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

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Key Words: fire, pyrophilous, culture, bacteria, fungi, soil.

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.

Introduction

In microbiological research, few 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, the hurdles of creating microbial culture collections are well documented and cannot be understated (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. Bearing all this in mind, when we set out to create a culture collection of pyrophilous (or “fire-loving”) microbes we knew we would face numerous technological and logistical challenges.

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). However, 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. 2022; 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 their traits and determine how they survived or thrived post-fire 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 Q1) the best methods to store post-fire soil to retain pyrophilous microbial culturable viability and Q2) 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 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 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.

Methods

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.

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 soil with glycerol to create a soil slurry and then frozen (-80ºC) (Figure 1).

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 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 S1**)**. 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.

Microbial Isolation Method

Culturing from Soil: 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.

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 with then stored at room temperature for future use.

<u>Soil Storage Method Experimental Design</u>

For each of 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 1).

<u>Effect of Media Type Experimental Design</u>

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 1).

<u>DNA Extraction & PCR</u>

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 supplements).

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.

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. 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 2.

Results

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 2) and all isolates are listed in Table S1. 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. 2021; 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 S2.

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 3A) and fungi (ANOVA: $F_{1,4} = 19.78$, $p < 0.001$; Figure 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).

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 3C) and fungi ($F_{1,2} = 7.77$, $p < 0.01$; Figure 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.

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 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 S2A and S3) and 64 bacterial species (Figure S2A and S4). 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 S3). 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 S4).

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 4A, 5A; S5). 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 S2B and S6) and 23 species (Figures S2B and S7) from the soil storage experiment, of which we found literature support as pyrophilous for 3 genera (Figures 4B and 5B) and 20 species (Figure S8). 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 S6). For species, fresh soil captured 52%, followed by 43% in frozen, and 30% in refrigerated and 26% in frozen with glycerol (Figure S7). 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 5B). For pyrophilous species, frozen soil captured 50%, followed closely by 45% in fresh, 35% in refrigerated and 30% in air-dried (Figure S8).

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.

<u>Effect of Media Selection on Diversity</u>

Choice of growth media strongly affected both bacterial and fungal isolate 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 S2C, S9 and S10). 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 4C, 6A; S11). 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 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 S2D, S12, and S13). 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 4D, 6B; S14). 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 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.

Discussion

Throughout our experiments we managed to obtain a remarkable array of bacterial and fungal morphotypes (Figure 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 4 & 5). Additionally, we found that the best media for culturing pyrophilous microbes was the oligotrophic R2A media (Figures 4 & 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 has informed our continual culturing efforts and resulted in a collection of over 500 post-fire microbial isolates (Supplementary File 2).

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 3A and 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. 2021; Pulido-Chavez et al. 2022; 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. 2022; 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 4B and 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 4D and 6B). Future work repeating the storage experiments utilizing R2A and PyOM might improve pyrophilous fungal isolate retention. 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, 4, and 5). This finding is significant 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). 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 4C) and fungal (Figure 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 4D) and 44% of bacteria (Figure 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 6). These taxa are particularly interesting because like *Massilia*, *Noviherbaspirillum* has been repeated observed increasing in abundance post-fire (Enright et al. 2021; Pulido-Chavez et al. 2022; 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 is 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.

In conclusion, 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 2). 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.

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 Marely Vega, 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.

Works Cited

Alster, Charlotte J, Steven D Allison, Nels G Johnson, Sydney I Glassman, and Kathleen K Treseder. 2021. "Phenotypic Plasticity of Fungal Traits in Response to Moisture and Temperature." *ISME Communications* 1 (1): 43. https://doi.org/10.1038/s43705-021-00045-9.

Aranda, Elisabet, Patricia Godoy, Rocío Reina, Marina Badia-Fabregat, Mònica Rosell, Ernest Marco-Urrea, and Inmaculada García-Romera. 2017. "Isolation of Ascomycota Fungi with Capability to Transform PAHs: Insights into the Biodegradation Mechanisms of Penicillium Oxalicum." *International Biodeterioration & Biodegradation* 122 (August):141–50. https://doi.org/10.1016/j.ibiod.2017.05.015.

