constraints, it is unlikely that one organism can express the traits for all strategies at once, and, therefore, microbes *trade off* the ability to perform one function versus another. While it is possible CSR triangle is a simplistic starting place (Treseder 2023) since we know that plants exist in n-dimensional trait space (Reich 2014; Díaz et al. 2016;

Laughlin 2014; Carmona, Pavanetto, and Puglielli 2024), this is nonetheless a good foundation upon which to build future understandings of pyrophilous microbial traits.

Using cultures to perform biophysical assays has advanced our understanding of fungal ecology and the ecosystem functions they impact. For example, using assays wood decomposing fungi have demonstrated trade-offs between hyphal extension rates and hyphal density (Lustenhouwer et al. 2020). Additionally, wood decomposing fungi have exhibited a wide range of competitive traits ranging from chemical antagonism to peaceful cohabitation of the same substrates (Boddy 2001; Boddy and Hiscox 2016; Lustenhouwer et al. 2020). Enough work has been done with wood decay fungi that researchers have even been able to synthesize the traits obtained across many studies into a framework of energy economics spectrum which categorize these fungi along a "slow" to "fast" continuum (Camenzind et al. 2024). A few studies have tested the traits of a small subset of pyrophilous fungi and found that the pyrophilous fungus *Pyronema* has the potential to degrade PyOM (Fischer et al 2021) and genomic studies have demonstrated that pyrophilous basidiomycetes possess an increased abundance of genes utilized in PyOM degredation and sporulation relative to closely related non-pyrophilous relatives. It is therefore likely that trade-offs exist amongst the traits of pyrophilous fungi that enable them to survive post-fire in a manner predictive of ecosystem impacts on C and N cycling.

Here, we leveraged our unique culture collection of pyrophilous fungi (Enright et al. 2024) to test for the first time if pyrophilous fungi fit into our proposed C-S-R trade-off conceptual model (Enright et al. 2022). We selected 20 fungal isolates representing 6

classes and 15 genera across the Ascomycota and Basidiomycota that were common after wildfires and that evidence from recent wildfires suggested likely represented our hypothesized trait suites (Pulido-Chavez et al. 2023; Caiafa et al. 2023; Enright et al. 2022). We used biophysical assays to test for post-fire resource acquisition (Grime's C) via extracellular enzyme assays and greenhouse gas emissions, thermotolerance (Grime's S) with maximum survivable temperature, and fast growth (Grime's R) with hyphal extension rate and hyphal density on nutrient rich versus pyrogenic organic matter (PyOM) derived media. Our findings suggest strong trade-offs exist amongst fast growth (Grime's R) versus post-fire resource acquisition and thermotolerance (C-S).

4.3 Methods

4.3.1 Isolates Used in this Study

20 fungal isolates were selected from a combination of the Glassman Lab pyrophilous fungal culture collection (Enright et al. 2024) and the Bruns Lab fungal culture collection. The fungi selected either had direct literature support describing them as pyrophilous, had demonstrated repeated observation post-fire, or had a combination of laboratory and field experiments (ex. soil heating, metabolomics, prescribed fire, etc.) that, in addition to being cultured from burned soil, merited their inclusion within the pyrophilous fungal definition. These fungi include isolates from 2 phyla, 6 classes, 8 orders and 15 genera and include both hyphal and yeast form fungi. A full listing of all isolates, their culture collection origin, and their literature support for inclusion within the

study can be found in Table 4.1. One fungus (*Peziza echinospora*) was omitted from thermotolerance, N_2O, and enzyme activity assays due to culturing issues.

4.3.2 Media Prep

Two medias were utilized throughout this study. For each liter of Malt-Yeast Agar (MYA) and broth 5g of powdered Malt Extract (Research Products International, Genessee, CAS# 8002-48-0) and 5g of powdered Yeast Extract (Sigma-Aldrich, Cat # 70161) was used. For solid agar media, 15g per liter of bacteriological grade agar (Apex Chemicals, Cat # 20-273) was added. For Pyrogenic Matter Media (PyOM) we utilized biochar harvested from the El Dorado Fire burn sites and processed it into liquid media as previously described in Chapter 2. For each liter of solid PyOM media, 13g of ultrapure noble agar (Thermo Scientific, CAS # 9002-18-0) was added. Both medias, after mixing with DI water to the previously stated volumes, were autoclaved at 121ºC for 45 minutes.

4.3.3 Hyphal Extension and Hyphal Density Assays

Preparation of Combined Assay Plates: Three 100mm replicate plates of each media (either MYA or PyOM) per isolate were used in tracking hyphal extension and density. Each plate was aseptically covered with autoclaved cellophane (Thermo Scientific, Cat # CM-20) prior to fungal inoculation (Lustenhouwer et al. 2020). Each plate was also marked with a 5mm straight line to use as a guide for hyphal extension measurements. For each plate, $5mm^2$ radius plugs of fungal tissue were inoculated into the center of each plate using autoclaved transfer tubes (Spectrum Scientific, Cat # 08-801-25).

Hyphal Extension Rate Assays: Plates were incubated at room temperature for 3 weeks. The growing edge of the hyphae was recorded every 24hrs using alternating-colored markers. At the conclusion of the recording period, plates were photographed, and images were imported into ImageJ where daily extension rates were quantified in millimeters per day.

Hyphal Density Assays: At the conclusion of the Hyphal Extension Rate Assay incubation, a 4mm x 4mm square of hyphae-covered cellophane was excised from the plate using an ethanol and flame-sterilized scalpel. Fungal tissue was then removed from the cellophane and placed into pre-weighed sterile 1.7mL centrifuge tubes. Tubes were then placed open into a dehydrator (NESCO, Cat# FD-1010) and desiccated at 60ºC for 48hrs (Lustenhouwer et al. 2020). Tubes were then closed and weighed again, and hyphal density was calculated as mg/cm^2 using the final net weights.

4.3.4 Yeast Growth Assays

Yeast cells were inoculated into 5mL MYA or PyOM broth cultures and placed into a shaking incubator and left to incubate at 25ºC for 24hrs. Once overnights were grown and well-mixed, 1mL of culture was removed and placed into a sterile 1.7mL centrifuge tube. Cells were pelleted by centrifugation at 12,000g force. Pellets were then washed 3x with sterile saline before resuspension into fresh broth. This served as the inoculant for the experiment. Triplicate replicates were made for each isolate. Then, a 48-

well clear well plate (Greiner Bio-One, Cat# 677102) was prepared by treating the lid with a 1% Triton X-100 solution. Outer wells of the plate were filled with sterile water and sample wells were filled with 490µL of liquid media (either MYA broth or PyOM). Then, 10µL cleaned inoculate was added to each sample well and the plate was covered and placed into a Tecan Infinite M Plex plate reader (Tecan, Mannedorf, Switzerland) set for 25ºC incubation with orbital shaking for 72hrs. Absorbance readings were taken at 600nm wavelength every 60 minutes. Growth was plotted and generation time was taken from at least 10 points along the exponential portion of the growth curve (Bergman 2001). Generation time was calculated using the equations below, beginning with calculating the number of generations and then the generation time afterwards.

Number of Generations:

$n = 3.3 (\log N - \log N_0)$

where:

N = Final cell concentration (or optical density value).

N_0 = Initial cell concentration (or optical density value).

n = number of generations.

Once the number of generations has been determined, the generation time was calculated by using:

$g = t / n$

where:

g = generation time.

t = time.

n = number of generations.

4.3.5 Thermotolerance Assays

Hyphal isolates: Isolates were inoculated into 45mL of MYA broth in a 50mL sterile Falcon Tube in triplicate. Tubes were then incubated for 1 week at 25°C in a shaking incubator set to 180rpm. After incubation, tissue was macerated and aliquoted into sterile 1.7mL centrifuge tubes. Tubes were then heated to 25°C, 30°C, 40°C, 50°C, 75°C and 100°C for 1 hour. After heating tissue was aseptically transferred to sterile 60mm MYA plates. Plates were then incubated for 1 week in the dark at room temperature. After 1-week, hyphal survivorship at each temperature was evaluated.

Yeast Isolates: Isolates were inoculated in triplicate into 5mL of MYA broth and allowed to grow for 24hrs in a 25°C shaking incubator. After incubation, 1mL of overnight culture per replicate was aliquoted into sterile 1.7mL centrifuge tubes. These tubes were then heated as described above. After heating, 10µL of each liquid culture was spotted onto 100mm MYA plates. Plates were incubated as described above and survivorship was assessed.

4.3.6 N_2O Emissions Assays

A defined broth containing per liter 1% glucose, 0.2% peptone, 0.02% $MgSO_4$ * 7 H_2O, 2 parts per million (ppm) $CaCl_2$ * 6 H_2O, 2ppm $FeSO_4$ * 7 H_2O, 0.01 moles KH_2PO_4, 10 millimoles of $NaNO_3$, and 5 millimoles of $NaNO_2$ was used for gas

emissions analysis to minimize abiotic N_2O production (Rohe et al. 2014; adapted from Shoun et al. 1992). For each replicate, 25mL of the defined broth was added to a 100mL narrow-necked bottle. After broth was added, a $5mm^2$ diameter plug of fungal tissue was aseptically added to each bottle and then the bottle was crimp sealed with a 20mm Agilent crimp cap with a rubber septum (Agilent Technologies, part no. 5183-447). For yeast form fungi, instead of a plug of hyphae a loop-full of cells were transferred aseptically to each bottle using sterile disposable loops. Triplicate replicates were prepared for each isolate. Cultures were grown in a shaking incubator at 25ºC for 3 days, after which the bottles were removed and the air within the bottle was replaced with dinitrogen (N_2) gas by flushing the bottles continuously for 40 minutes at a flow rate of 300 mL/min using a manifold fitted with sterile 0.2µm filters (Whatman Puradisc 25, part no. 6780-2502) to prevent contamination. Liquid media was swirled after 20 minutes to release dissolved oxygen and generate micro-anaerobic conditions conducive to denitrification (Zhou et al. 2001). The bottles were then incubated under N_2 headspace at room temp for an additional 5 days in order to allow N_2O to accumulate. Because nitrous oxide reductase (Nos) has not been found in fungi (Aldossari and Ishii 2021), we assumed there was little to no reduction of N_2O over the incubation period. After the secondary incubation, 20mL of headspace was removed with a gas-tight syringe (Hamilton, part no. 86326) and transferred to 20mL pre-evacuated glass vials (Agilent Technologies, part no. 5182-0837) fitted with 20mm gas-tight septa (Agilent Technologies, part no. 5183-447). Gas samples were measured within 72hrs on a GC-MS (Agilent Technologies; Headspace Sampler 7697A connected to a gas chromatograph GC

System 7890B) and N_2O concentrations were referenced to a standard curve (0.2-500 ppm N_2O) and normalized for abiotic N_2O production by subtracting a blank (containing sterile broth only).

4.3.7 Enzymatic Activity Assays

Hyphal fungi were grown on MYA solid media until sufficient hyphal area was produced in order to obtain 4 5mm^2 plugs of tissue could be obtained per replicate. Once plugs were obtained, the tissue was lysed and crude enzyme was extracted in a manner similar to previous fungal enzymatic work from pure culture isolates (Maynard et al. 2019; Baldrian et al. 2011; Lustenhouwer et al. 2020) . Briefly, tissue was placed into 3mL of 50mM acetate buffer, and the sonicated at 60hZ for 30s on ice using 6 second bursts. Then the enzymes were allowed to extract at 4°C for four hours under constant agitation. For yeast-form fungi, 1mL of a 5mL of 48hr liquid culture was used per replicate. These cells were pelleted by centrifugation, then washed 3 times with cold 50mM acetate buffer. Once washed, cells were sonicated and enzyme extracted as previously described. Once extracted, the lysate was filtered through a Whatman filter paper (Whatman Qualitative, Cat# 1001-070). The resultant crude enzyme extract was then used for assaying of β-Glucosidase (BG) and N-Acetylglutamate synthase (NAG) activity levels using methylumbelliferone (MUF)-bound substrates for each respective enzyme. For each replicate, 100µL of crude enzyme was mixed with 100µL of MUF-linked substrate in a COSTAR flat black 96-well plate (Corning Inc., Ref # 3603). After mixing, the plate was placed in a Tecan plate reader pre-heated to 40°C and read at an

excitation wavelength of 355nm and an emission wavelength of 450nm at 5-minute intervals for 125 minutes. Control wells of MUF-substrate and buffer were used to calculate background fluorescence and was subtracted off from sample well measurements. A standard curve of 4-methylumbelliferone salt in molecular-grade water was used to calculate the nanomoles of fluorescently-bound substrate cleaved per minute and activity was quantified using peak enzyme activity for each enzyme.

4.3.8 Data Analysis

All statistical analyses and figures were produced in R 4.0.2 (R Core Team 2020) and all scripts are available on GitHub. To evaluate whether there was statistical non-independence of samples due to relatedness of species within our group, Bloomberg's K was calculated for all trait datasets using the "MultiPhyloSignal" command from Picante. For this test a tree of all species within our group was created using prior obtained genomes and conservation of BUSCO genes. All trait datasets were found to not have statistically significant phylogenetic signal other than the N_2O emissions data. To calculate statistical differences among taxa within each assay, ANOVA was employed followed by a post-hoc Tukey honest significant difference (HSD) test. In the case of the N_2O emissions data, a phylogenetic ANOVA was employed using the "phylANOVA" command in Phytools in order to account for the significant phylogenetic signal found in that dataset, and post-hoc Tukey HSD lettering was calculated at the order level. To visualize the differences within the N_2O emissions for each taxa without the significantly

higher emissions by *Neurospora discreta* skewing the plotting, a y axis break was introduced using the "ggbreak" package in R (Xu et al. 2021). To visualize the distribution of traits driving post-fire adaptation, the Funspace package in R was used (Carmona, Pavanetto, and Puglielli 2024). Briefly, data tables of the trait measurements and phylogenetic information were constructed for the Basidiomycetes, Ascomycetes, and yeast-form fungi. Trait measurements were normalized using Tukey's ladders of power transformations for all measurements, except for those which resulted in infinite values following transformation. In the case of those traits, a cube-root transformation was found to be the best normalization which did not result in an infinite value. The data tables were loaded into Funspace to compute trait probability models based on axis derived from iterative PCAs. Significant dimensions were tested using the FunspaceDIM function and then the Funspace calculations were grouped phylogenetically by family. Once the Funspace objects were fully calculated and grouped, they were plotted both at the full global object level and plotted again at the family level, as much of the prior research on pyrophilous fungi occur within specific families of fungi such as the Pyronemataceae (Fox et al. 2022). Significant peaks of trait space probability distribution were bounded within the 0.5 and 0.25 regions of the plot.

4.4 Results

4.4.1 Hyphal Extension Rates

Hyphal extension rates varied significantly among species grown on MYA ($F_{1,16} = 51.52$, $p < 0.001$), among species grown on PyOM ($F_{1,16} = 38.90$, $p < 0.001$), and when

compared between the two medias ($F_{1,166} = 211.34$, $p < 0.001$). Extension rates on MYA varied wildly, ranging from 0.167 mm/day to 30.92 mm/ day, with a median extension rate of 2.675 mm/day (Figure 4.1A). Isolates separated out statistically into 5 statistically different groupings (Figure C.1.1). The groupings with the highest rates of extension consisted of *Pyronema domesticum, Neurospora discreta, Pyronema omphalodes,* and *Morchella eximia* (Figure 4.1A). The two groupings that collectively aligned at or below the median extension rate consisted of *Anthracobia macrocystis, Penicillium restrictum, Peziza echinospora, Geopyxis carbonaria, Pholiota brunnescens, Aspergillus fumigatus, Tricharina praecox, Aspergillus galapagensis, Pholiota highlandensis, Penicillium chrysogenum, Rasamsonia columbensis,* and *Lyophyllum atratum* (Figure 4.1A). Extension rates on PyOM ranged from 0.569 mm/day to 26.97 mm/day, with a median rate of 3.197 mm/day (Figure 4.1A). The isolates separated out into 3 statistically significant groupings, with two groups being significantly faster than the remaining grouping (Figure C.1.2). Though the number of significantly different groupings changed, the isolates that grew fastest did not change regardless of media (Figure 4.1A).

4.4.2 Hyphal Density

Hyphal densities varied significantly among species grown on MYA ($F_{1, 15} = 12.9$, $p < 0.001$), among species grown on PyOM ($F_{1, 15} = 10.36$, $p < 0.001$), and to a lesser degree when compared between the two medias ($F_{1, 42} = 0.702$, $p < 0.05$). Hyphal densities on MYA varied widely, ranging from 0.1 mg/cm^2 to 6.15 mg/cm^2 with a median density of 0.8 mg/cm^2 (Figure 4.1B). Among hyphal densities of isolates grown on MYA,

only *Rasamsonia columbensis* was significantly higher than other isolates and *Pyronema omphalodes* was the lowest than other isolates (Figure C.1.3). Hyphal densities of isolates grown on PyOM varied much less, ranging from 0.025 mg/cm^2 to 0.35 mg/cm^2 with a median density of 0.175 mg/cm^2 (Figure 4.1B). Hyphal densities of isolates grown on PyOM separated out into more significantly different groupings, with *Lyophyllum atratum, Aspergillum fumigatus,* and *Pholiota highlandensis* being significantly higher than most isolates. By contrast *Rasamsonia columbensis*, *Penicillium chrysogenum, Pyronema omphalodes,* and *Tricharina praecox* had significantly less dense hyphae on PyOM than most isolates (Figure C.1.4). Extension rates of isolates grown on MYA negatively correlated with their MYA hyphal densities ($R = -0.61$, $p < 0.001$) (Figure C.1.5A). There was no correlation found among PyOM hyphal extension rates vs. PyOM hyphal densities (Figure C.1.5B).

4.4.3 Yeast Generation Time in MYA vs. PyOM

Generation times of yeast-form fungi differed significantly when grown in MYA ($F_{1,2} = 6.453$, $p < 0.01$). Among the three yeast-form isolates, *Coniochaeta hoffmanii* had the fastest generation time (average of 20.23 mins) while *Holtermaniella festucosa* had the longest generation time (average of 26.00 mins) (Figure C.1.6). *Basidioascus undulatus* was not significantly different from either of the other two isolates, having an average generation time of 23.63 minutes. No detectable growth was found for any of the three isolates in PyOM media.

4.4.4 Thermotolerance

Thermal maxima of the fungal isolates ranged from 30°C to 50°C (Figure 4.2A). Most isolates assayed (14/19 total) had a thermal maximum of 40°C. Only 4 isolates (*Aspergillus galapagensis, Neurospora discreta, Penicillium chrysogenum,* and *Rasamsonia columbensis*) exhibited a thermal maximum of 50°C. Only one isolate (*Geopyxis carbonaria*) had a thermal maximum of 30°C.

4.4.5 N_2O Emissions

Emission of N_2O was the only trait to exhibit a statistically significant phylogenetic signal within the measurements obtained (*Bloomberg's K*= 0.906, $p < 0.01$). As such differences among emissions could not be calculated at the species level, but rather were calculated at genus, family, and order level using a phylogenetic ANOVA. Emissions varied significantly among cultures at the family level ($F_{1613.54,\ 28.88} = 83.79$, $p = 0.01$) and more significantly at the order level ($F_{1601.03,\ 41.39} = 154.71$, $p < 0.001$), but were not significantly different at the genus level ($F_{1638.42,\ 4.00} = 148.80$, $p = 0.08$) though there was still a trend. Emissions ranged from no detectable emissions to 47.85 ppm/day (Figure 4.2B), with a median emission rate of 1.55 ppm/day. Other than the Sordariales which emitted the highest rate (average of 43.06 ppm/day) and far higher than other isolates, orders separated into two statistically distinct groupings with the Holtermanialles being higher than all the remaining orders (Figure 4.2B).

4.4.6 β-Glucosidase Expression

β-Glucosidase expression (BG) varied significantly among cultures ($F_{1,18}$ = 62.46, $p < 0.001$) ranging from 0.56 to 557.43 nM of substrate cleaved per minute with a median expression of 190.30 nM of substrate cleaved per minute (Figure 4.3A). The BG expression of almost every isolate was statistically distinct from each other (Figure 4.3A), though some isolates (*Coniochaeta hoffmannii, Rasamsonia columbensis, Aspergillus galapagensis, Penicillium restrictum, Anthracobia macrocystis, Tricharina praecox,* and *Morchella eximia*) showed much higher expression levels than the median activity level. Conversely, some isolates (*Holtermaniella festucosa, Pholiota brunnescens, Lyophyllum atratum, Pholiota highlandensis, Coprinellus radians,* and *Neurospora discreta*) exhibited much lower enzymatic activity than the median expression level.

4.4.7 N-Acetylglutamate Synthase Expression

NAG expression varied significantly among isolates ($F_{1,18}$ = 45.25, $p < 0.001$) ranging from below detectable limits to 489.68 nM of substrate cleaved per minute with a median expression of 39.96 nM of substrate cleaved per minute (Figure 4.3B). The NAG enzyme activity among isolates sorted into many statistically separate groupings that resolve into 2 tiers of expression (Figure 4.3B). The high activity tier included *Pyronema domesticum, Rasamsonia columbensis, Morchella eximia, Basidioascus undulatus,* and *Anthracobia macrocystis* with the rest of the isolates being of the lower tier of expression.