Arnold, A. Elizabeth, and F. Lutzoni. 2007. "Diversity and Host Range of Foliar Fungal Endophytes: Are Tropical Leaves Biodiversity Hotspots?" *Ecology* 88 (3): 541–49. https://doi.org/10.1890/05-1459.

Bárcenas-Moreno, Gema, and Erland Bååth. 2009. "Bacterial and Fungal Growth in Soil Heated at Different Temperatures to Simulate a Range of Fire Intensities." *Soil Biology and Biochemistry* 41 (12): 2517–26. https://doi.org/10.1016/j.soilbio.2009.09.010.

Bárcenas-Moreno, Gema, Fuensanta García-Orenes, Jorge Mataix-Solera, Jorge Mataix-Beneyto, and Erland Bååth. 2011. "Soil Microbial Recolonisation after a Fire in a Mediterranean Forest." *Biology and Fertility of Soils* 47 (3): 261–72. https://doi.org/10.1007/s00374-010-0532-2.

Ben-Jacob, Eshel, Inon Cohen, and David L. Gutnick. 1998. "COOPERATIVE ORGANIZATION OF BACTERIAL COLONIES: From Genotype to Morphotype."

Annual Review of Microbiology 52 (Volume 52, 1998): 779–806. https://doi.org/10.1146/annurev.micro.52.1.779.

Brown, Shawn, Allison Veach, Jonathan Horton, Emerald Ford, Ari Jumpponen, and Richard Baird. 2019. "Context Dependent Fungal and Bacterial Soil Community Shifts in Response to Recent Wildfires in the Southern Appalachian Mountains." *Forest Ecology and Management*, August. https://doi.org/10.1016/j.foreco.2019.117520.

Caiafa, Marcos V., Amelia R. Nelson, Thomas Borch, Holly K. Roth, Timothy S. Fegel, Charles C. Rhoades, Michael J. Wilkins, and Sydney I. Glassman. 2023. "Distinct Fungal and Bacterial Responses to Fire Severity and Soil Depth across a Ten-Year Wildfire Chronosequence in Beetle-Killed Lodgepole Pine Forests." *Forest Ecology and Management* 544 (September):121160. https://doi.org/10.1016/j.foreco.2023.121160.

Caktu, Kivilcim, and Emir Turkoglu. 2011. "Microbial Culture Collections: The Essential Resources for Life." *Gazi University Journal of Science* 24 (2): 175–80.

Caon, Lucrezia, V. Ramón Vallejo, Coen J. Ritsema, and Violette Geissen. 2014. "Effects of Wildfire on Soil Nutrients in Mediterranean Ecosystems." *Earth-Science Reviews* 139 (December):47–58. https://doi.org/10.1016/j.earscirev.2014.09.001.

Carini, Paul. 2019. "A 'Cultural' Renaissance: Genomics Breathes New Life into an Old Craft." *mSystems* 4 (3). https://doi.org/10.1128/mSystems.00092-19.

Castaño, Carles, Javier Parladé, Joan Pera, Juan Martínez de Aragón, Josu G. Alday, and José Antonio Bonet. 2016. "Soil Drying Procedure Affects the DNA Quantification of Lactarius Vinosus but Does Not Change the Fungal Community Composition." *Mycorrhiza* 26 (8): 799–808. https://doi.org/10.1007/s00572-016-0714-3.

Černohlávková, Jitka, Jiří Jarkovský, Michala Nešporová, and Jakub Hofman. 2009. "Variability of Soil Microbial Properties: Effects of Sampling, Handling and Storage." *Ecotoxicology and Environmental Safety* 72 (8): 2102–8. https://doi.org/10.1016/j.ecoenv.2009.04.023.

Chase, Alexander B., Ulas Karaoz, Eoin L. Brodie, Zulema Gomez-Lunar, Adam C. Martiny, and Jennifer B. H. Martiny. 2017. "Microdiversity of an Abundant Terrestrial Bacterium Encompasses Extensive Variation in Ecologically Relevant Traits." *mBio* 8 (6): 10.1128/mbio.01809-17. https://doi.org/10.1128/mbio.01809-17.