4.4.8 Trait Space Ordination

Three trait space ordinations were calculated using all hyphal Basidiomycota, all hyphal Ascomycota, and all yeast-form isolates as the groupings for each ordination. Basidiomycota trait space dimensional analysis found that the first dimension of the aggregated PCAs explained the most variation (52.4%). When plotted, three distinct peaks of trait space probability were bounded within 95% confidence intervals (Figure 4.4A). When the total model was visualized at the family level, each peak was found to correspond to each of the 3 families that the hyphal Basidiomycota fungi belonged to (Figure C.1.7).

Ascomycota trait space dimensional analysis found that the first two dimensions of the aggregated PCAs explained the most variation (36.17% and 28.63%, respectively). When plotted, two distinct peaks of trait space probability resolved within the 95% confidence intervals (Figure 4.4B). The first peak was correlated with hyphal extension rates on both MYA and PyOM, NAG enzyme expression, and hyphal density on PyOM. The second significant peak correlated with N_2O gas emissions, thermotolerance, and to a lesser extent MYA hyphal density and BG enzyme expression. When this model was visualized at the family level (Figure C.1.8), the Pyronemataceae fell strongly in the first peak with the Morchellaceae sharing some of the significant region to a lesser extent. The second peak was associated with the Aspergillaceae. The Sordariacaeae did not align well with either significant peak, but instead were correlated with hyphal extension rates on both medias and N_2O emissions.

Yeast form fungi followed a pattern much more similar to the Basidiomycota. Dimensional analysis found that only the first dimension of the aggregated PCAs explained the most variation (62.21%). Unlike the other two plots, the yeast plots have the thermotolerance and PyOM growth axes removed as all yeast fungi exhibited a thermal maximum of 40°C and all yeasts did not grow within detectable limits in the PyOM media. When plotted, three distinct peaks of trait space probability were bounded within 95% confidence intervals (Figure 4.4C). When separated into family-level plots, each peak corresponded to each of the 3 families represented within the yeast-form fungi (Figure C.1.9).

4.5 Discussion

In this study we were able to obtain trait measurements on a wide range of growth, nutrient acquisition, and stress tolerance traits on taxa that have never had these phenotypes measured in such a way before. While a significant phylogenetic signal was not present in almost every trait dataset, we found that in many of our assays, the divide between high performers and low performers within a given trait often followed phylogenetic patterns. While two species of the same genus often had slightly different trait expression patterns, overall expression among our pyrophilous isolates of a given trait was often conserved at the order level at least within members of the Ascomycota. For growth related metrics, we found that members of the Pezizales were more likely to be present in the "fast" group regardless of media, relative to members of the Eurotiales being more likely to be in the "slow" group (Figure 4.1A). This is surprising in many

ways as members of the Eurotiales, like *Aspergillus*, are often considered fast-growing molds (Meletiadis et al. 2001), but relative to other pyrophilous fungi would therefore be considered slow growing. Their relatively slower growth rate could be due to a preference for a more intensive nutrient acquisition strategy (Veresoglou et al. 2018) than the "fast growing" members of the Pezizales. Additionally, our data supports that pyrophilous members of the Eurotiales may be favoring more energetically expensive lifestyles than their Pezizales counterparts, as evidenced by a higher N_2O emissions rate (Figure 4.2B) and higher thermotolerance (Figure 4.2A) relative to the Pezizales fungi. This is also evidence of utilization of unique post-fire soil chemistry, as post-fire soils have been repeatedly observed to be very rich in inorganic nitrogen (Wan, Hui, and Luo 2001). Fungi may undergo denitrification as a means to acquire energy (Kobayashi et al. 1996; Aldossari and Ishii 2021), which could then be used for more energetically expensive traits such as the aforementioned thermotolerance. Our data fully supports the pyrophilous members of the Pezizales as being more fast-growing both in their increased hyphal extension rates (Figure 4.1A), and in their overall higher NAG enzyme activity compared to Eurotiales fungi (Figure 4.3B). It has been previously demonstrated that NAG activity in fungi functions to acquire organic nitrogen which results in biomass production (Sequeira et al. 2016), which in this case might support a proposed ecological function of pyrophilous fungi in the formation of soil aggregates (Filialuna and Cripps 2021). Several of our Pezizales pyrophilous fungi are the same species that were observed to improve soil aggregation post-fire (Filialuna and Cripps 2021).

This observed trade-off of pyrophilous Eurotiales being relatively more nutrient acquisitive and stress tolerant at the expense of being more fast-growing and the pyrophilous Pezizales fungi being relatively more nutrient acquisitive and fast-growing at the expense of stress tolerance traits holds true not only in many of the individual assays, but also in the overall trait space model as well (Figure 4.5). We found that among the Ascomycota trait space ordination, that two significant and distinct hotspots of trait probability emerged. The first hotspot is characterized by hyphal extension rates on either MYA or PyOM media, high NAG enzyme activity, and to a lesser extent high hyphal density on PyOM (Figure 4.4B). When visualized at the family level, we find that the Pyronemataceae matches this first hotspot very well (Figure C.1.8) and likely drives its formation as the Pyronemataceae capture the majority of the Pezizales fungi within our tested isolates. This finding is also corroborated with previous work studying a few of these species in culture (Dzhagan et al. 2023) and also previous work characterizing their genomes (Steindorff et al. 2020; 2022). The second hotspot is explained more by N_2O production, thermotolerance, and to a lesser extent BG enzyme activity and hyphal density on MYA (Figure 4.4B). When the ordination is broken down by family groupings the Aspergillaceae aligns almost perfectly with the second hotspot (Figure C.1.8), and similarly likely drives the hotspot's formation as the Aspergillaceae includes all of our Eurotiales fungi. Some members of the Eurotiales, especially *Aspergillus*, do have previous work showing capability for fungal denitrification (Aldossari and Ishii 2021; Shoun et al. 1992). Additionally, there is early work profiling some *Penicillium* species as being thermotolerant and having very diverse metabolic capabilities (Aranda et al.

2017; Leitão 2009; Seaver and Clark 1910). The genus *Rasamsonia*, which includes one of our most thermotolerant taxa and belongs to the Eurotiales, was originally described as being not only thermotolerant but also thermophilic (Houbraken, Spierenburg, and Frisvad 2012).

These observed trade-offs within the Ascomycota isolates allow us to make some conclusions regarding our initial hypothesis that most pyrophilous fungi would likely exist somewhere between a stress tolerant lifestyle and a nutrient acquisitive lifestyle with clear trade-offs for fast growth. The first trait hotspot that we identified, characterized by growth metrics and organic nitrogen acquisition (Figure 4.4B) somewhat contradicts our initial hypotheses of expected trait ordination. While the trait syndrome is still characterized by nutrient acquisition strategies, in the form of organic N acquisition and high hyphal density on PyOM, the hotspot leans more in a ruderal fast-growth lifestyle than hypothesized. The alignment of the Pezizales fungi to this hotspot allows us to place that group between a competitive and ruderal lifestyle in both Grime's original CSR framework (J. P. Grime 1977) and between the "fast-growth" and "post-fire nutrient acquisitive" trait syndromes in the proposed pyrophilous adaptation of Grime's triangle (Enright et al. 2022) (Figure 4.5), relative to other pyrophilous fungi. More aligned to our hypothesis, the hotspot of trait probability that is explained by thermotolerance, inorganic N acquisition, and dense hypha in MYA would be more aligned with competitive and stress tolerant trait syndromes in Grime's CSR triangle (J. Philip Grime 1988) and the close alignment of the Aspergillaceae would allow us to place the Eurotiales between a thermotolerant and post-fire nutrient acquisitive lifestyle in the

pyrophilous triangle (Enright et al. 2022), relative to the other pyrophilous fungi tested. These distinctions place themselves reasonably well into other recent trait frameworks with our Eurotiales placement aligning with Malik's YAS Stress Tolerant-Resource Limited (S-A) framework region (Malik et al. 2020), though the placement of the Pezizales once again would need to be placed in between the gradients of two categorized trait suits, the Resource Limited (A) and the Resource Abundant (Y) lifestyles which might appear contradictory to one another. Perhaps the better placements would instead be found in Camenzind's slow – fast fungal economic spectrum (Camenzind et al. 2024) where the Eurotiales would place closer to the "slow" end of the spectrum and the Pezizales would place closer to the "fast" end of the spectrum, though both would likely fall somewhere in the middle of the total spectrum due to both species having unique nutrient acquisition lifestyle components. Additionally, as these hypothesized placements are derived from comparisons within pyrophilous fungi, future tests will need to compare these against the non-pyrophilous fungi to determine if a given species is truly slow-growing or low-thermotolerant or is only slow-growing relative to the pyrophilous fungi. However, these comparisons are often hard to accomplish as data like that obtained here is very rare, and even among studies which have collected similar trait data the reported measurements of each trait are often not reported and instead only correlations between traits or some other internally consistent ratio is presented in the literature (Lustenhouwer et al. 2020; Maynard et al. 2019; Camenzind et al. 2024). It is my hope that by presenting the direct measurements of isolates within this study that future comparisons will be easier to achieve.

Unlike the two trait syndromes that emerged from the pyrophilous Ascomycota, the Basidiomycota and yeast-form fungi did not lend themselves to clear interpretable placements (Figure 4.4 A & C). In both cases, each model suggested 3 distinct hotspots, but when broken down to the family level each hotspot corresponded directly to each of the 3 families to which the Basidiomycota isolates or the yeast-isolates belonged to (Figures C.1.7 and C.1.9). This suggests a limitation within our modelling that is likely due to sample size. Of the 20 isolates we assayed, only 3 were yeast-form fungi and 4 were hyphal Basidomycetes (Table 4.1). This means the remaining 13 isolates were hyphal Ascomycetes which could explain why that model was more informative and why the other two models were less smooth and thus less informative (Figure 4.4). However, even with the less-smooth model some insights can be gleaned about the lifestyles exhibited within each trait hotspot. Among the Basidiomycetes, the Strophariaceae were strongly correlated with high expression of both assayed enzymes along with relatively high extension rates on PyOM (C.1.7). The Lyophyllaceae exhibited a much more relatively slow lifestyle, growing densely and producing N_2O while the Psathyrellaceae were characterized by relatively faster growth in rich media while still producing N_2O (C.1.7).

Within the yeast isolates assayed, each isolate appears to align almost directly to a single trait (C.1.9). *Holtermanniella festucosa*'s trait distribution correlated strongly with N_2O production, while *Coniochaeta hoffmannii* correlated strongly with BG enzyme activity. *Basidioascus undulatus* was explained by a combination of relatively fast-

growth in rich media and acquisition of organic nitrogen, which is similar to the more ruderal pyrophilous hyphal isolates assayed in this study.

The uneven distribution of taxa within the study is in part due to a reflection of the pyrophilous isolates that exist to work with likely due to the impacts of fire on post-fire fungal communities (Pressler, Moore, and Cotrufo 2019). It has been repeatedly observed that post-fire communities are largely dominated by Ascomycetes and that many dominant Basidiomycete groups are lost post-fire (Enright et al. 2022; Pulido-Chavez et al. 2023; Fox et al. 2022; Brown et al. 2019). Additionally, early work on pyrophilous fungi were done based on sporocarp assays (Seaver and Clark 1910; Petersen 1970) which have still been a measurement of pyrophilous fungal abundance even as recent as 2020 (Hughes et al. 2020). This means that for almost 100 years of post-fire fungal ecology, non-fruiting fungi such as yeasts were missed entirely and it is only due to recent community DNA sequencing work that we have been able to observe the blooming of pyrophilous yeasts post-fire (Enright et al. 2022; Pulido-Chavez et al. 2023; Caiafa et al. 2023). Therefore, in both the pyrophilous Basidiomycetes and yeasts researchers have a more limited pool of taxa to work with.

It is also important to acknowledge that while this work forms a foundation upon which to build our knowledge of pyrophilous traits, there are some limitations to these assays which might be missing some of the traits that these pyrophilous fungi express in natural settings. For example, it is well documented that *Pyronema* species in soil produce large amounts of sclerotia (Moore 1962), and that those sclerotia are very thermotolerant and are certainly one of the mechanisms by which these pyrophilous fungi

are adapted to wildfires (Bruns et al. 2020). Our assays can only measure hyphal thermotolerance as we did not force sclerotia production in culture and thus we may be missing a biologically relevant aspect of thermotolerance in pyrophilous fungi. Additionally, while we probed BG and NAG enzyme activity there are additional enzymes that are relevant that we did not assess such as Catechol and Protocatechuate monooxygenases which are involved in the breakdown of polycyclic aromatic hydrocarbons (PAHs) which are a major component of PyOM (Michelotti and Miesel 2015; Vergnoux et al. 2011; Reyes-César et al. 2014). It has been previously demonstrated through genomic and transcriptomic work that *Pyronema* species have the metabolic pathways necessary for the production of these enzymes and the potential breakdown of PyOM (Steindorff et al. 2020; Fischer et al. 2021). It is possible that future pyrophilous assay work that includes enzymes such as these could demonstrate a wider expression of these metabolic pathways among pyrophilous fungi and thus change the dimensions of the predicted trait space that these pyrophilous fungi would inhabit. Certainly, there is also evidence that members of the Eurotiales can also utilize PAHs metabolically (Reyes-César et al. 2014; Aranda et al. 2017; Leitão 2009), and including these fire-relevant enzymes could help bring more nuance to the trait space predictions we can therefore make. Overall, inclusion of more trait assays will improve the accuracy with which we can place any given pyrophilous fungal taxon and will help to establish parity with other trait assessments such as those done in wood-decay fungi (Camenzind et al. 2024; Lustenhouwer et al. 2020).

The insights which we gleaned from these assays of pyrophilous fungal traits should ultimately improve our predictive modelling for post-fire ecosystem dynamics and function. Though our observation of variation among traits within species of the same genus might mean that some hypothesized conservation of functions at the genus level may not hold true for pyrophilous fungi (Carini 2019), it does support previous statistical testing that response to fire is conserved at the order or class levels in fungi (Enright et al. 2022). This might make some predictive modelling regarding the traits an individual taxa might employ more difficult with pyrophilous fungi as opposed to what has been attempted in other soil microbes (Goberna and Verdú 2016; A. C. Martiny, Treseder, and Pusch 2013). However, even more the more coarse order-level pattern should improve our predictions of ecosystem function post-fire when a certain clade is present much like what has been done with the DEMENT model and drought-tolerant microbes(Allison and Goulden 2017). Predictive modelling of this nature will greatly expand our understanding of post-fire ecological interactions and post-fire ecosystem functions. This work alongside future studies which include additional trait assays and more species (especially among the Basidiomycota and yeast fungi) will bring light to a previously understudied group of fungi that are becoming more ecologically relevant with each passing year as global wildfires continue to persist and become more devastating.

4.6 Conclusion

In this study we assessed a wide range of traits including growth patterns on rich media vs. PyOM, thermotolerance, organic and inorganic nitrogen acquisition strategies,

and carbon acquisition enzyme activity and found a wide range of statistically diverse phenotypes among the 20 pyrophilous fungi assessed (Figure 4.1-4.3). Our trait space modeling found 2 distinct statistically significant hotspots of trait probability among the Ascomycota hyphal fungi (Figure 4.4B) that align with a post-fire nutrient acquisition and fast-growth lifestyle and a post-fire nutrient and stress tolerant lifestyle which the Pezizales and Eurotiales fungi within the study exemplify, respectively (Figure 4.5). This work represents a meaningful contribution to fungal trait work as a field and is the first study of its kind to engage in live culture trait characterization of pyrophilous fungi. This work sheds light on some of the ecological functions these fungi may engage in within the post-fire environment and will help to improve both our understanding of pyrophilous fungal ecological interactions, and predictive modeling of post-fire ecosystem function.

4.7Acknowledgements

I would like to thank Monika Fisher for providing isolates from the Bruns lab pyrophilous fungal isolate collection. Additionally, we would like to thank undergraduate students and trainees of the Glassman and Holmyak labs including Renee Rincon, Yareli Olazabal, and Omar Valencia that have assisted with the laboratory preparations necessary for us to collect this data. We would also like to thank Dr. Kathy Borkovich for guidance on working with *Neurospora*, and Dr. Ehsan Sari for guidance derived from his genomic work on these isolates including the creation of phylogenetic trees. I would also like to thank Dr. Daniel Moen for assistance with phylogenetic ANOVA analyses of the nitrous oxide emission data. Finally, we would like to thank Dylan Enright's dissertation

committee advisors Jason Stajich and Marko Spasojevic for advice on the writing of this work and for guidance with statistical analyses including implementation of the Funspace R package.

3.8 Competing Interests

The authors declare no conflicts of interest or competing financial interests in relation to the work described.

3.9 Data Accessibility and Benefit-Sharing:

All scripts used to analyze the data will be available on GitHub at time of publication.

4.10 References

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4.11 Tables

Table 4.1: Table of all isolates used in this study including the culture collected they originated from, phylogenetic information for the organism, and 3-5 references as support for inclusion in this study. Attempts were made to include both classic and recent references.

Isolate Name	Culture Origin	Phylum	Class	Order	Family	References
Pholiota brunnescens	Glassman Culture Collection	Basidiomycota	Agaricomycetes	Agaricales	Strophariacaeae	(Caiafa et al. 2023) (Enright et al. 2024) (Raudabaugh et al. 2020) (Fox et al. 2022) (Petersen 1970)
Pholiota highlandensis	Bruns Collection (CBS KNAW 144467)	Basidiomycota	Agaricomycetes	Agaricales	Strophariacaeae	(Steindorff et al. 2020) (Caiafa et al. 2023) (Enright et al. 2024) (Raudabaugh et al. 2020) (Fox et al. 2022)
Penicillium restrictum	Glassman Culture Collection	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	(Aranda et al. 2017)(Seaver and Clark 1910) (Enright et al. 2022) (Enright et al. 2024) (Caiafa et al. 2023)
Penicillium chrysogenum	Glassman Culture Collection	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	(Enright et al. 2022) (Seaver and Clark 1910) (Petersen 1970) (Brown et al. 2013)(Fox et al. 2022)
Aspergillus fumigatus	Glassman Culture Collection	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	(Pulido-Chavez et al. 2023) (Caiafa et al. 2023) (Fox et al. 2022) (Seaver and Clark 1910) (Enright et al. 2024)
Aspergillus galapagensis	Glassman Culture Collection	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	(Pulido-Chavez et al. 2023) (Caiafa et al. 2023) (Fox et al. 2022) (Seaver and Clark 1910) (Enright et al. 2024)
Rasamsonia columbensis	Glassman Culture Collection	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	(Caiafa et al. 2023) (Enright et al. 2024) (Pulido-Chavez et al. 2023) (Houbraken, Spierenburg, and Frisvad 2012) (Witfeld, Begerow, and Guerreiro 2021)
Peziza echinospora	Bruns Collection (CBS KNAW 144458)	Ascomycota	Pezizomycetes	Pezizales	Pezizaceae	(Petersen 1970) (Warcup and Baker 1963)(Enright et al. 2022) (Pulido-Chavez et al. 2023) (Caiafa et al. 2023)
Geopyxis carbonaria	Bruns Collection (CBS KNAW 144460)	Ascomycota	Pezizomycetes	Pezizales	Pyronemataceae	(Petersen 1970) (Warcup and Baker 1963) (Seaver and Clark 1910) (Steindorff et al. 2020) (Fox et al. 2022)

Isolate Name	Culture Origin	Phylum	Class	Order	Family	References
Pyronema domesticum	Glassman Culture Collection	Ascomycota	Pezizomycetes	Pezizales	Pyronemataceae	(Seaver and Clark 1910) (Seaver 1909) (Petersen 1970) (Warcup and Baker 1963) (Fischer et al. 2021)
Pyronema omphalodes	Bruns Collection (CBS KNAW 144459)	Ascomycota	Pezizomycetes	Pezizales	Pyronemataceae	(Seaver and Clark 1910) (Seaver 1909) (Petersen 1970) (Warcup and Baker 1963) (Fischer et al. 2021)
Tricharina praecox	Bruns Collection (CBS KNAW 144465)	Ascomycota	Pezizomycetes	Pezizales	Pyronemataceae	(Petersen 1970) (Steindorff et al. 2020) (Fox et al. 2022) (Enright et al. 2022) (Steindorff et al. 2022)
Coprinellus radians	Bruns Collection (CBS KNAW 144469)	Basidiomycota	Agaricomycetes	Agaricales	Psathyrellaceae	(Caiafa et al. 2023) (Pulido-Chavez et al. 2023) (Fox et al. 2022) (Petersen 1970) (Hughes et al. 2020)
Lyophyllum atratum	Bruns Collection (CBS KNAW 144462)	Basidiomycota	Agaricomycetes	Agaricales	Lyophyllaceae	(Petersen 1970) (Enright et al. 2022) (Pulido-Chavez et al. 2023) (Caiafa et al. 2023) (Steindorff et al. 2020)
Morchella eximia	Bruns Collection (CBS KNAW 144461)	Ascomycota	Pezizomycetes	Pezizales	Morchellaceae	(Petersen 1970) (Warcup and Baker 1963) (Steindorff et al. 2020) (Fox et al. 2022) (Pulido-Chavez et al. 2023)
Neurospora discreta	Glassman Culture Collection	Ascomycota	Sordariomycetes	Sordariales	Sordariaceae	(Fox et al. 2022) (Enright et al. 2024) (Perkins 1992) (Gladieux et al. 2015) (Powell et al. 2003)
Anthracobia macrocystis	Bruns Collection (CBS KNAW 144468)	Ascomycota	Pezizomycetes	Pezizales	Pyronemataceae	(Petersen 1970) (Warcup and Baker 1963) (Enright et al. 2022) (Caiafa et al. 2023) (Fox et al. 2022)
Holtermaniella festucosa	Glassman Culture Collection	Basidiomycota	Tremellomycetes	Holtermaniales	Holtermaniales*	(Caiafa et al. 2023) (Pulido-Chavez et al. 2023) (Enright et al. 2024)
Basidioascus undulatus	Canadian DAOMC (Strain 241956)	Basidiomycota	Wallemiomycetes	Geminibasidiales	Geminibasidiaceae	(Nguyen, Nickerson, and Seifert 2013)(Enright et al. 2022) (Pulido-Chavez et al. 2023) (Caiafa et al. 2023)
Coniochaeta hoffmannii	Glassman Culture Collection	Ascomycota	Sordariomycetes	Coniochaetales	Coniochaetaceae	(Caiafa et al. 2023) (Pulido-Chavez et al. 2023) (Enright et al. 2024) (Wicklow 1975) (Mahoney and LaFavre 1981)

4.12 Figures

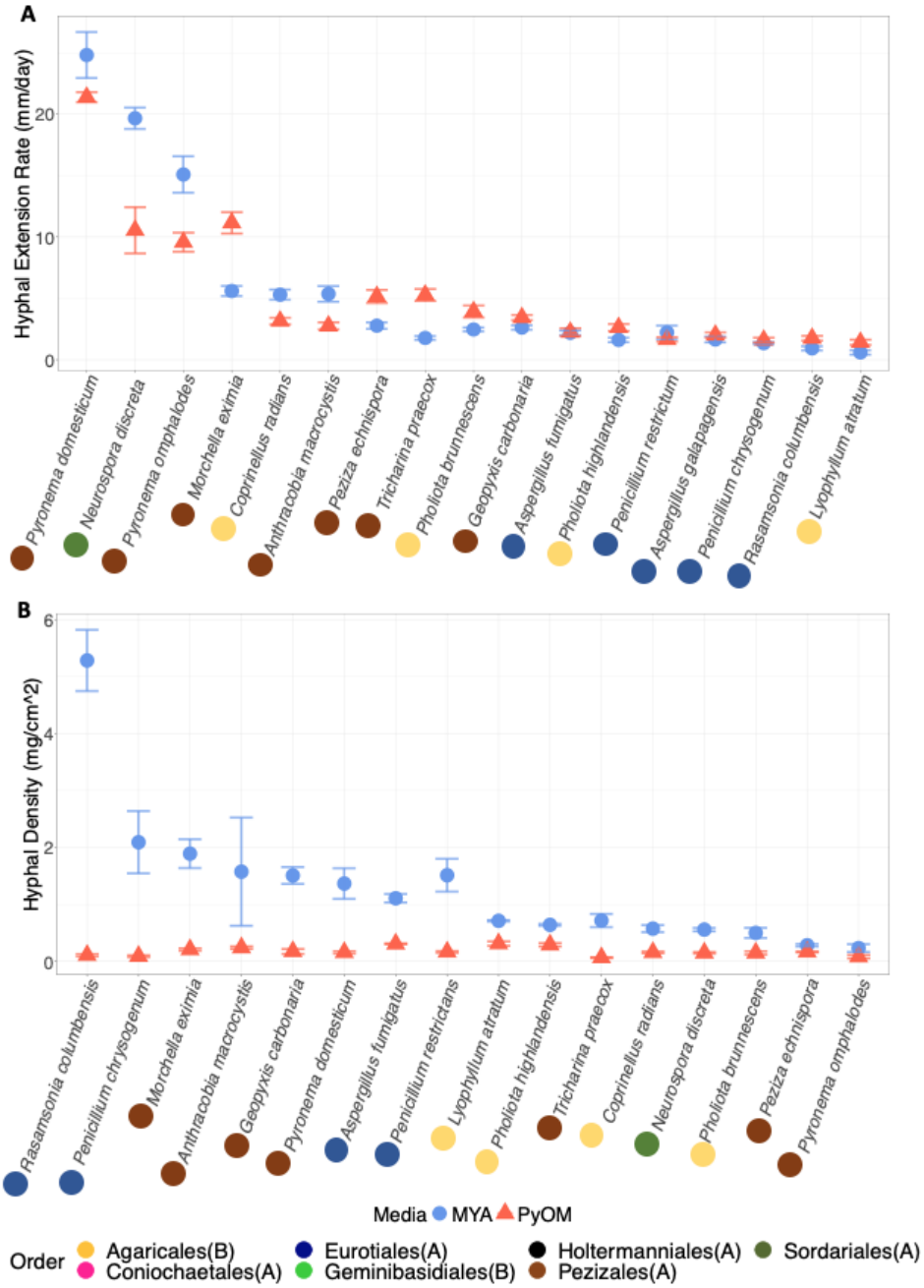

Figure 4.1 A) Hyphal extension rates of all hyphal isolates on MYA vs. PyOM media in mm/day. Points are colored and shaped by media utilized, and colored circles are assigned to each isolate based on the isolate's corresponding phylogenetic order. B) Hyphal densities of all hyphal isolates on MYA vs. PyOM media in mg/cm^2. Points are colored and shaped by media utilized, and colored circles are assigned to each isolate based on the isolate's corresponding phylogenetic order.

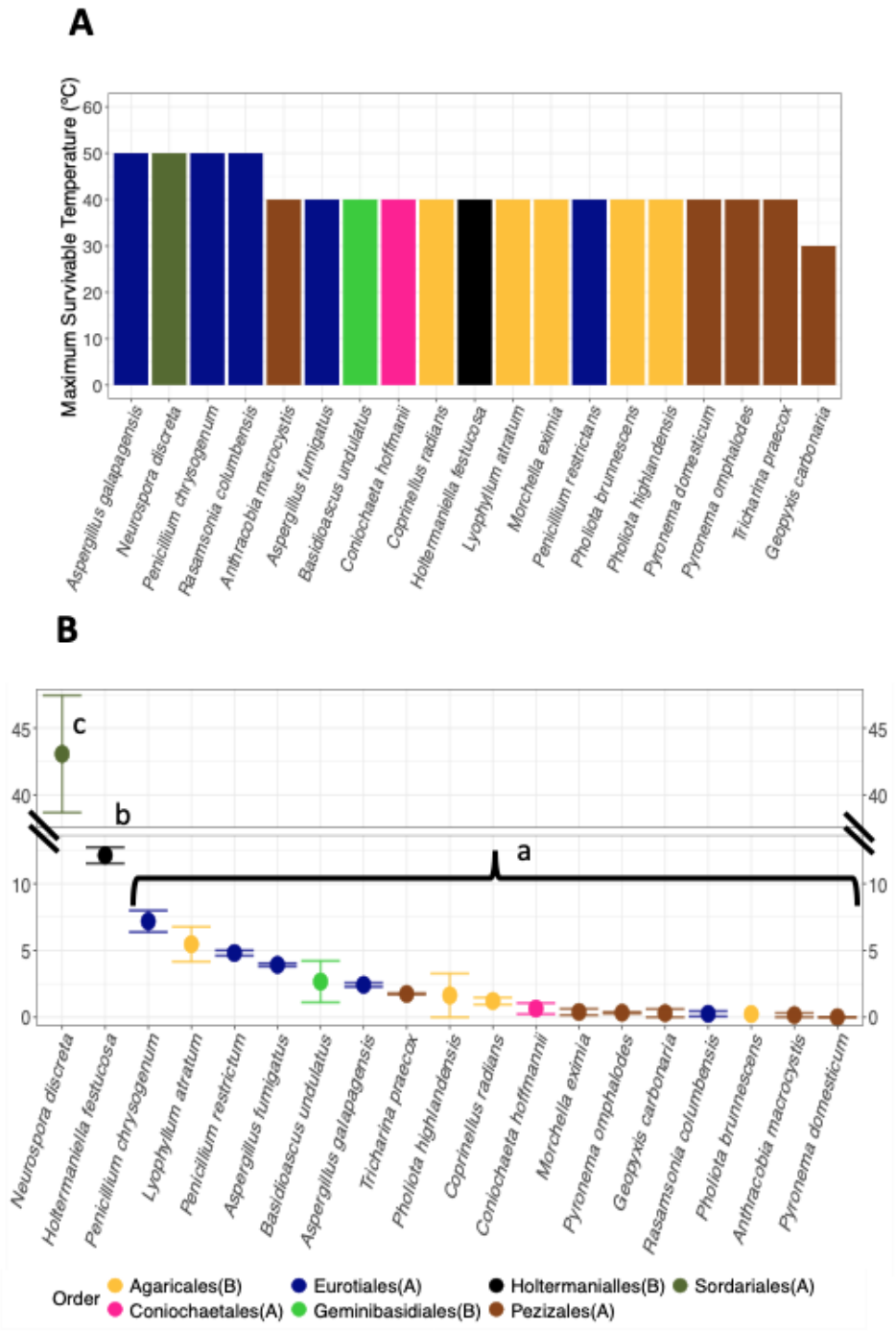

Figure 4.2: A) Thermotolerance of fungal isolates calculated from maximum survivable temperature (ºC) following heat shocked from 25ºC to 100ºC. Bars are colored according to the phylogenetic order of the isolates. B) Emission rates of N_2O per isolate expressed in parts per million N_2O produced per day. The y axis has been broken from 15 to 35ppm in order to visualize the variation among isolates other than *Neurospora discreta*. Points are colored according to the corresponding phylogenetic order of the isolate.

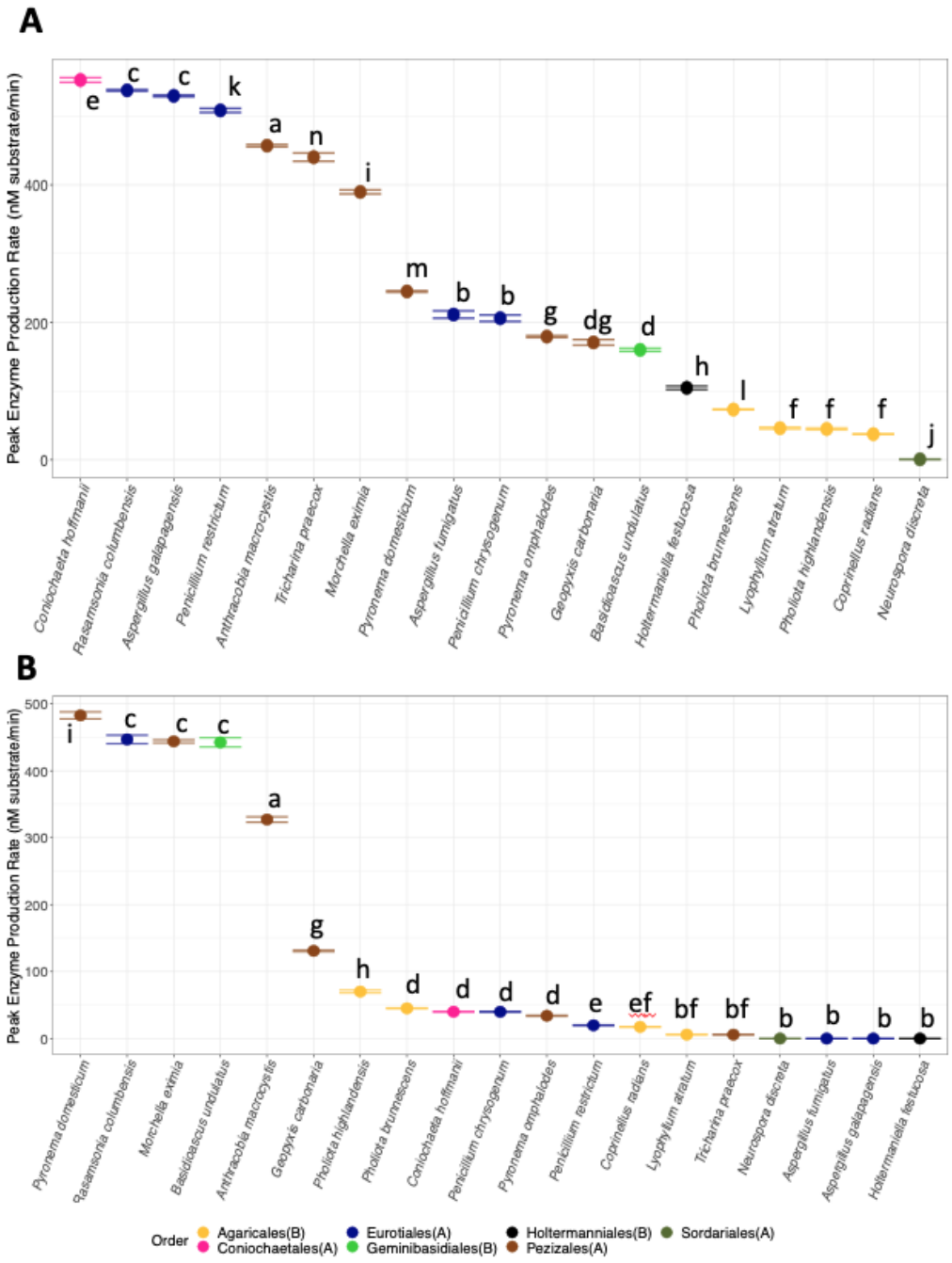

Figure 4.3: Enzyme expression of β-Glucosidase (A) and N-Acetylglutamate synthase (B) of all fungal isolates expressed as peak conversion of substrate in nM per minute. Points are colored according to the corresponding isolate's phylogenetic order.

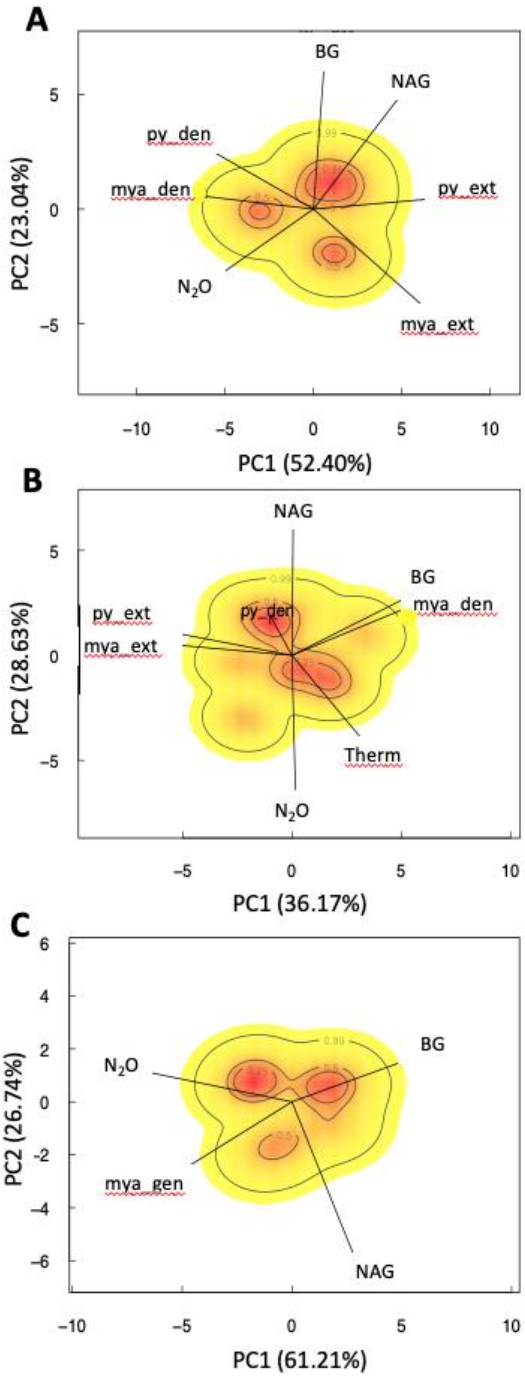

Figure 4.4: Trait space ordinations of all hyphal Basidiomycetes (A), Ascomycetes (B), and yeast-form fungi (C). Statistically significant peaks of trait probability are bounded by 0.5 and 0.25 intervals. The distribution of trait probability is colored from yellow to red as the probability of the isolates within the group are explained by corresponding trait axes increases. Axes for thermotolerance and PyOM generation time are removed from the yeast trait space ordination as the values for all isolates within those trait measurements were the same.

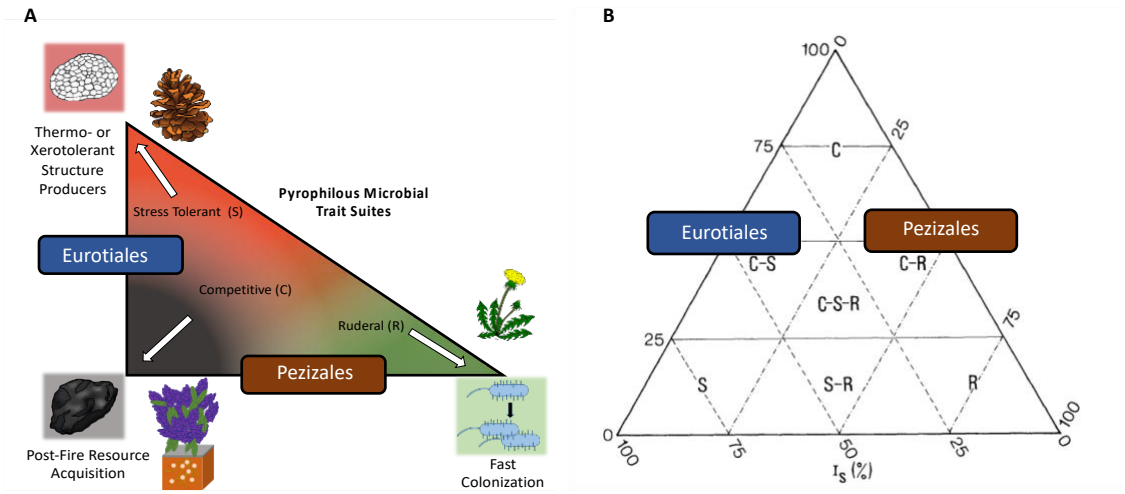

Figure 4.5: Placement of the Eurotiales and Pezizales pyrophilous fungal groups in the hypothetical pyrophilous trait framework adapted from Enright et al 2022 (A) and in the Grime C-S-R triangle adapted from Grime 1988 (B) according to the results of the trait assays and their corresponding trait space ordinations.

Chapter V

Conclusion to the Dissertation

Throughout the course of this dissertation, I have endeavored to deepen scientific understanding of the ecology of pyrophilous microbes. In pursuit of this goal, I leveraged an exceptionally fortuitous set of circumstances whereby we were able to obtain pre- and post-fire samples from the exact same plots following a catastrophic mega-fire. This experimental set up had the rare advantage of possessing true unburned controls (rather than space-for-time substitutions commonly found in wildfire studies) which allowed us a BACI experimental design (Conquest 2000). Furthermore, this mega-fire took place in a redwood-tanoak forest, which not only allowed us to profile the effects of severe wildfire on the soil microbiome an unstudied ecosystem, but also gave us a non-Pinaceae dataset to compare to other studies and identify patterns in the post-fire pyrophilous microbial community. This in turn led us to stronger hypotheses regarding the traits, which likely govern pyrophilous microbial response to fire which we could compare to both classic (Grime 1977) and recent (Whitman et al. 2019) hypothetical frameworks. Then over the

course of nearly 5 years, we created a large collection of pyrophilous microbial cultures that resulted in over 500 isolates and the creation of a new culture media targeted at pyrophilous microbes. In the creation of this collection, we also learned the best method for storing burned soil to retain cultural viability of pyrophilous microbes and what culture medias were most successful in obtaining the largest diversity of pyrophilous microbes. Finally, we then used these cultures as part of a battery of phenotypic assays to ascertain the traits that govern pyrophilous fungal adaptations to fire and the post-fire environment. We then leveraged cutting edge statistical modelling of trait space (Carmona, Pavanetto, and Puglielli 2024) to test if the hypothetical pyrophilous trait frameworks that we and others proposed were supported, and to enable an ecological understanding of pyrophilous fungi and the functions they support in the post-fire environment.

In the second chapter of this dissertation, we examined the immediate effects of a mega-fire on both bacterial and fungal communities using pre-and post-fire samples from the exact same plots. This was the first study to comprehensively characterize the soil microbial communities of a redwood–tanoak forest. At the time of its publication, only two other instances where pre- and post-fire samples from the same locations existed (Baar et al. 1999; Glassman et al. 2016), but both were in pine forests, focused only on ectomycorrhizal communities, and both lacked a true unburned control. With our study taking place in a non-Pinaceae forest, we were able to identify some generalized patterns of pyrophilous microbiomes. We identified a massive increase in the Basidiomycete yeast *Basidioascus* and the bacterial phylum Firmicutes post-fire, which underscored the

importance of DNA-sequencing in pyrophilous microbial studies as *Basidioascus* would have be missed by purely sporocarp-driven surveys despite being the most abundant fungal taxon post-fire and all bacterial conclusions would otherwise be impossible. We also found a dominance of described pyrophilous fungal taxa post-fire (such as *Pyronema, Tricharina, Lyophyllum*, *Penicillium*, etc.), which corroborated patterns seen in other systems. We showed that pyrophilous bacterial and fungal response to fire were phylogenetically conserved at the class level, which improves our potential predictive power regarding changes in post-fire communities. By comparing our work to other recent characterizations of post-fire microbes in Pinaceae forests (Bruns et al. 2020; Glassman et al. 2016; Whitman et al. 2019), we were able to hypothesize generalized traits of post-fire microbes and compare them to analogous traits suites in plants (Grime 1977). These hypotheses regarding pyrophilous traits and the hypothetical placement of pyrophilous taxa in trait space provided a foundation for our continuing work in pyrophilous microbial traits.

In the third chapter of this dissertation, our experiments used soil samples from high-severity burn scars in a chaparral ecosystem to test the effects of soil storage and laboratory media selection on the culturing of pyrophilous microbes abundance and diversity. We found that storing soil samples at -80°C without glycerol preserved the greatest diversity of culturable pyrophilous bacteria and fungi and is thus the recommended long-term storage method for soil intended for pyrophilous culturing. We found that the best laboratory media for culturing pyrophilous bacteria and fungi was the oligotrophic media Reasoner's 2 Agar (R2A) (Reasoner and Geldreich 1985), and the

newly created solid agar media created from field-harvested pyrogenic organic matter (PyOM) also captured a diverse community of isolates including some taxa of interest that were only captured on PyOM such as the bacterial species *Pseudonocardia saturnea* (Enright et al. 2022; Caiafa et al. 2023; Pulido-Chavez et al. 2023) and the fungal species *Neurospora discreta* (Kuo et al. 2014; Perkins 1992). The methods we employed captured many groups of interest from previous pyrophilous microbial studies, which garnered many potential isolates for our continuing pyrophilous traits work. Additionally, this study was also an examination of prudent laboratory practices when considering future culturing efforts from soil, which adds to our understanding of the creation of culture collections which are a vital resource for microbiology (Caktu and Turkoglu 2011; Malik and Claus 1987). Finally, the culmination of this work in conjunction with our lab's previous culturing efforts resulted in the creation of a large and diverse culture collection of pyrophilous bacteria and fungi that leveraged 7 different wildfires, culturing from soil, smoke, and fungal fruiting bodies, and contains over 500 isolates of bacteria and fungi. These cultures gave us many isolates to choose from when engaging in our ultimate goal of interogating pyrophilous microbial traits.

Finally, in the last chapter of this dissertation, we assessed 20 isolates of pyrophilous fungi from 2 phyla and 15 genera for a wide range of traits including growth patterns on rich media vs. PyOM, thermotolerance, organic and inorganic nitrogen acquisition strategies, and carbon acquisition enzyme activity. We found widely varying phenotypes among the traits of our 20 fungi that were statistically significant in their differences from one another. Our trait space modeling using the recently developed

Funspace tool (Carmona, Pavanetto, and Puglielli 2024) found 2 distinct statistically significant hotspots of trait probability among the Ascomycota hyphal fungi that aligned with a post-fire nutrient acquisition and fast-growth lifestyle and a post-fire nutrient and stress tolerant lifestyle. When these models were visualized at the family-level the Pezizales aligned closest to the fast-growth and nutrient acquisitive hotspot while the Eurotiales aligned closest to the stress tolerant and nutrient acquisitive hotspot. These significant hotspots of pyrophilous fungal trait probability allowed us to corroborate some of the hypothetical placements we made in the hypothetical trait framework of the second chapter (Enright et al. 2022) and provide laboratory evidence of some of the hypothesized traits of pyrophilous fungi (Fox et al. 2022). This work is a major step in our understanding of pyrophilous fungal traits and is a meaningful contribution to the field of fungal ecology and microbail traits as whole.

Although the work presented in this dissertation makes significant strides in our understanding of the ecology of pyrophilous microbes, future research is still needed to fully understand the traits which govern pyrophilous microbial response to fire and how they interact in the post-fire environment. One of the most important future areas of focus should be the inclusion of more pyrophilous taxa, particularly from Basidiomycete and yeast-form fungi. While our study was able to profile the traits of a few of these fungi, we did not have sufficient numbers of either of these groups to detect meaningful patterns within them and our trait space modelling was not smooth enough to detect overarching patterns. Additionally, while our assays quantified labile carbon assimilation through β-glucosidase enzyme activity we were unable to profile carbon acquisition enzymes that

might target the more recalcitrant carbon forms present in PyOM. Therefore, a meaningful line of research would be to profile these same fungi for catechol monooxygenase, protocatechuate monooxygenase, or peroxidases that have been demonstrated through genomic and transcriptomic means as being potentially involved in the degradation of PyOM (Steindorff et al. 2020; Fischer et al. 2021). With continued research, we hope that our understanding of the ecology of pyrophilous microbes will one day gain parity to that of our knowledge of pyrophilous plant traits, and allow us to implement restoration techniques such as targeted microbial applications to improve post-fire recovery.

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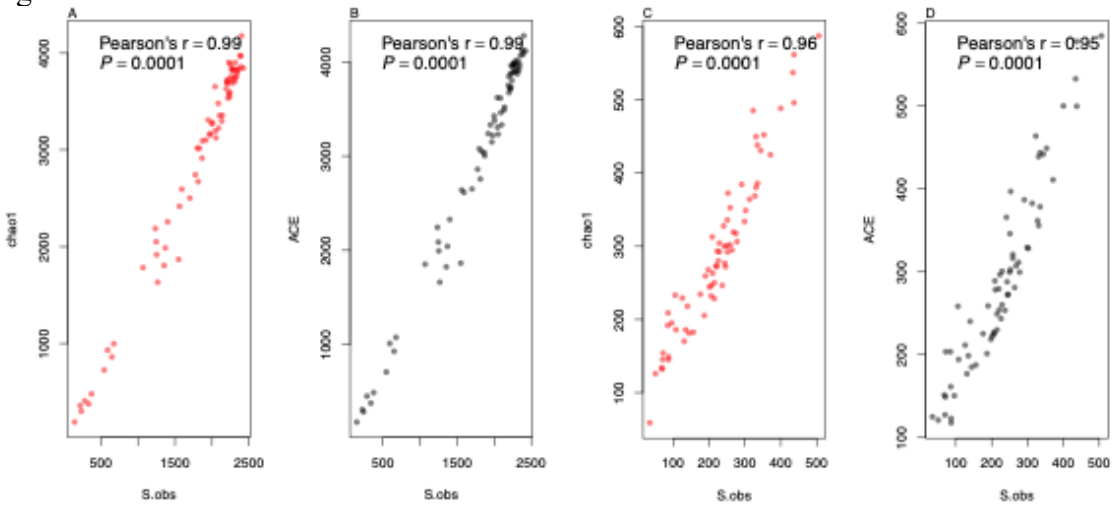
Appendix A:

Supplemental Materials for Chapter II

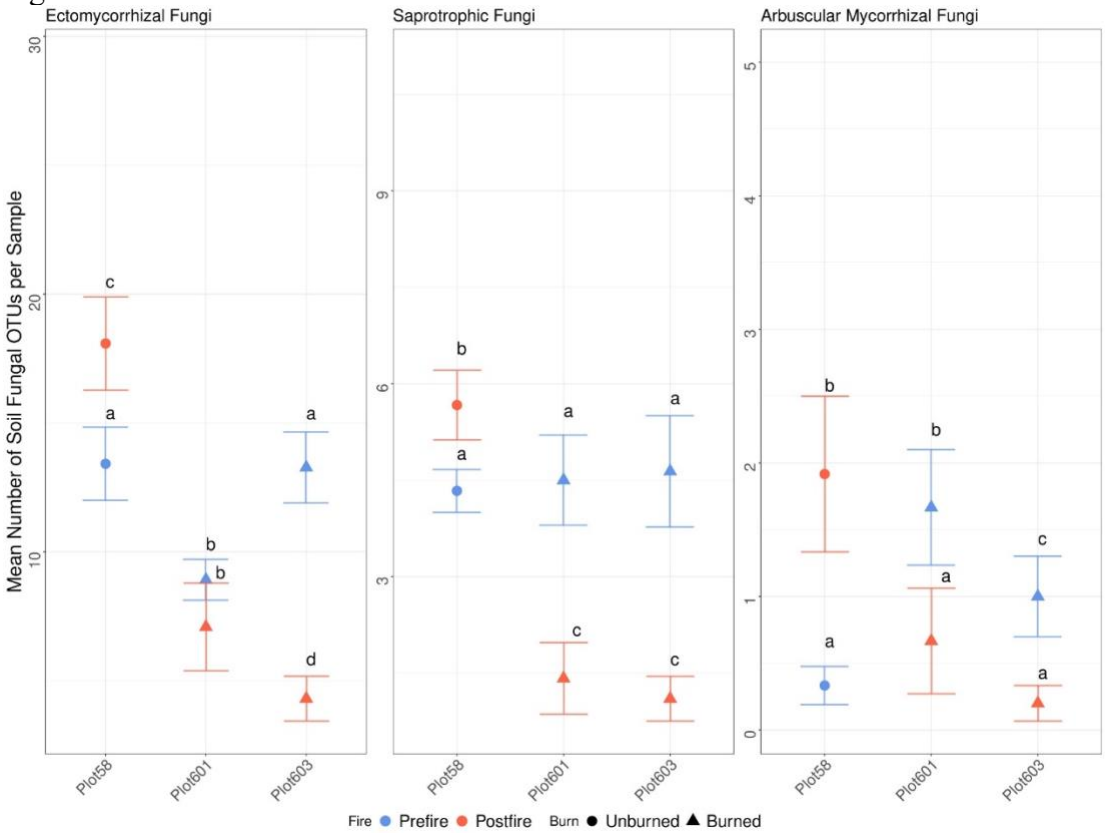
A.1 Figures

Figure A.1.1



Correlation of bacterial (A & B) and fungal (C & D) species observed with ACE and Chao1 richness indices. Due to high correlation of metrics, observed species was chosen.

Figure A.1.2



Mean per sample number of observed species for Ectomycorrhizal, Saprotrophic, and Arbuscular mycorrhizal fungi. Colors differentiate pre- and post-fire and shapes differentiate burned and unburned plots. Statistically significant difference in richness was tested using ANOVA (for burned plots, $F_{1,1} = 84.91$, $p < 0.001$, for unburned $F_{1,1} = 4.119$. $p = 0.0547$). Letters represent Tukey HSD differences.

A.2 Tables

Table A.2.1

Plot information for each of the three plots sampled before and after the Soberanes fire. All plots are in UTM Zone 10.

Plot	Slope	Aspect	Elevation	Land Ownership
58	19°	180	744m	University of California Landels-Hill Big Creek Reserve
601	34°	62	603m	Monterey Peninsula Regional Park District
603	31°	116	491m	Monterey Peninsula Regional Park District

Plot	Distance to Road	Distance to Coast	Distance Between Plots
58	0.04km	4.1km	To 601: 42.02km, To 603: 43.04km
601	0.84km	6.4km	To 58: 42.02km, To 603: 1.03km
603	0.62km	6.3km	To 58: 43.04km, To 601: 1.03km

Table A.2.2.
Summary of mean per-sample richness estimated by observed species, Shannon and Simpson diversity indices, and percent change in each plot before and after the Soberanes Fire. P value relates to the result of ANOVA analysis on each observed metric of alpha diversity.

Bacteria	Mean Per-Sample richness				
Plots	Burn status	Pre-fire	Post-fire	Percent change	P value
58	unburned	2,183	2,188	2.0	0.30
601	burned	2,082	1,255	40.1	<0.01
603	burned	2,188	843	52.2	<0.01

	Mean Per-Sample Shannon Diversity				
Plots	Burn status	Pre-fire	Post-fire	Percent change	P value
58	unburned	775	742	4.3	0.12
601	burned	763	264	65.4	<0.001
603	burned	782	171	78.2	<0.001

	Mean Per-Sample Simpson Diversity^{-1}				
Plots	Burn status	Pre-fire	Post-fire	Percent change	P value
58	unburned	0.0037	0.005	18.0	0.49
601	burned	0.0034	0.014	75.7	<0.001
603	burned	0.0038	0.021	81.6	<0.001

Fungi	Mean Per-Sample Richness				
Plots	Burn status	Pre-fire	Post-fire	Percent change	P value
58	unburned	267	286	0.1	0.78
601	burned	229	96	37.6	<0.01
603	burned	321	143	70.0	<0.01

	Mean Per-Sample Shannon Diversity				
Plots	Burn status	Pre-fire	Post-fire	Percent change	P value
58	unburned	39	42.01	4.7	0.87
601	burned	42	7.31	82.7	<0.001
603	burned	46	8.46	81.6	<0.001

	Mean Per-Sample Simpson Diversity^{-1}				
Plots	Burn status	Pre-fire	Post-fire	Percent change	P value
58	unburned	0.059	0.05	10.5	0.70
601	burned	0.056	0.26	78.3	<0.001
603	burned	0.059	0.20	70.5	<0.001

Table A.2.3.
Summary of all PERMANOVA statistics done on community composition shifts. PERMANOVA was done on Bray-Curtis dissimilarity matrices with 999 permutations. Df = degrees of freedom, Sum of Sqs = Sum of Squares, Mean Sqs = Mean Squares. Significance displayed using asterisks: * < 0.05, ** < 0.01, *** < 0.001.

Summary of PERMANOVA Statistics Results

All Bacterial Plots (058, 601, 603) Together

	Df	Sum of Sqs	Mean Sqs	F Model	R2	P Value
Fire	1	2.3667	2.3667	11.744	0.144	0.001***
Residuals	70	14.106	0.202		0.856	
Total	71	16.473		1.000		

Bacteria Unburned Plot (058) Only

	Df	Sum of Sqs	Mean Sqs	F Model	R2	P Value
Fire	1	0.215	0.215	2.054	0.085	0.02*
Residuals	22	2.304	0.105		0.915	
Total	23	2.519		1.000		

Bacteria Burned Plots (601 & 603) Only

	Df	Sum of Sqs	Mean Sqs	F Model	R2	P Value
Fire	1	3.146	3.146	16.589	0.265	0.001***
Residuals	46	8.723	0.190		0.735	
Total	47	11.869		1.000		

All Fungal Plots (058, 601, 603) Together

	Df	Sum of Sqs	Mean Sqs	F Model	R2	P Value
Fire	1	2.741	2.741	7.162	0.097	0.001***
Residuals	67	25.646	0.383		0.903	
Total	68	28.387		1.000		

Fungi Unburned Plot (058) Only

	Df	Sum of Sqs	Mean Sqs	F Model	R2	P Value
Fire	1	0.421	0.421	1.191	0.051	0.171
Residuals	22	7.77	0.353		0.949	
Total	23	8.192		1.000		

Fungi Burned Plots (601 & 603) Only

	Df	Sum of Sqs	Mean Sqs	F Model	R2	P Value
Fire	1	4.161	4.161	13.399	0.238	0.001***
Residuals	43	13.353	0.311		0.762	
Total	44	17.514		1.000		

Table A.2.4
Table showing all bacterial OTUs (with taxonomic identification) with at least 1% sequence abundance in the burned plots either pre or post-fire (Plots 601 and 603). Percent sequences refers to the percentage of total pre-fire or post-fire sequences that OTU utilized.

ID	Phylum	Genus	Species	% Sequences	Fire
Otu5	Proteobacteria	*Bradyrhizobium*	*spp.*	2.3	Pre-fire
Otu14	Proteobacteria	*Rhodoplanes*	*spp.*	1.4	Pre-fire
Otu18	Proteobacteria	*Betaproteobacteria spp.*	*spp.*	1.3	Pre-fire
Otu24	Proteobacteria	*Sinobacteraceae*	*spp.*	1.2	Pre-fire
Otu15	Acidobacteria	*Acidobacteria spp.*	*spp.*	1.1	Pre-fire
Otu6	Firmicutes	*Sporosarcina*	*spp.*	6.9	Post-fire
Otu7	Firmicutes	*Sporosarcina*	*spp.*	6.9	Post-fire
Otu8	Firmicutes	*Sporosarcina*	*spp.*	6.9	Post-fire
Otu9	Actinobacteria	*Micromonospora*	*spp.*	2.7	Post-fire
Otu13	Firmicutes	*Bacillus*	*spp.*	1.9	Post-fire
Otu1421	Actinobacteria	*Micromonosporaceae*	*spp.*	1.2	Post-fire
Otu17024	Firmicutes	*Bacillus*	*muralis*	1.1	Post-fire
Otu2032	Firmicutes	*Solibacillus*	*spp.*	1.1	Post-fire
Otu61	Actinobacteria	*Pseudonocardia*	*spp.*	1.1	Post-fire
Otu49	Firmicutes	*Cohnella*	*spp.*	1.1	Post-fire

Table A.2.5
Table summarizing which fungal OTUs (with taxonomic identification) utilized at least 1% of the sequence abundance in the burned plots pre-fire or post-fire (Plots 601 and 603). Percent sequences refers to the percentage of total pre-fire or post-fire sequences that OTU utilized.

ID	Phylum	Genus	Species	% Sequences	Fire
Otu13	*Basidiomycota*	*Hygrocybe*	*acutoconica var. microspora*	13.0	Pre-fire
Otu23	*Mucoromycota*	*Mortierella*	*bainieri*	10.7	Pre-fire
Otu57	*Basidiomycota*	*Inocybe*	*flocculosa*	3.5	Pre-fire
Otu40	*Basidiomycota*	*Cystolepiota*	*bucknallii*	1.8	Pre-fire
Otu32	*Ascomycota*	*Cladophialophora*	*spp.*	1.8	Pre-fire
Otu89	*Basidiomycota*	*Xeromphalina*	*cauticinalis*	1.8	Pre-fire
Otu78	*Basidiomycota*	*Protomerulius*	*spp.*	1.6	Pre-fire
Otu63	*Basidiomycota*	*Tomentella*	*fuscocinerea*	1.3	Pre-fire
Otu41	*Unidentified*	*Fungus Spp.*	*spp.*	1.2	Pre-fire
Otu53	*Ascomycota*	*Polyphilus*	*spp.*	1.1	Pre-fire
Otu49	*Ascomycota*	*Cylindrodendrum*	*spp.*	1.1	Pre-fire
Otu156	*Basidiomycota*	*Lyophyllum*	*semitale*	1.0	Pre-fire
Otu70	*Basidiomycota*	*Clavaria*	*fragilis*	1.0	Pre-fire
Otu100	*Basidiomycota*	*Ramariopsis*	*spp.*	1.0	Pre-fire
Otu1	*Basidiomycota*	*Basidioascus*	*undulatus*	28.2	Post-fire
Otu2	*Ascomycota*	*Tricharina*	*spp.*	13.6	Post-fire
Otu6	*Ascomycota*	*Penicillium*	*decumbens*	13.1	Post-fire
Otu8	*Ascomycota*	*Peziza*	*spp.*	10.2	Post-fire

Otu4	*Ascomycota*	*Anthracobia*	*spp.*	8.8	Post-fire
Otu5	*Ascomycota*	*Scutellinia*	*vitreola*	7.2	Post-fire
Otu7	*Ascomycota*	*Pyronema*	*domesticum*	4.2	Post-fire
Otu16	*Basidiomycota*	*Lyophyllum*	*semitale*	2.6	Post-fire
Otu20	*Ascomycota*	*Fusarium*	*acutatum*	1.2	Post-fire

Table A.2.6
Bacterial Indicator Species Analysis results. For pre-fire bacteria the top 10 taxa are listed and identified (out of 86 identified indicators).

Pre-fire

ID	p Value	Phylum	Best Taxonomic ID
Otu147	0.001	Proteobacteria	Nitrosomonadales
Otu99	0.001	Bacteroidetes	Cytophagaceae
Otu215	0.001	Actinobacteria	Gaiellaceae
Otu107	0.001	Proteobacteria	Sinobacteraceae
Otu51	0.001	Proteobacteria	Rhodospirillaceae
Otu13706	0.001	Proteobacteria	Kaistobacter
Otu273	0.001	Acidobacteria	Acidobacteria
Otu13215	0.001	Chloroflexi	Chloroflexi
Otu962	0.001	Verrucomicrobia	Verrucomicrobia
Otu355	0.001	Proteobacteria	Betaproteobacteria

Post-fire

ID	p Value	Phylum	Best Taxonomic ID
Otu84	0.001	Firmicutes	Thermoactinomyceteae
Otu4	0.001	Firmicutes	*Thermoflavimicrobium*
Otu2	0.001	Firmicutes	*Fictibacillus*
Otu34	0.001	Actinobacteria	Thermomonosporaceae
Otu12829	0.001	Firmicutes	*Cohnella*
Otu40	0.001	Firmicutes	*Tepidibacterium infernus*
Otu3319	0.001	Firmicutes	*Paenibacillus*
Otu1987	0.001	Actinobacteria	*Streptomyces* (Actinobacteria)
Otu3360	0.001	Firmicutes	*Bacillus*
Otu33	0.001	Firmicutes	Thermoactinomyceteae
Otu840	0.001	Firmicutes	*Lysinibacillus massiliensis*
Otu2099	0.001	Actinobacteria	Micromonosporaceae
Otu209	0.001	Firmicutes	*Paenibacillus sp.*
Otu1104	0.001	Firmicutes	*Paenibacillus sp.*
Otu689	0.001	Actinobacteria	Gaiellaceae
Otu87	0.001	Firmicutes	*Paenibacillus*
Otu122	0.001	Proteobacteria	Betaproteobacteria
Otu6430	0.001	Firmicutes	*Paenibacillus*
Otu215	0.001	Actinobacteria	Gaiellaceae
Otu13215	0.001	Chloroflexi	Chloroflexi
Otu1713	0.001	Firmicutes	*Aneurinibacillus*

Table A.2.7
Pre-fire fungal Indicator Species Analysis results.

Pre-fire

ID	p Value	Phylum	Best Taxonomic ID
Otu60	0.001	Mucuromycota	*Mortierella horticola*
Otu23	0.001	Mucuromycota	*Mortierella baineri*
Otu53	0.001	Ascomycota	*Polyphilus frankenii*
Otu433	0.001	Ascomycota	*Cladophialophora sp.*
Otu113	0.001	Ascomycota	*Pycnopeziza sympodialis*
Otu3750	0.001	Mucuromycota	*Mortierella amoeboidea*
Otu424	0.001	Ascomycota	*Cladiophialophora sp.*
Otu432	0.001	Ascomycota	*Thelonectria nodosa*
Otu597	0.001	Ascomycota	*Pectenia plumbea*
Otu3759	0.001	Mucuromycota	*Mortierella elongata*
Otu772	0.001	Ascomycota	Pezizamycotina (sub-phylum)
Otu487	0.001	Ascomycota	*Phomopsis sp.*
Otu275	0.001	Ascomycota	Helotiales

Post-fire

ID	p Value	Phylum	Best Taxonomic ID
Otu2	0.001	Ascomycota	*Tricharina praecox*
Otu8	0.001	Ascomycota	*Peziza vacinii*
Otu4	0.001	Ascomycota	*Anthracobia sp.*
Otu6	0.001	Ascomycota	*Penicillium decumbens*
Otu180	0.001	Ascomycota	*Geopyxis alpina*

Table A.2.8
All significant bacterial interactions to fire as reported by DeSEQ2 analysis. Bacterial taxonomic groupings at phylum or order taxonomic levels whose response was more positive (blue) or negative (orange) than expected by chance (two-tailed exact test; $P < 0.05$). "Increase" and "Decrease" refer to the number of OTUs that changed significantly and "p Value" refers to the results of the two-tailed exact test.

Phylum	Order	Increase	Decrease	p Value	Stat
Acidobacteria	-	6	0	0.031	Increase
Acidobacteria	unidentified	6	0	0.031	Increase
Acidobacteria	unidentified	6	0	0.031	Increase
Acidobacteria	unidentified	6	0	0.031	Increase
Actinobacteria	Actinomycetales	6	0	0.031	Increase
Actinobacteria	Actinomycetales	6	0	0.031	Increase
Actinobacteria	Gaiellales	61	31	0.002	Increase
Actinobacteria	Gaiellales	54	19	0.000	Increase
Actinobacteria	Gaiellales	54	19	0.000	Increase
Firmicutes	-	127	2	0.000	Increase
Firmicutes	Bacillales	125	2	0.000	Increase
Firmicutes	Bacillales	11	0	0.001	Increase
Firmicutes	Bacillales	12	1	0.003	Increase
Firmicutes	Bacillales	78	1	0.000	Increase
Firmicutes	Bacillales	6	0	0.031	Increase
Firmicutes	Bacillales	15	0	0.000	Increase
Firmicutes	Bacillales	10	0	0.002	Increase
Firmicutes	Bacillales	9	1	0.021	Increase
Firmicutes	Bacillales	61	1	0.000	Increase
Firmicutes	Bacillales	9	0	0.004	Increase
Firmicutes	Bacillales	8	0	0.008	Increase
Firmicutes	-	13	1	0.002	Increase
Firmicutes	Clostridiales	13	1	0.002	Increase
Acidobacteria	-	16	97	0.000	Decrease
Acidobacteria	24-Nov	0	16	0.000	Decrease
Acidobacteria	DS-100	0	10	0.002	Decrease
Acidobacteria	RB41	10	55	0.000	Decrease
Acidobacteria	unidentified	1	9	0.021	Decrease
Acidobacteria	24-Nov	0	16	0.000	Decrease
Acidobacteria	DS-100	0	10	0.002	Decrease
Acidobacteria	RB41	7	22	0.008	Decrease

Acidobacteria	RB41		3	33	0.000	Decrease
Acidobacteria	unidentified		1	9	0.021	Decrease
Acidobacteria		24-Nov	0	16	0.000	Decrease
Acidobacteria	DS-100		0	10	0.002	Decrease
Acidobacteria	RB41		7	22	0.008	Decrease
Acidobacteria	RB41		3	33	0.000	Decrease
Acidobacteria	unidentified		1	9	0.021	Decrease
Acidobacteria	-		5	19	0.007	Decrease
Acidobacteria	unidentified		5	19	0.007	Decrease
Acidobacteria	unidentified		5	19	0.007	Decrease
Acidobacteria	unidentified		5	19	0.007	Decrease
Acidobacteria	-		55	89	0.006	Decrease
Acidobacteria	iii1-15		43	79	0.001	Decrease
Acidobacteria	iii1-15		27	62	0.000	Decrease
Acidobacteria	iii1-15		27	62	0.000	Decrease
Acidobacteria	-		6	24	0.001	Decrease
Acidobacteria	Acidobacteriales		6	24	0.001	Decrease
Acidobacteria	Acidobacteriales		4	17	0.007	Decrease
Acidobacteria	Acidobacteriales		2	11	0.022	Decrease
Acidobacteria	-		3	18	0.001	Decrease
Acidobacteria	DS-18		1	16	0.000	Decrease
Acidobacteria	DS-18		1	16	0.000	Decrease
Acidobacteria	DS-18		1	16	0.000	Decrease
Acidobacteria	-		10	72	0.000	Decrease
Acidobacteria	Solibacterales		10	72	0.000	Decrease
Acidobacteria	Solibacterales		2	25	0.000	Decrease
Acidobacteria	Solibacterales		7	46	0.000	Decrease
Acidobacteria	Solibacterales		1	20	0.000	Decrease
Acidobacteria	Solibacterales		7	46	0.000	Decrease
Actinobacteria	Solirubrobacterales		5	17	0.017	Decrease
Actinobacteria	Solirubrobacterales		4	16	0.012	Decrease
Armatimonadetes	-		1	16	0.000	Decrease
Armatimonadetes	[Fimbriimonadales]		1	16	0.000	Decrease
Armatimonadetes	[Fimbriimonadales]		1	15	0.001	Decrease
Armatimonadetes	[Fimbriimonadales]		1	13	0.002	Decrease
Armatimonadetes	-		5	26	0.000	Decrease
Armatimonadetes	unidentified		5	26	0.000	Decrease
Armatimonadetes	unidentified		5	26	0.000	Decrease
Armatimonadetes	unidentified		5	26	0.000	Decrease

Armatimonadetes	-	0	6	0.031	Decrease
Armatimonadetes	-	1	9	0.021	Decrease
Armatimonadetes	SJA-22	1	8	0.039	Decrease
Armatimonadetes	SJA-22	1	8	0.039	Decrease
Armatimonadetes	SJA-22	1	8	0.039	Decrease
Bacteroidetes	-	26	179	0.000	Decrease
Bacteroidetes	[Saprospirales]	26	179	0.000	Decrease
Bacteroidetes	[Saprospirales]	20	164	0.000	Decrease
Bacteroidetes	[Saprospirales]	2	10	0.039	Decrease
Bacteroidetes	[Saprospirales]	0	9	0.004	Decrease
Bacteroidetes	[Saprospirales]	18	137	0.000	Decrease
Bacteroidetes	-	11	75	0.000	Decrease
Bacteroidetes	Cytophagales	11	75	0.000	Decrease
Bacteroidetes	Cytophagales	11	72	0.000	Decrease
Bacteroidetes	Cytophagales	9	58	0.000	Decrease
Bacteroidetes	-	5	19	0.007	Decrease
Bacteroidetes	Flavobacteriales	5	19	0.007	Decrease
Bacteroidetes	Flavobacteriales	3	12	0.035	Decrease
Bacteroidetes	-	11	100	0.000	Decrease
Bacteroidetes	Sphingobacteriales	11	100	0.000	Decrease
Bacteroidetes	Sphingobacteriales	1	14	0.001	Decrease
Bacteroidetes	Sphingobacteriales	10	86	0.000	Decrease
Bacteroidetes	Sphingobacteriales	1	13	0.002	Decrease
Bacteroidetes	Sphingobacteriales	10	86	0.000	Decrease
Chlamydiae	-	13	28	0.028	Decrease
Chlamydiae	Chlamydiales	12	27	0.024	Decrease
Chlamydiae	Chlamydiales	7	24	0.003	Decrease
Chlorobi	-	0	6	0.031	Decrease
Chlorobi	unidentified	0	6	0.031	Decrease
Chlorobi	unidentified	0	6	0.031	Decrease
Chlorobi	unidentified	0	6	0.031	Decrease
Chloroflexi	-	24	74	0.000	Decrease
Chloroflexi	SBR1031	11	39	0.000	Decrease
Chloroflexi	SBR1031	8	28	0.001	Decrease
Chloroflexi	SBR1031	2	11	0.022	Decrease
Chloroflexi	SBR1031	8	28	0.001	Decrease
Chloroflexi	SBR1031	2	11	0.022	Decrease
Chloroflexi	-	6	23	0.002	Decrease
Chloroflexi	Chloroflexales	0	6	0.031	Decrease

Chloroflexi	-	7	39	0.000	Decrease
Chloroflexi	AKYG885	1	19	0.000	Decrease
Chloroflexi	unidentified	0	12	0.000	Decrease
Chloroflexi	AKYG885	0	7	0.016	Decrease
Chloroflexi	AKYG885	1	12	0.003	Decrease
Chloroflexi	unidentified	0	12	0.000	Decrease
Chloroflexi	AKYG885	0	7	0.016	Decrease
Chloroflexi	AKYG885	1	12	0.003	Decrease
Chloroflexi	unidentified	0	12	0.000	Decrease
Cyanobacteria	-	1	11	0.006	Decrease
Cyanobacteria	MLE1-12	1	8	0.039	Decrease
Cyanobacteria	MLE1-12	1	8	0.039	Decrease
Cyanobacteria	MLE1-12	1	8	0.039	Decrease
Elusimicrobia	-	7	36	0.000	Decrease
Elusimicrobia	FAC88	1	15	0.001	Decrease
Elusimicrobia	IIb	2	14	0.004	Decrease
Elusimicrobia	FAC88	1	15	0.001	Decrease
Elusimicrobia	IIb	2	14	0.004	Decrease
Elusimicrobia	FAC88	1	15	0.001	Decrease
Elusimicrobia	IIb	2	14	0.004	Decrease
Elusimicrobia	-	0	9	0.004	Decrease
Elusimicrobia	unidentified	0	9	0.004	Decrease
Elusimicrobia	unidentified	0	9	0.004	Decrease
Elusimicrobia	unidentified	0	9	0.004	Decrease
FBP	-	0	8	0.008	Decrease
FBP	unidentified	0	8	0.008	Decrease
FBP	unidentified	0	8	0.008	Decrease
FBP	unidentified	0	8	0.008	Decrease
Gemmatimonadetes	-	10	28	0.005	Decrease
Gemmatimonadetes	unidentified	10	28	0.005	Decrease
Gemmatimonadetes	unidentified	10	28	0.005	Decrease
Gemmatimonadetes	unidentified	10	28	0.005	Decrease
Gemmatimonadetes	-	1	10	0.012	Decrease
Gemmatimonadetes	unidentified	1	10	0.012	Decrease
Gemmatimonadetes	unidentified	1	10	0.012	Decrease
Gemmatimonadetes	unidentified	1	10	0.012	Decrease
Gemmatimonadetes	-	33	91	0.000	Decrease
Gemmatimonadetes	Ellin5290	7	26	0.001	Decrease
Gemmatimonadetes	Gemmatimonadales	5	23	0.001	Decrease

Gemmatimonadetes	Ellin5290	7	26	0.001	Decrease
Gemmatimonadetes	Gemmatimonadales	0	6	0.031	Decrease
Gemmatimonadetes	Ellin5290	7	26	0.001	Decrease
Gemmatimonadetes	Gemmatimonadales	0	6	0.031	Decrease
OP3	-	3	23	0.000	Decrease
OP3	GIF10	1	10	0.012	Decrease
OP3	unidentified	2	13	0.007	Decrease
OP3	GIF10	1	10	0.012	Decrease
OP3	unidentified	2	13	0.007	Decrease
OP3	GIF10	1	10	0.012	Decrease
OP3	unidentified	2	13	0.007	Decrease
Planctomycetes	-	33	169	0.000	Decrease
Planctomycetes	WD2101	10	120	0.000	Decrease
Planctomycetes	WD2101	10	120	0.000	Decrease
Planctomycetes	WD2101	10	120	0.000	Decrease
Planctomycetes	-	98	463	0.000	Decrease
Planctomycetes	Gemmatales	71	387	0.000	Decrease
Planctomycetes	Pirellulales	21	49	0.001	Decrease
Planctomycetes	Planctomycetales	5	23	0.001	Decrease
Planctomycetes	Gemmatales	66	335	0.000	Decrease
Planctomycetes	Gemmatales	5	52	0.000	Decrease
Planctomycetes	Pirellulales	21	49	0.001	Decrease
Planctomycetes	Planctomycetales	5	23	0.001	Decrease
Planctomycetes	Gemmatales	8	76	0.000	Decrease
Planctomycetes	Gemmatales	58	259	0.000	Decrease
Planctomycetes	Gemmatales	5	51	0.000	Decrease
Planctomycetes	Pirellulales	19	38	0.016	Decrease
Planctomycetes	Planctomycetales	5	23	0.001	Decrease
Planctomycetes	-	0	19	0.000	Decrease
Planctomycetes	DH61	0	8	0.008	Decrease
Planctomycetes	p04_C01	0	11	0.001	Decrease
Planctomycetes	DH61	0	8	0.008	Decrease
Planctomycetes	p04_C01	0	11	0.001	Decrease
Planctomycetes	DH61	0	8	0.008	Decrease
Planctomycetes	p04_C01	0	11	0.001	Decrease
Proteobacteria	-	100	452	0.000	Decrease
Proteobacteria	Caulobacterales	2	24	0.000	Decrease
Proteobacteria	Ellin329	3	20	0.000	Decrease
Proteobacteria	Rhizobiales	26	161	0.000	Decrease

Proteobacteria	Rhodobacterales	5	19	0.007	Decrease
Proteobacteria	Rhodospirillales	37	138	0.000	Decrease
Proteobacteria	Sphingomonadales	10	39	0.000	Decrease
Proteobacteria	unidentified	6	28	0.000	Decrease
Proteobacteria	Caulobacterales	2	24	0.000	Decrease
Proteobacteria	Ellin329	3	20	0.000	Decrease
Proteobacteria	Rhizobiales	0	7	0.016	Decrease
Proteobacteria	Rhizobiales	14	64	0.000	Decrease
Proteobacteria	Rhizobiales	0	13	0.000	Decrease
Proteobacteria	Rhizobiales	0	12	0.000	Decrease
Proteobacteria	Rhizobiales	3	29	0.000	Decrease
Proteobacteria	Rhizobiales	0	7	0.016	Decrease
Proteobacteria	Rhodobacterales	4	15	0.019	Decrease
Proteobacteria	Rhodospirillales	4	27	0.000	Decrease
Proteobacteria	Rhodospirillales	30	104	0.000	Decrease
Proteobacteria	Sphingomonadales	8	36	0.000	Decrease
Proteobacteria	unidentified	6	28	0.000	Decrease
Proteobacteria	Caulobacterales	2	16	0.001	Decrease
Proteobacteria	Ellin329	3	20	0.000	Decrease
Proteobacteria	Rhizobiales	0	7	0.016	Decrease
Proteobacteria	Rhizobiales	0	8	0.008	Decrease
Proteobacteria	Rhizobiales	7	39	0.000	Decrease
Proteobacteria	Rhizobiales	0	12	0.000	Decrease
Proteobacteria	Rhizobiales	0	6	0.031	Decrease
Proteobacteria	Rhizobiales	3	29	0.000	Decrease
Proteobacteria	Rhodobacterales	4	15	0.019	Decrease
Proteobacteria	Rhodospirillales	4	24	0.000	Decrease
Proteobacteria	Rhodospirillales	30	97	0.000	Decrease
Proteobacteria	Sphingomonadales	1	11	0.006	Decrease
Proteobacteria	Sphingomonadales	0	13	0.000	Decrease
Proteobacteria	unidentified	6	28	0.000	Decrease
Proteobacteria	-	47	133	0.000	Decrease
Proteobacteria	Burkholderiales	3	42	0.000	Decrease
Proteobacteria	unidentified	12	26	0.034	Decrease
Proteobacteria	Burkholderiales	0	20	0.000	Decrease
Proteobacteria	Burkholderiales	2	14	0.004	Decrease
Proteobacteria	unidentified	12	26	0.034	Decrease
Proteobacteria	Burkholderiales	0	11	0.001	Decrease
Proteobacteria	Burkholderiales	2	10	0.039	Decrease

Proteobacteria	unidentified	12	26	0.034	Decrease
Proteobacteria	-	114	491	0.000	Decrease
Proteobacteria	Bdellovibrionales	8	53	0.000	Decrease
Proteobacteria	FAC87	0	10	0.002	Decrease
Proteobacteria	MIZ46	6	50	0.000	Decrease
Proteobacteria	Myxococcales	65	276	0.000	Decrease
Proteobacteria	Syntrophobacterales	18	55	0.000	Decrease
Proteobacteria	unidentified	4	24	0.000	Decrease
Proteobacteria	Bdellovibrionales	4	50	0.000	Decrease
Proteobacteria	FAC87	0	10	0.002	Decrease
Proteobacteria	MIZ46	6	50	0.000	Decrease
Proteobacteria	Myxococcales	9	28	0.003	Decrease
Proteobacteria	Myxococcales	2	15	0.002	Decrease
Proteobacteria	Myxococcales	5	24	0.001	Decrease
Proteobacteria	Myxococcales	39	190	0.000	Decrease
Proteobacteria	Syntrophobacterales	18	55	0.000	Decrease
Proteobacteria	unidentified	4	24	0.000	Decrease
Proteobacteria	Bdellovibrionales	4	47	0.000	Decrease
Proteobacteria	FAC87	0	10	0.002	Decrease
Proteobacteria	MIZ46	6	50	0.000	Decrease
Proteobacteria	Myxococcales	8	28	0.001	Decrease
Proteobacteria	Myxococcales	1	11	0.006	Decrease
Proteobacteria	Myxococcales	3	21	0.000	Decrease
Proteobacteria	Myxococcales	39	190	0.000	Decrease
Proteobacteria	Syntrophobacterales	18	55	0.000	Decrease
Proteobacteria	unidentified	4	24	0.000	Decrease
Proteobacteria	-	39	124	0.000	Decrease
Proteobacteria	Legionellales	14	40	0.001	Decrease
Proteobacteria	Xanthomonadales	14	64	0.000	Decrease
Proteobacteria	Legionellales	6	26	0.001	Decrease
Proteobacteria	Xanthomonadales	8	51	0.000	Decrease
Proteobacteria	Legionellales	0	8	0.008	Decrease
Proteobacteria	Legionellales	6	18	0.023	Decrease
Proteobacteria	Xanthomonadales	1	9	0.021	Decrease
Proteobacteria	Xanthomonadales	7	41	0.000	Decrease
TM7	-	0	10	0.002	Decrease
TM7	unidentified	0	10	0.002	Decrease
TM7	unidentified	0	10	0.002	Decrease
TM7	unidentified	0	10	0.002	Decrease

Verrucomicrobia	-	1	12	0.003	Decrease
Verrucomicrobia	S-BQ2-57	1	10	0.012	Decrease
Verrucomicrobia	S-BQ2-57	1	10	0.012	Decrease
Verrucomicrobia	S-BQ2-57	1	10	0.012	Decrease
Verrucomicrobia	-	39	118	0.000	Decrease
Verrucomicrobia	[Pedosphaerales]	39	117	0.000	Decrease
Verrucomicrobia	[Pedosphaerales]	3	35	0.000	Decrease
Verrucomicrobia	[Pedosphaerales]	20	48	0.001	Decrease
Verrucomicrobia	[Pedosphaerales]	3	35	0.000	Decrease
Verrucomicrobia	[Pedosphaerales]	20	48	0.001	Decrease
Verrucomicrobia	-	9	80	0.000	Decrease
Verrucomicrobia	[Chthoniobacterales]	9	80	0.000	Decrease
Verrucomicrobia	[Chthoniobacterales]	9	80	0.000	Decrease
Verrucomicrobia	[Chthoniobacterales]	0	6	0.031	Decrease
Verrucomicrobia	[Chthoniobacterales]	2	21	0.000	Decrease
Verrucomicrobia	[Chthoniobacterales]	0	14	0.000	Decrease
Verrucomicrobia	[Chthoniobacterales]	3	28	0.000	Decrease
Verrucomicrobia	-	1	18	0.000	Decrease
Verrucomicrobia	Opitutales	0	16	0.000	Decrease
Verrucomicrobia	Opitutales	0	16	0.000	Decrease
Verrucomicrobia	Opitutales	0	11	0.001	Decrease
Verrucomicrobia	-	8	22	0.016	Decrease
Verrucomicrobia	Verrucomicrobiales	8	22	0.016	Decrease
Verrucomicrobia	Verrucomicrobiales	8	22	0.016	Decrease
Verrucomicrobia	Verrucomicrobiales	6	19	0.015	Decrease

Table A.2.9
All significant fungal interactions to fire as reported by DeSEQ2 analysis. Fungal taxonomic groupings at phylum or order taxonomic levels whose response was more positive (blue) or negative (orange) than expected by chance (two-tailed exact test; $P < 0.05$). "Increase" and "Decrease" refer to the number of OTUs that changed significantly and "p Value" refers to the results of the two-tailed exact test. (see corresponding .xlsx file)

Phylum	Order	Increase	Decrease	p Value	Stat
Ascomycota	Eurotiales	53	21	0.000	Increase
Ascomycota	Onygenales	14	3	0.013	Increase
Ascomycota	Eurotiales	44	17	0.001	Increase
Ascomycota	Eurotiales	31	13	0.010	Increase
Ascomycota	-	55	34	0.033	Increase
Ascomycota	Pezizales	55	34	0.033	Increase
Ascomycota	Pezizales	39	17	0.005	Increase
Ascomycota	Pezizales	16	3	0.004	Increase
Basidiomycota	Russulales	9	1	0.021	Increase
Basidiomycota	Russulales	8	0	0.008	Increase
Basidiomycota	-	24	6	0.001	Increase
Basidiomycota	Geminibasidiales	24	6	0.001	Increase
Basidiomycota	Geminibasidiales	24	6	0.001	Increase
Basidiomycota	Geminibasidiales	19	0	0.000	Increase
Ascomycota	-	103	158	0.001	Decrease
Ascomycota	Capnodiales	21	38	0.036	Decrease
Ascomycota	Capnodiales	5	16	0.027	Decrease
Ascomycota	Pleosporales	0	6	0.031	Decrease
Ascomycota	Chaetothyriales	51	96	0.000	Decrease
Ascomycota	Chaetothyriales	34	76	0.000	Decrease
Ascomycota	Chaetothyriales	9	36	0.000	Decrease
Ascomycota	-	101	151	0.002	Decrease
Ascomycota	Helotiales	95	130	0.023	Decrease
Ascomycota	unidentified	1	10	0.012	Decrease
Ascomycota	Helotiales	31	52	0.028	Decrease
Ascomycota	unidentified	1	10	0.012	Decrease
Ascomycota	Helotiales	31	52	0.028	Decrease
Ascomycota	unidentified	1	10	0.012	Decrease
Ascomycota	Orbiliales	1	9	0.021	Decrease

Ascomycota	-	20	37	0.033	Decrease
Ascomycota	unidentified	20	37	0.033	Decrease
Ascomycota	unidentified	20	37	0.033	Decrease
Ascomycota	unidentified	20	37	0.033	Decrease
Basidiomycota	Agaricales	2	10	0.039	Decrease
Basidiomycota	Boletales	1	8	0.039	Decrease
Basidiomycota	Boletales	1	8	0.039	Decrease
Glomeromycota	-	1	13	0.002	Decrease
Glomeromycota	Glomerales	1	13	0.002	Decrease
Glomeromycota	Glomerales	1	13	0.002	Decrease
Glomeromycota	Glomerales	0	9	0.004	Decrease
Mortierellomycota	-	37	60	0.025	Decrease
Mortierellomycota	Mortierellales	37	60	0.025	Decrease
Mortierellomycota	Mortierellales	37	60	0.025	Decrease
Mortierellomycota	Mortierellales	37	60	0.025	Decrease
Mucoromycota	-	1	9	0.021	Decrease
Mucoromycota	Umbelopsidales	1	9	0.021	Decrease
Mucoromycota	Umbelopsidales	1	9	0.021	Decrease
Mucoromycota	Umbelopsidales	1	9	0.021	Decrease

Appendix B:

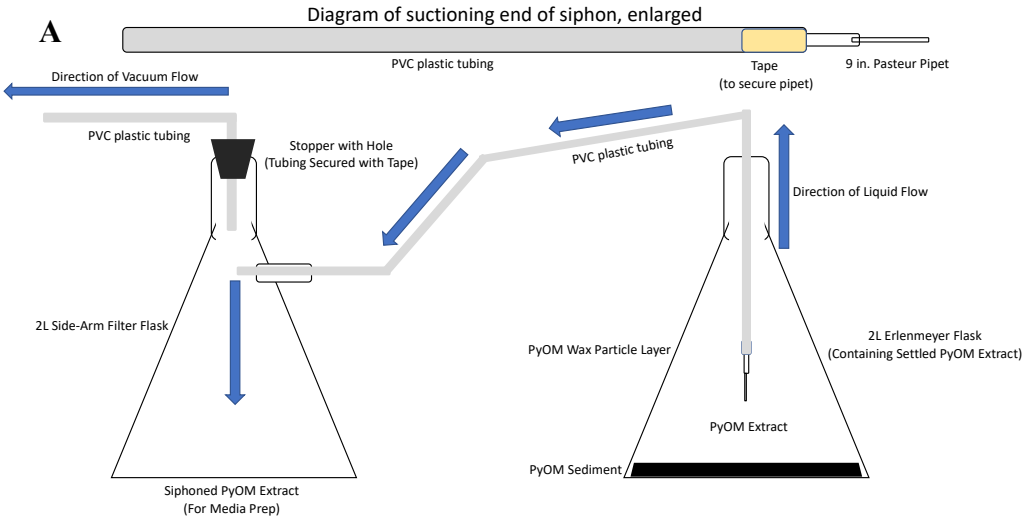
Supplemental Materials for Chapter III

B.1.1 Supplemental Methods

Extract-N-Amp Extraction Solution Recipe:

1. 10mL of 1M pH 8.0 Tris-HCl
2. 1.86g Potassium Chloride
3. 0.37g EDTA
4. 80mL DI Water
5. Add 1M Sodium Hydroxide until pH = 9.5
6. Dilute to total volume of 100mL
7. Filter sterilze

B.2 Figures



Vacuum Siphon Apparatus Set-Up for PyOM Liquid Extraction

Figure B.2.1

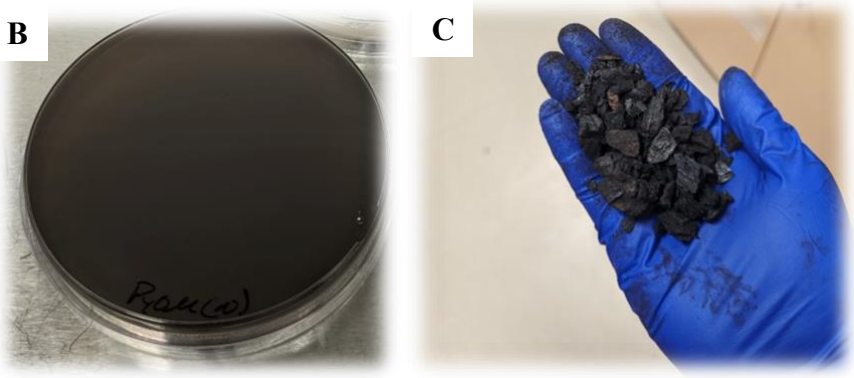


Figure B.2.1: A) Schematic diagram visualizing the vacuum siphoning set up for the creation of PyOM media. Prior to siphoning pyrogenic matter was ground, added to water, and heated at 40ºC overnight. After allowing 1 day of settling, the solution was vacuum siphoned as above. This extract would then be filtered through cheesecloth and coffee filters and allowed to settle one additional time before sterilization. B) And example of the field-sourced pyrogenic organic matter used to make PyOM media. C) An example plate of solid PyOM agar media.

Figure B.2.2

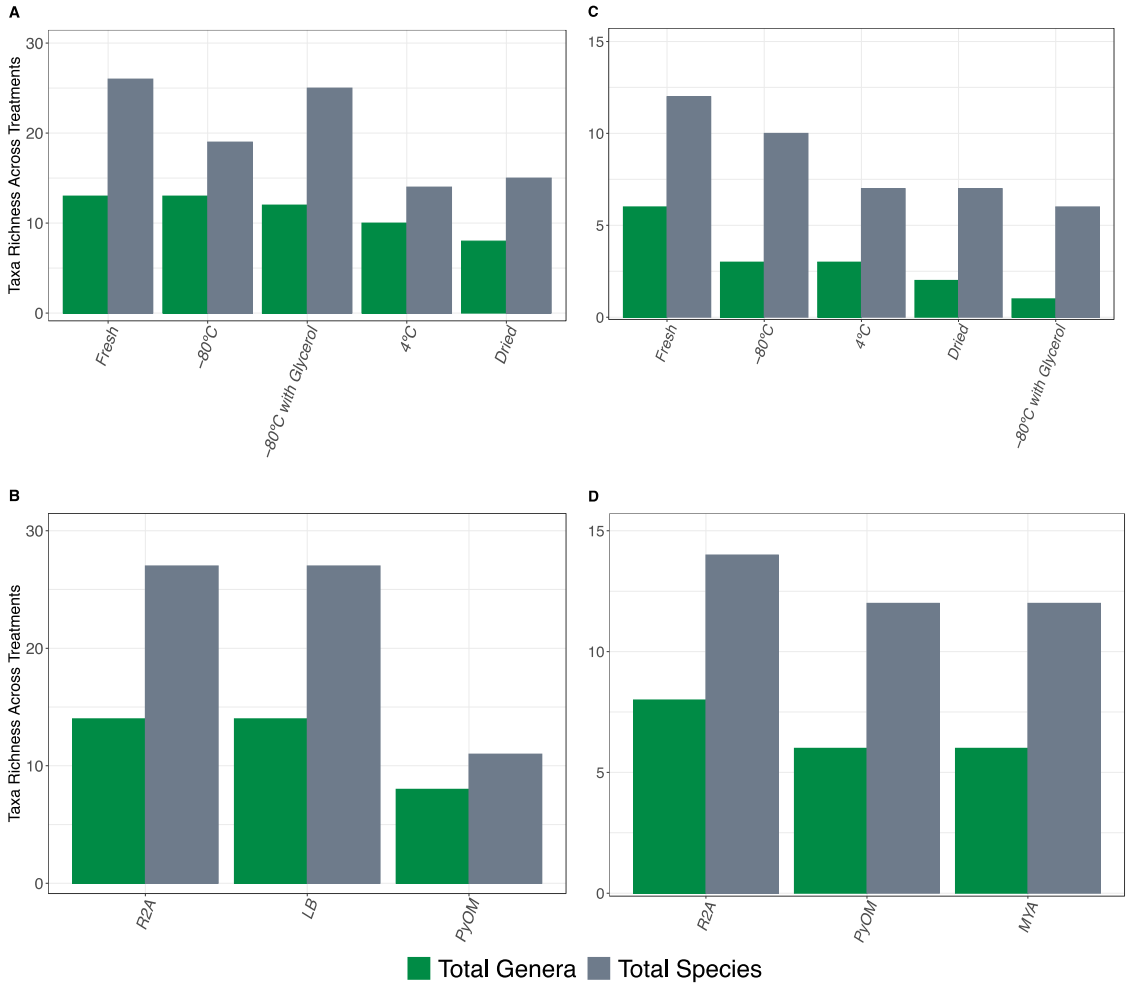


Figure B.2.2: Summarized counts of all bacterial and fungal genera (green) and species (grey) obtained from the soil storage experiment (A, Bacteria & B, Fungi) and from the media types experiments (C, Bacteria & D, Fungi).

Figure B.2.3

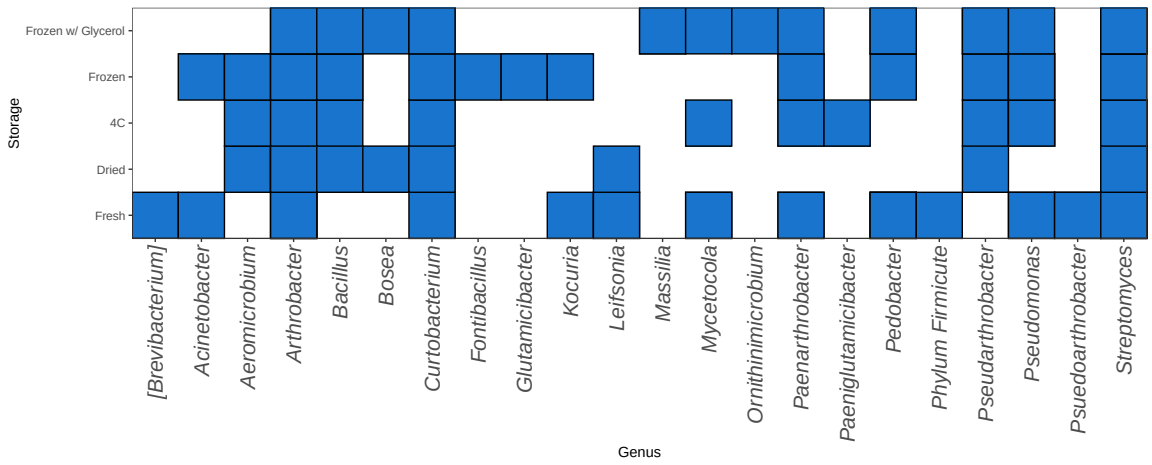


Figure B.2.3: Heatmap-like visualization of the diversity of all bacterial genera across all storage method treatments. Filled blue squares indicate the presence of the genus on the x axis in the treatment on the y axis.

Figure B.2.4

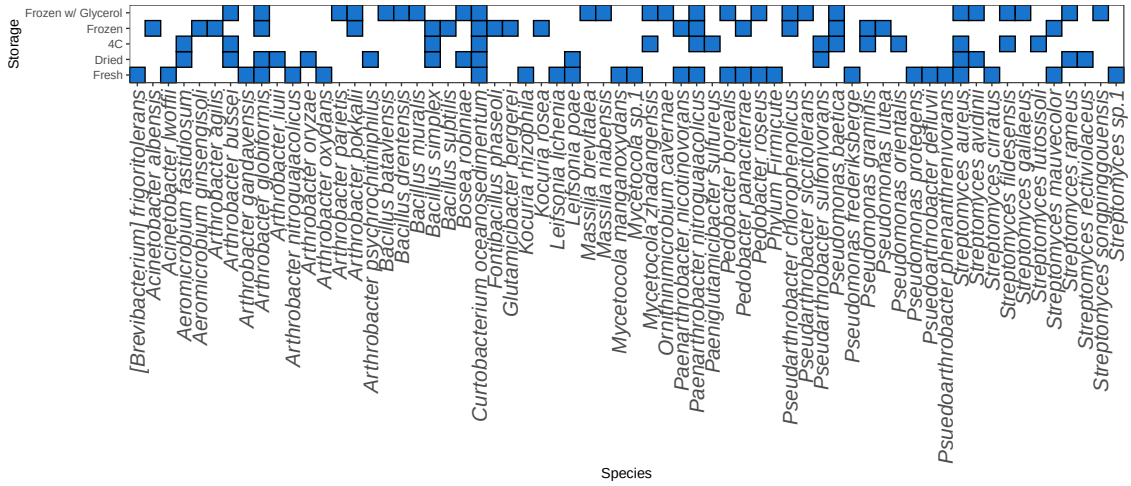


Figure B.2.4: Heatmap-like visualization of the diversity of all bacterial species across all storage method treatments. Filled blue squares indicate the presence of the species on the x axis in the treatment on the y axis.

Figure B.2.5

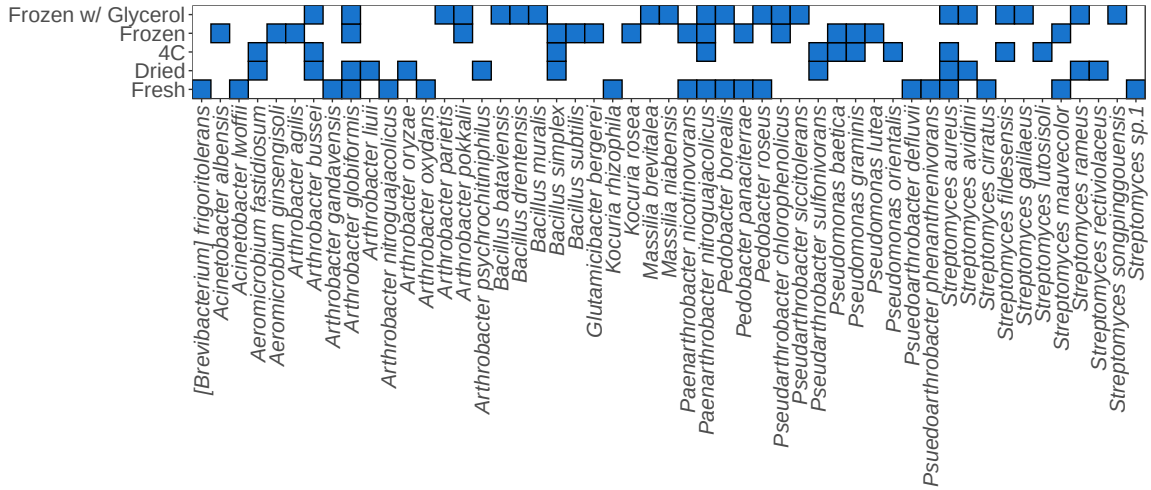


Figure B.2.5: Heatmap-like visualization of the diversity of all pyrophilous bacterial species across all storage method treatments. Filled blue squares indicate the presence of the species on the x axis in the treatment on the y axis.

Figure B.2.6

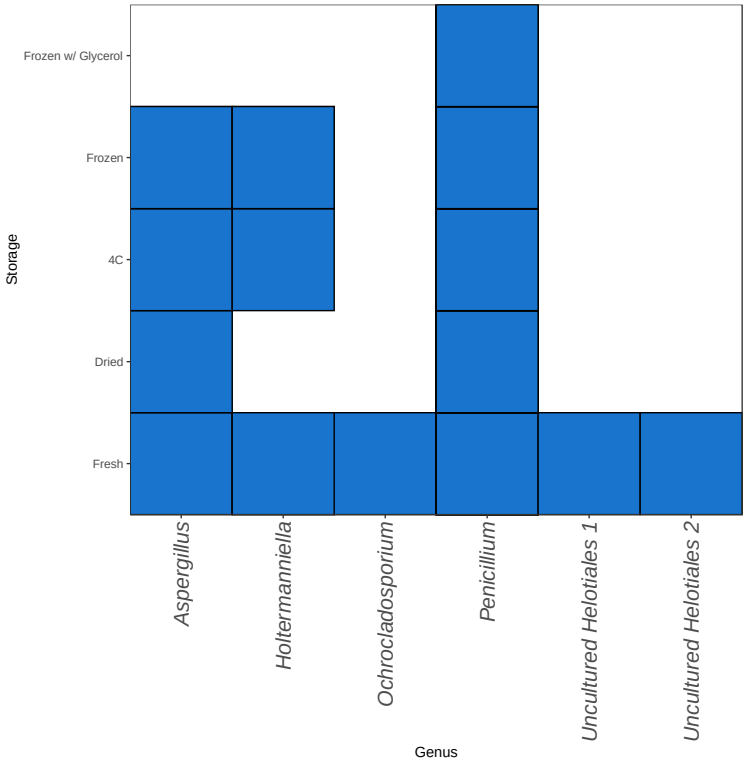


Figure B.2.6: Heatmap-like visualization of the diversity of all fungal genera across all storage method treatments. Filled blue squares indicate the presence of the genus on the x axis in the treatment on the y axis.

Figure B.2.7

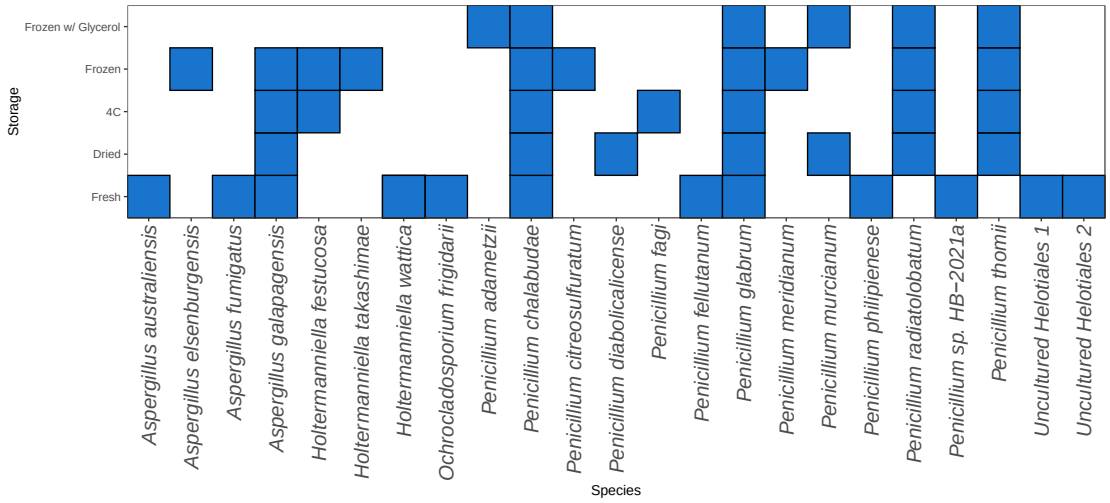


Figure B.2.7: Heatmap-like visualization of the diversity of all fungal species across all storage method treatments. Filled blue squares indicate the presence of the species on the x axis in the treatment on the y axis.

Figure B.2.8

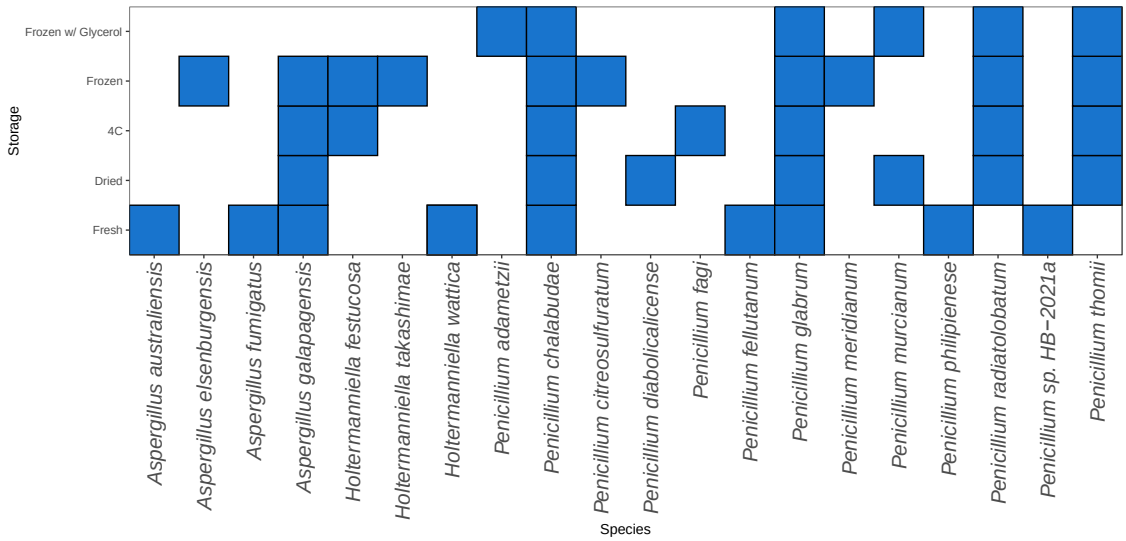


Figure B.2.8: Heatmap-like visualization of the diversity of all pyrophilous fungal species across all storage method treatments. Filled blue squares indicate the presence of the species on the x axis in the treatment on the y axis.

Figure B.2.9

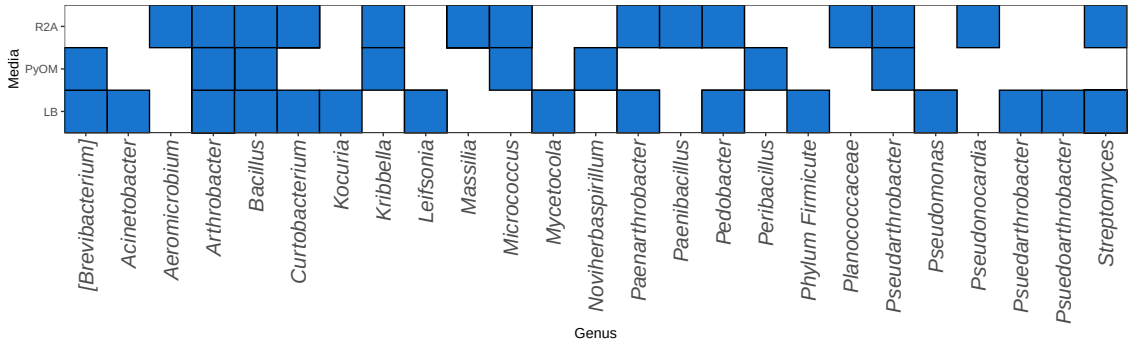


Figure B.2.9: Heatmap-like visualization of the diversity of all bacterial genera across all media type treatments. Filled blue squares indicate the presence of the genus on the x axis in the treatment on the y axis.

Figure B.2.10

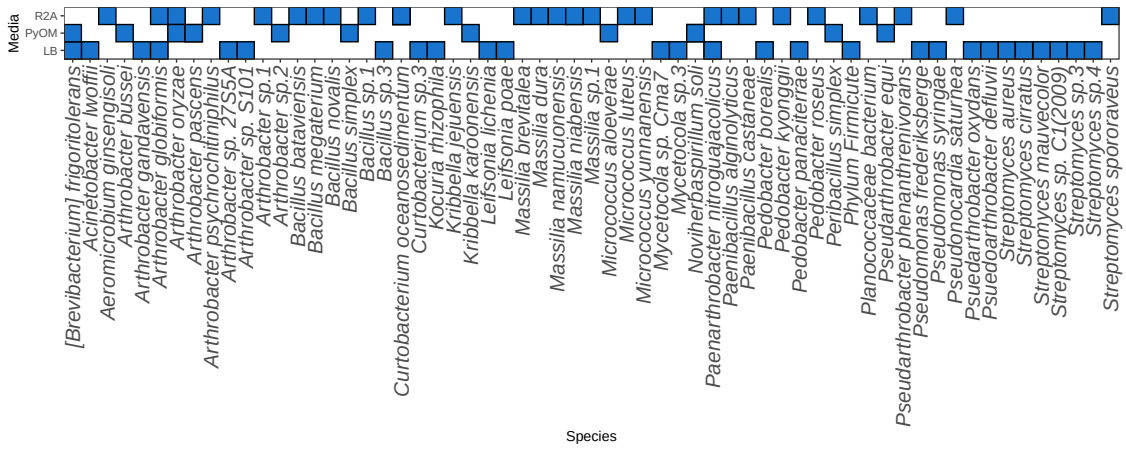


Figure B.2.10: Heatmap-like visualization of the diversity of all bacterial species across all media type treatments. Filled blue squares indicate the presence of the species on the x axis in the treatment on the y axis.

Figure B.2.11

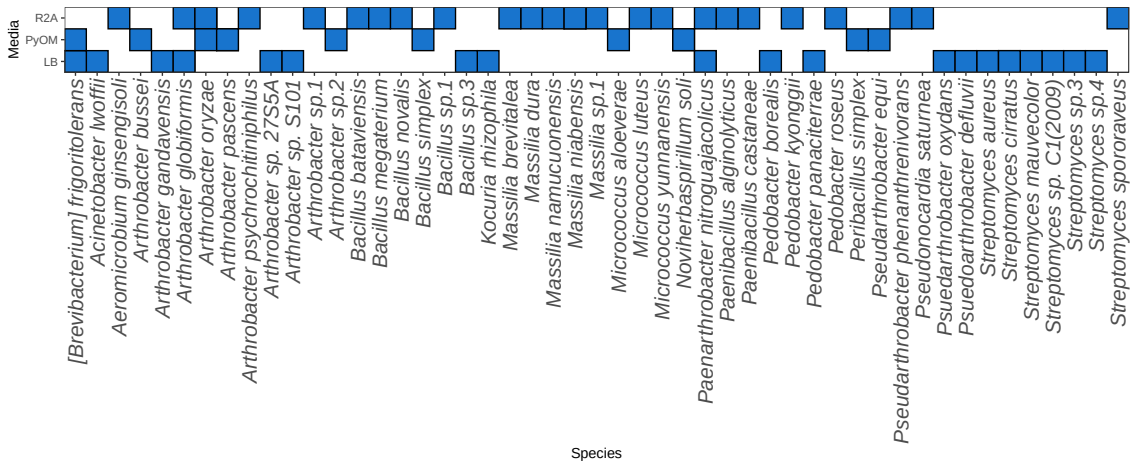


Figure B.2.11: Heatmap-like visualization of the diversity of all pyrophilous bacterial species across all media type treatments. Filled blue squares indicate the presence of the species on the x axis in the treatment on the y axis.

Figure B.2.12

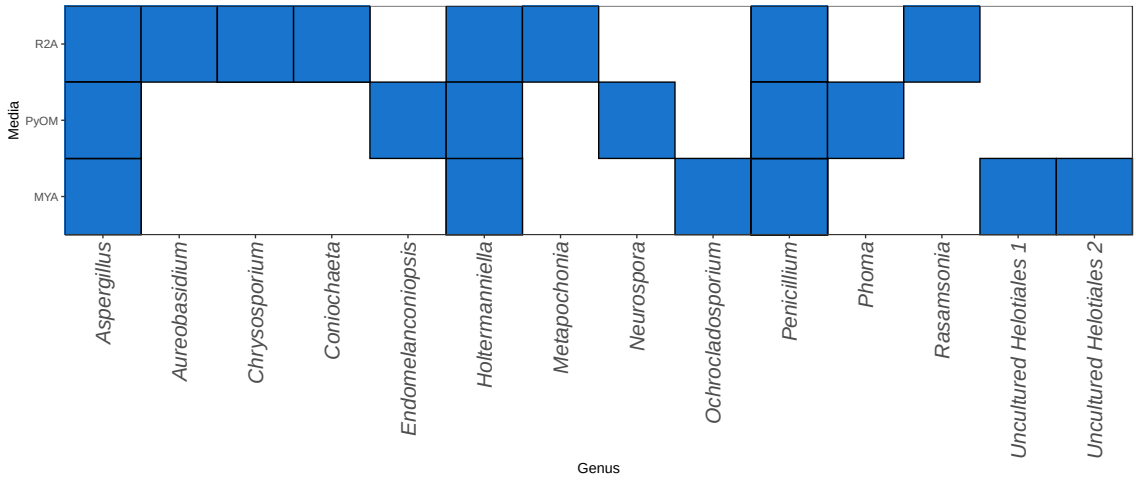


Figure B.2.12: Heatmap-like visualization of the diversity of all fungal genera across all media type treatments. Filled blue squares indicate the presence of the genus on the x axis in the treatment on the y axis.

Figure B.2.13

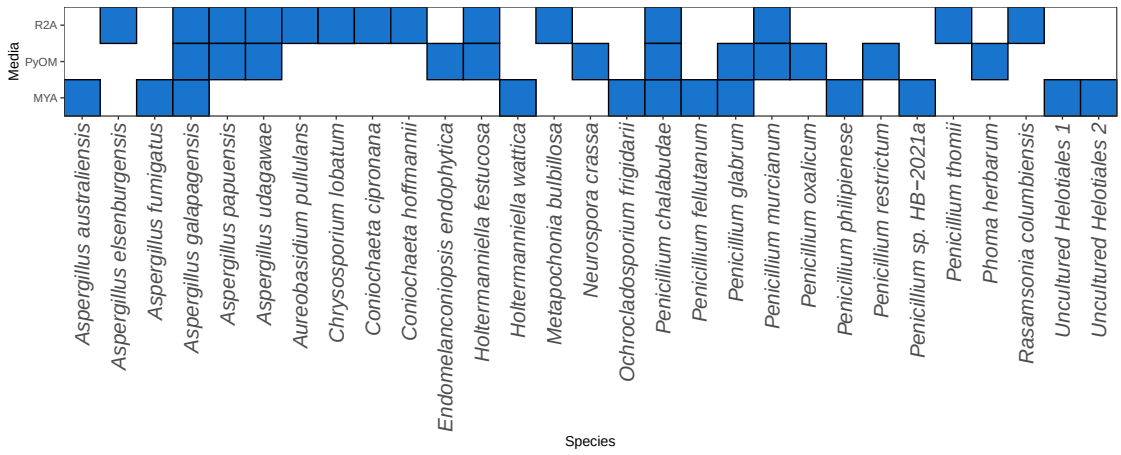


Figure B.2.13: Heatmap-like visualization of the diversity of all fungal species across all media type treatments. Filled blue squares indicate the presence of the species on the x axis in the treatment on the y axis.

Figure B.2.14

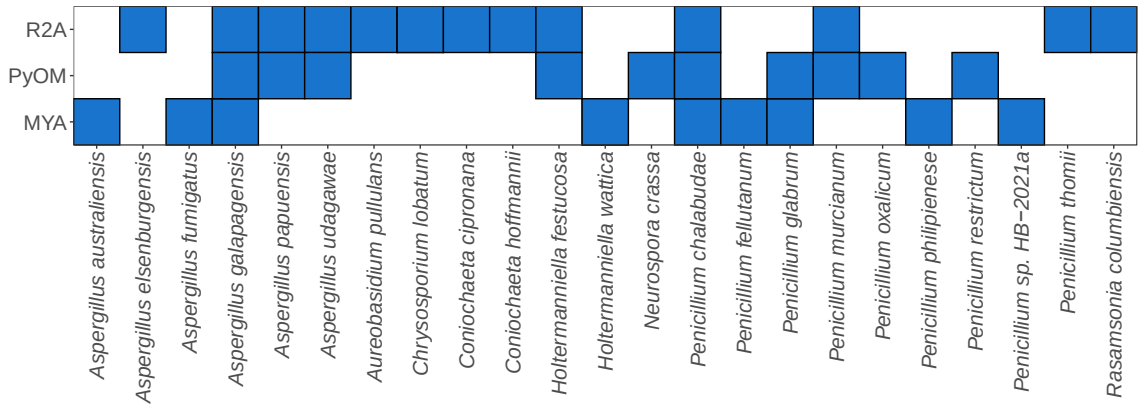


Figure B.2.14: Heatmap-like visualization of the diversity of all pyrophilous fungal species across all media type treatments. Filled blue squares indicate the presence of the species on the x axis in the treatment on the y axis.

B.3 Tables

Table B.3.1: All isolates obtained across both experiments.
For supplementary table B.3.1, please see the Microsoft Excel spreadsheet titled “Table B.3.1.xlsx”.
Table B.3.1 shows all isolates obtained from the El Dorado Fire 6 months post-fire across all experimental treatments.

Table B.3.2: All pyrophilous isolates obtained across both experiments.
For supplementary table B.3.2, please see the Microsoft Excel spreadsheet titled “Table B.3.2.xlsx”.
Table B.3.2 shows all pyrophilous isolates obtained from the El Dorado Fire 6 months post-fire across all experimental treatments.

Appendix C:

Supplemental Materials for Chapter IV

C.1 Figures

Figure C.1.1

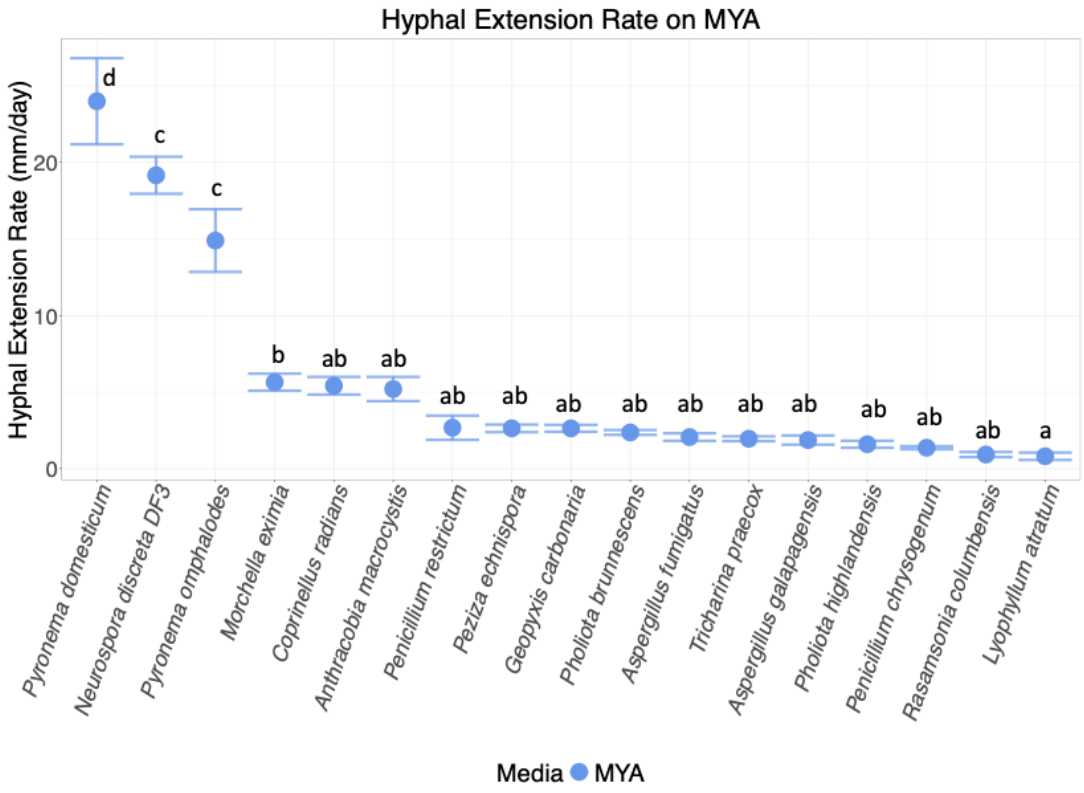


Figure C.1.1: Hyphal extension rates of hyphal fungal isolates grown on MYA in millimeters per day. Significance tested using ANOVA ($F_{1,16} = 51.52$, $p < 0.001$) and Tukey Post-Hoc analysis.

Figure C.1.2

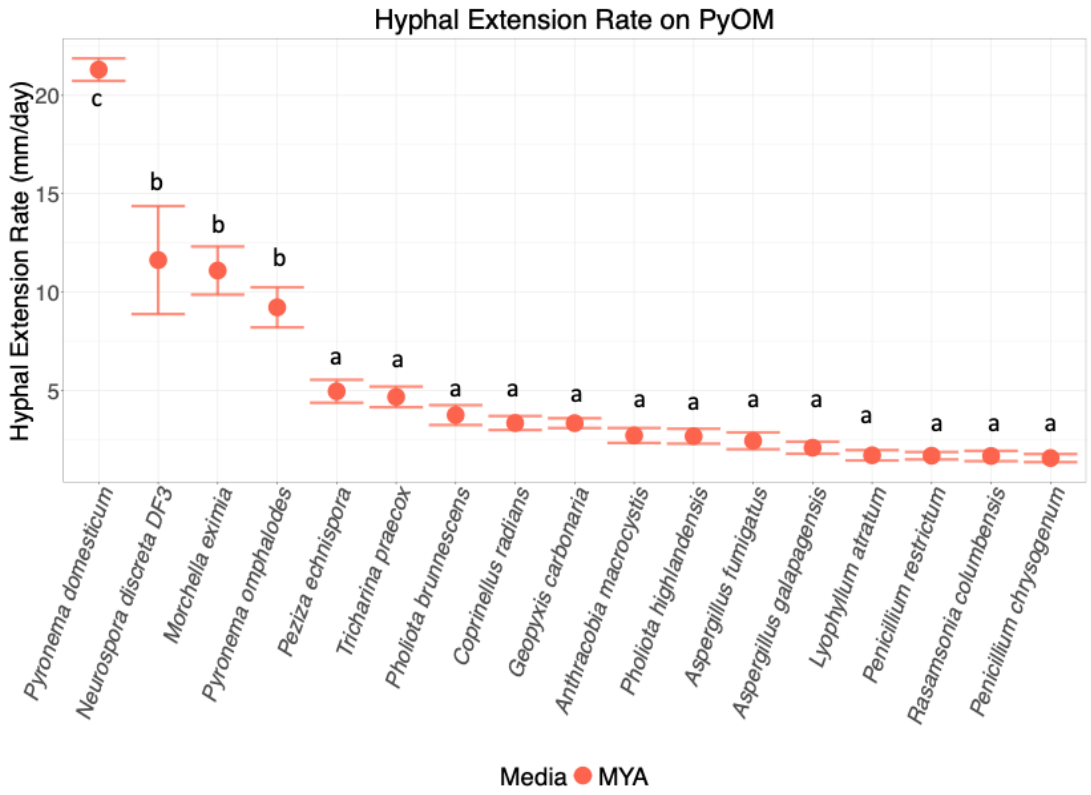


Figure C.1.2: Hyphal extension rates of hyphal fungal isolates grown on PyOM in millimeters per day. Significance tested using ANOVA ($F_{1,16} = 38.90$, $p < 0.001$), and Tukey Post-Hoc analysis.

Figure C.1.3

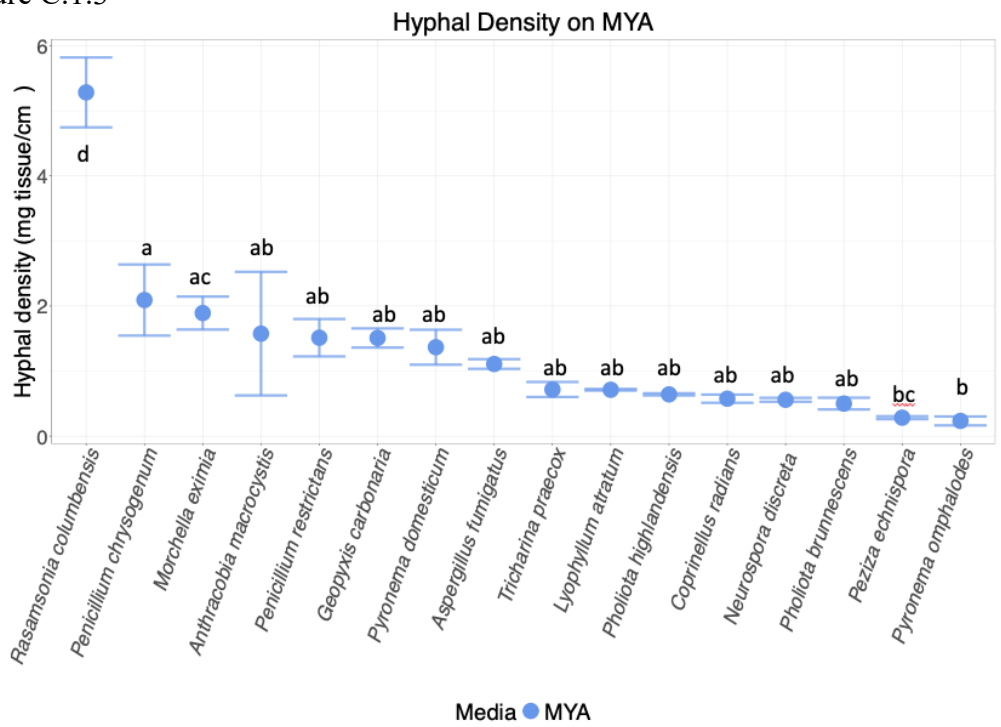


Figure C.1.3: Hyphal densities of isolates grown on MYA expressed in mg tissue / cm^2. Significance tested using ANOVA ($F_{1,15} = 12.9$, $p < 0.001$), and Tukey Post-Hoc analysis.

Figure C.1.4:

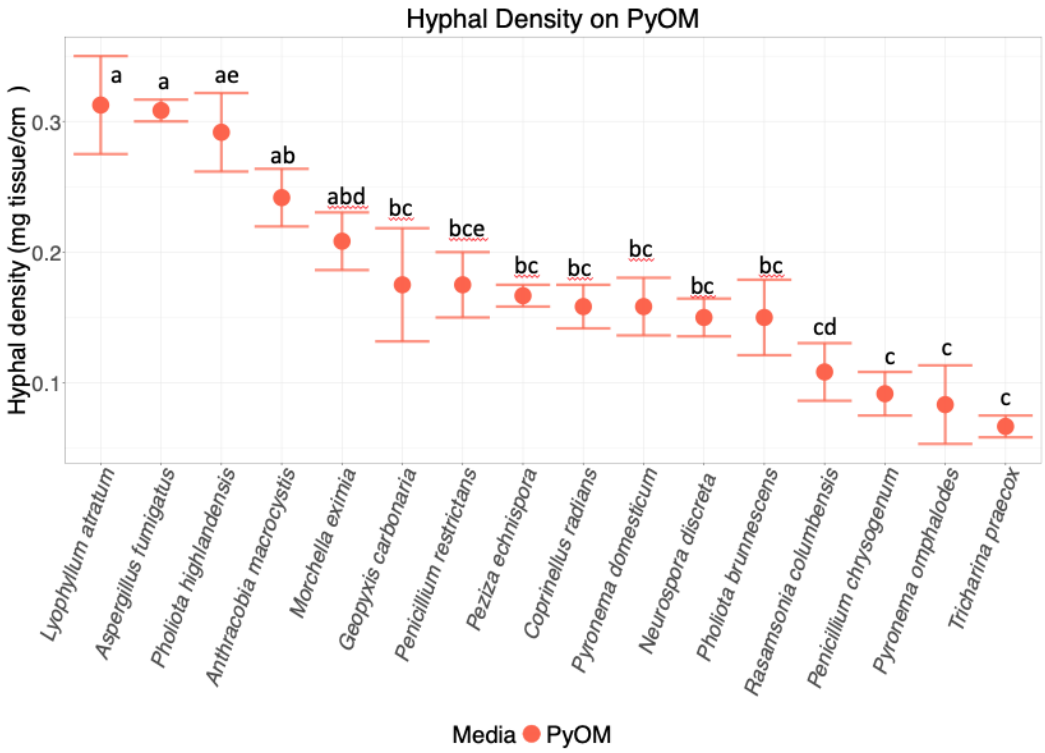


Figure C.1.4: Hyphal densities of isolates grown on MYA expressed in mg tissue / cm^2. Significance tested using ANOVA ($F_{1,15} = 10.36$, $p < 0.001$), and Tukey Post-Hoc analysis.

Figure C.1.5

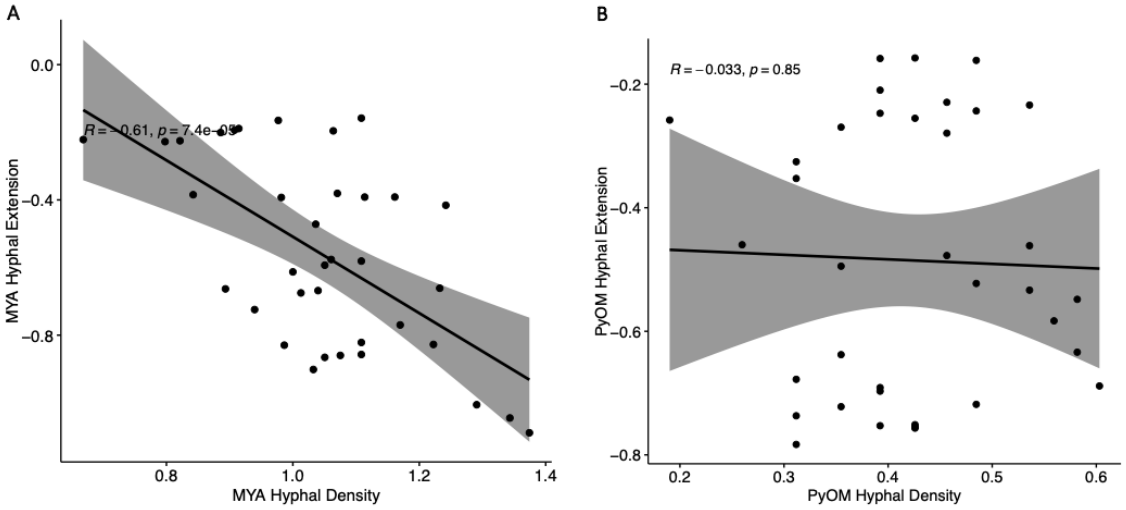


Figure C.1.5 Correlation between hyphal densities and hyphal extension rates. A) Linear correlation of MYA hyphal extension rate vs. MYA hyphal density. Correlations were calculated using the Pearson method. B) Linear correlation of PyOM hyphal extension rate vs. PyOM hyphal density. Correlations were calculated using the Pearson method

Figure C.1.6

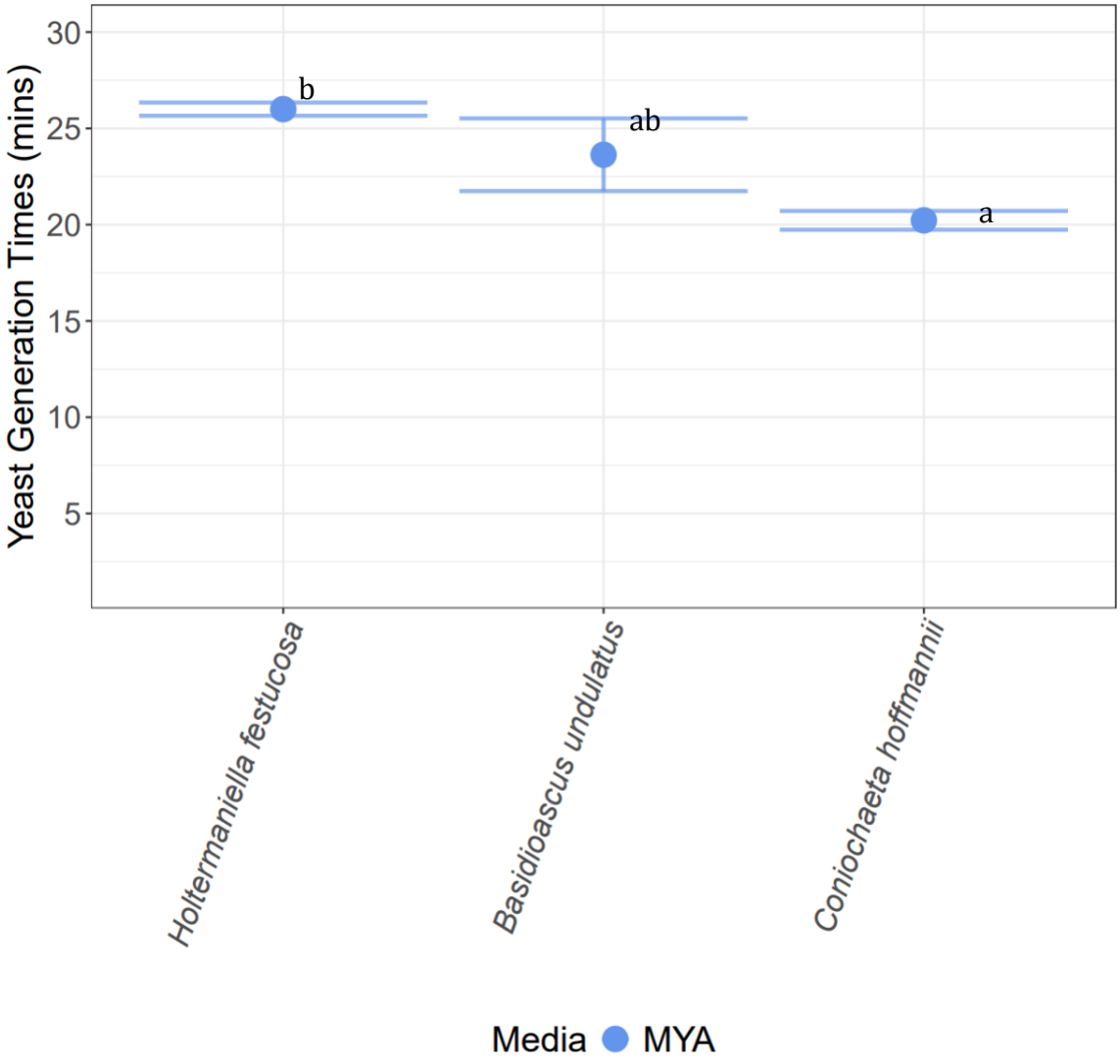


Figure C.1.6: Generation times of yeast isolates grown in MYA. Generation times are expressed in minutes, and are calculated from 10 OD_{600} measurements during each isolate's exponential growth phase. Significant differences between generation times was tested using ANOVA ($F_{2,27} = 6.453$) and a post-hoc Tukey's HSD test were used for group assignment.

Figure C.1.7

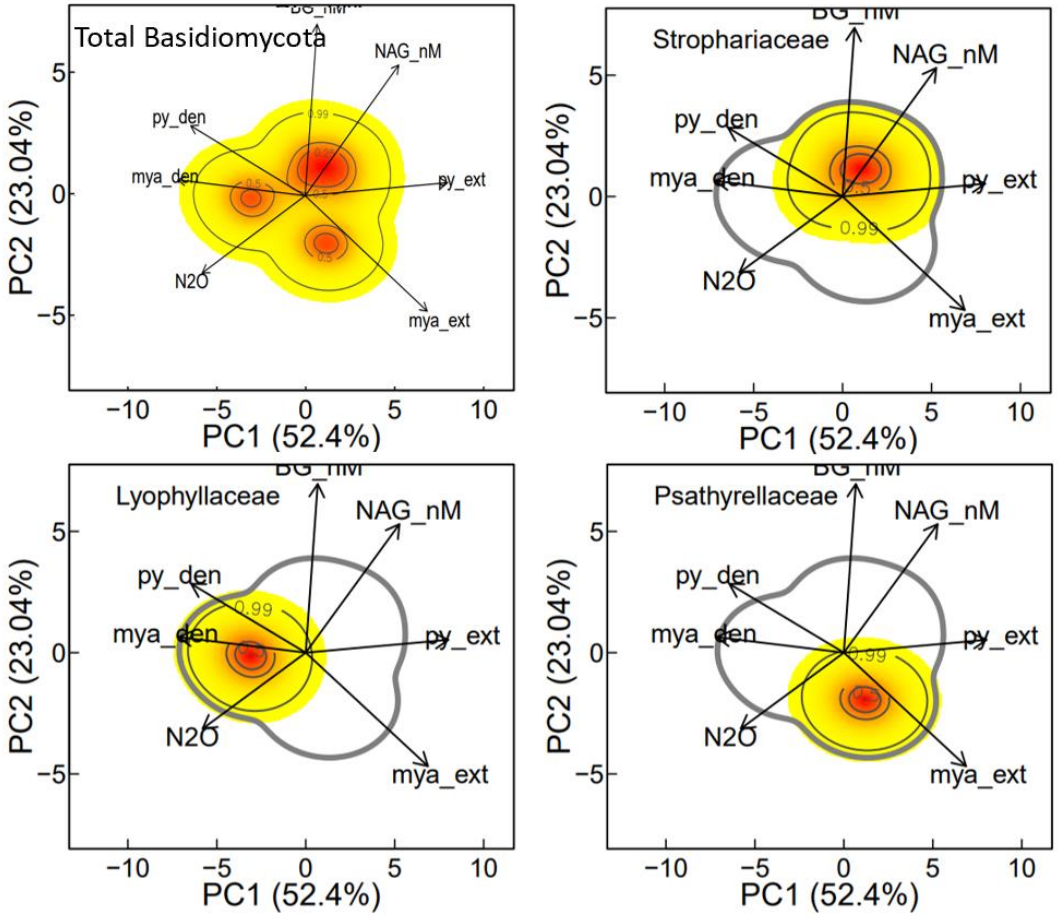


Figure C.1.7: Trait space ordinations of all hyphal Basidiomycota isolates (top left) and the trait space separated by the phylogenetic families of the isolates that were used in the total trait space ordination. Probability that a given isolate's trait will be determined by the trait axes are colored from yellow to red as probability increases. Peaks of significant trait space probability accumulation are bounded by 0.5 and 0.25 regions while total distribution is bounded within the 0.99 probability region.

Figure C.1.8

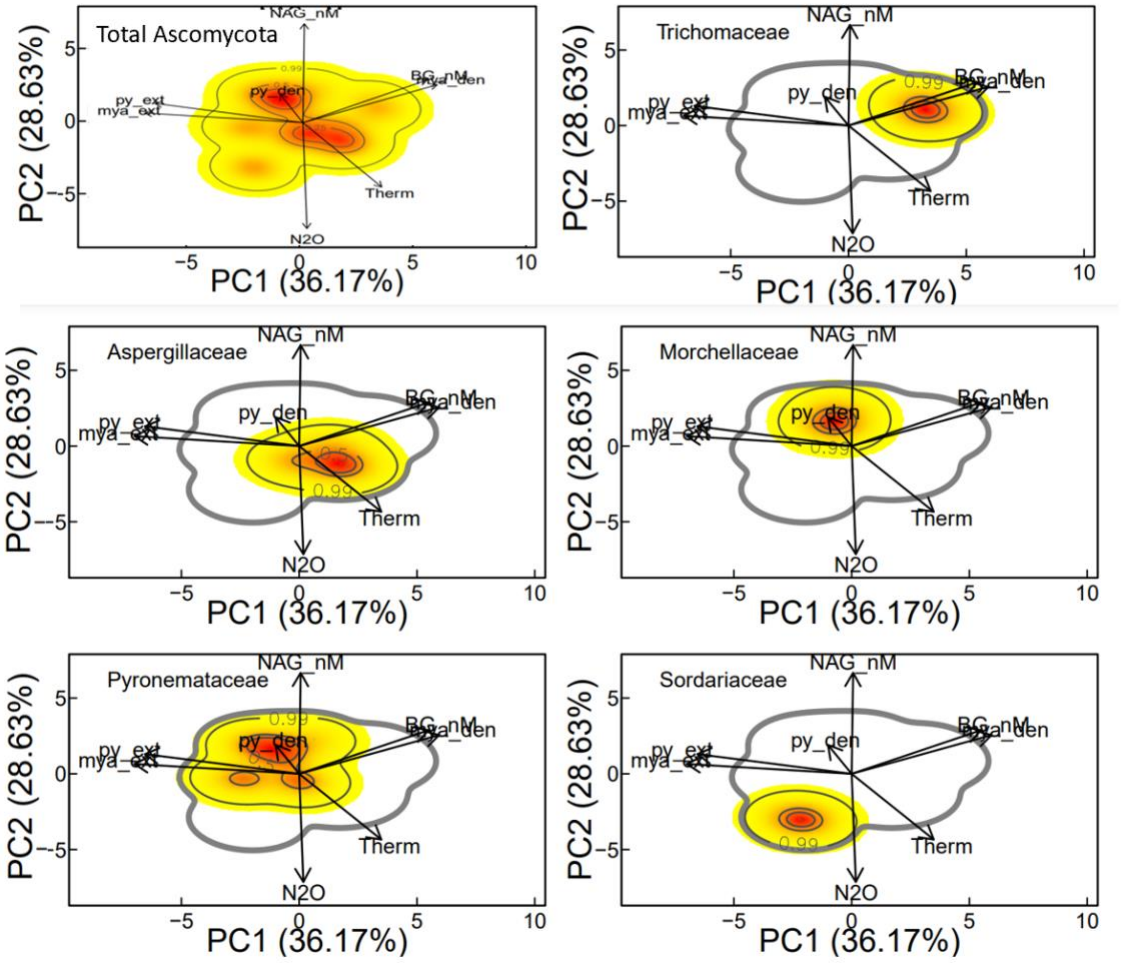


Figure C.1.8: Trait space ordinations of all hyphal Ascomycota isolates (top left) and the trait space separated by the phylogenetic families of the isolates that were used in the total trait space ordination. Probability that a given isolate's trait will be determined by the trait axes are colored from yellow to red as probability increases. Peaks of significant trait space probability accumulation are bounded by 0.5 and 0.25 regions while total distribution is bounded within the 0.99 probability region.

Figure C.1.9:

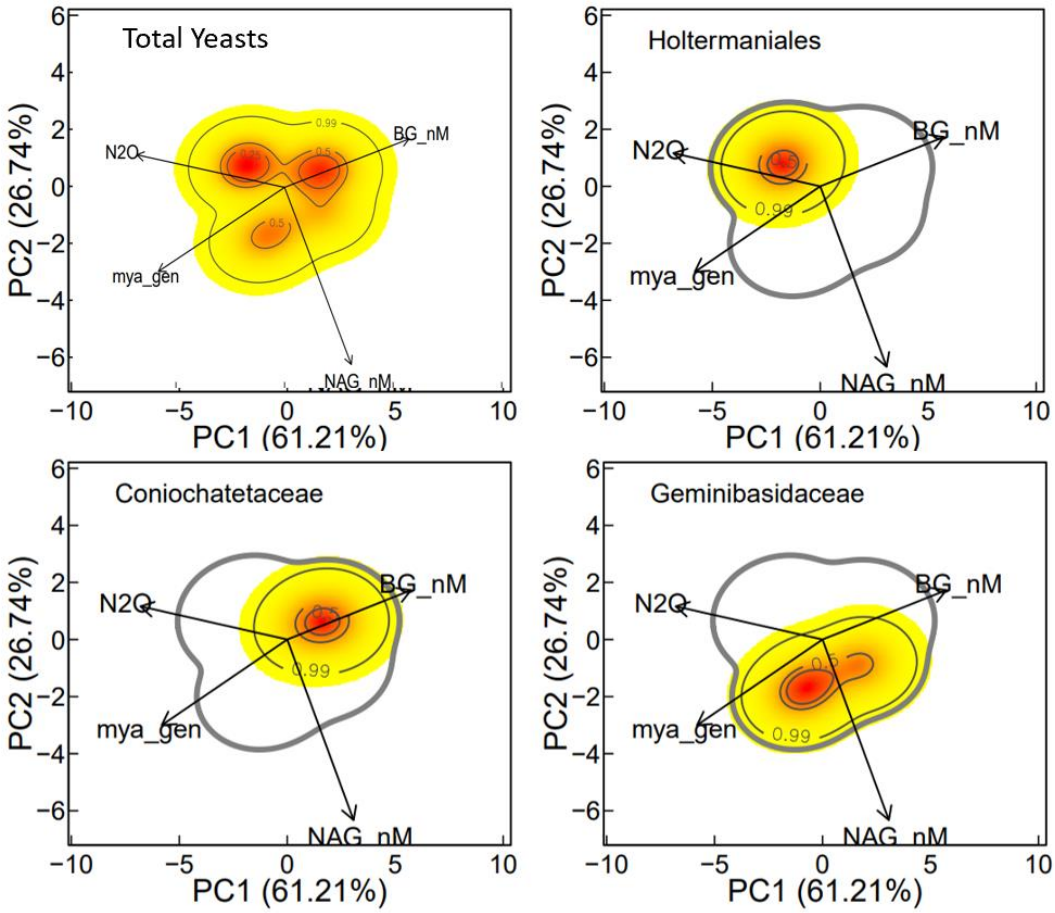


Figure C.1.9: Trait space ordinations of all yeast isolates (top left) and the trait space separated by the phylogenetic families of the isolates that were used in the total trait space ordination. Probability that a given isolate's trait will be determined by the trait axes are colored from yellow to red as probability increases. Peaks of significant trait space probability accumulation are bounded by 0.5 and 0.25 regions while total distribution is bounded within the 0.99 probability region.