Collado, Javier, Gonzalo Platas, Barbara Paulus, and Gerald F. Bills. 2007. "High-Throughput Culturing of Fungi from Plant Litter by a Dilution-to-Extinction Technique." *FEMS Microbiology Ecology* 60 (3): 521–33. https://doi.org/10.1111/j.1574-6941.2007.00294.x.

Cui, Hang, Caihuan Wang, Zhenhong Gu, Honghui Zhu, Shenlei Fu, and Qing Yao. 2014. "Evaluation of Soil Storage Methods for Soil Microbial Community Using Genetic and Metabolic Fingerprintings." *European Journal of Soil Biology* 63 (July):55–63. https://doi.org/10.1016/j.ejsobi.2014.05.006.

Davis, Kathryn E. R., Shayne J. Joseph, and Peter H. Janssen. 2005. "Effects of Growth Medium, Inoculum Size, and Incubation Time on Culturability and Isolation of Soil Bacteria." *Applied and Environmental Microbiology* 71 (2): 826–34. https://doi.org/10.1128/AEM.71.2.826-834.2005.

Enright, Dylan J., Kerri M. Frangioso, Kazuo Isobe, David M. Rizzo, and Sydney I. Glassman. 2021. "Mega-Fire in Redwood Tanoak Forest Reduces Bacterial and Fungal Richness and Selects for Pyrophilous Taxa and Traits That Are Phylogenetically Conserved." https://doi.org/10.1101/2021.06.30.450634.

Falconer, Ruth E., Wilfred Otten, and Nia A. White. 2015. "Chapter One - Toward Modeling the Resistance and Resilience of 'Below-Ground' Fungal Communities: A Mechanistic and

Trait-Based Approach." In *Advances in Applied Microbiology*, edited by Sima Sariaslani and Geoffrey Michael Gadd, 93:1–44. Academic Press. https://doi.org/10.1016/bs.aambs.2015.08.001.

Ferrenberg, Scott, Sean P. O'Neill, Joseph E. Knelman, Bryan Todd, Sam Duggan, Daniel Bradley, Taylor Robinson, et al. 2013. "Changes in Assembly Processes in Soil Bacterial Communities Following a Wildfire Disturbance." *The ISME Journal* 7 (6): 1102–11. https://doi.org/10.1038/ismej.2013.11.

Fischer, Monika S., Frances Grace Stark, Timothy D. Berry, Nayela Zeba, Thea Whitman, and Matthew F. Traxler. 2021. "Pyrolyzed Substrates Induce Aromatic Compound Metabolism in the Post-Fire Fungus, Pyronema Domesticum." *Frontiers in Microbiology* 12. https://www.frontiersin.org/article/10.3389/fmicb.2021.729289.

Franzosa, Eric A., Tiffany Hsu, Alexandra Sirota-Madi, Afrah Shafquat, Galeb Abu-Ali, Xochitl C. Morgan, and Curtis Huttenhower. 2015. "Sequencing and beyond: Integrating Molecular 'omics' for Microbial Community Profiling." *Nature Reviews Microbiology* 13 (6): 360–72. https://doi.org/10.1038/nrmicro3451.

González-Pérez, José A., Francisco J. González-Vila, Gonzalo Almendros, and Heike Knicker. 2004. "The Effect of Fire on Soil Organic Matter—a Review." *Environment International* 30 (6): 855–70. https://doi.org/10.1016/j.envint.2004.02.003.

Haridas, Sajeet, Colette Breuill, Joerg Bohlmann, and Tom Hsiang. 2011. "A Biologist's Guide to *de Novo* Genome Assembly Using next-Generation Sequence Data: A Test with Fungal Genomes." *Journal of Microbiological Methods* 86 (3): 368–75. https://doi.org/10.1016/j.mimet.2011.06.019.

Hernandez, Damian J, Aaron S David, Eric S Menges, Christopher A Searcy, and Michelle E Afkhami. 2021. "Environmental Stress Destabilizes Microbial Networks." *The ISME Journal* 15 (6): 1722–34. https://doi.org/10.1038/s41396-020-00882-x.

Ho, Adrian, D. Paolo Di Lonardo, and Paul L. E. Bodelier. 2017. "Revisiting Life Strategy Concepts in Environmental Microbial Ecology." *FEMS Microbiology Ecology* 93 (3): fix006. https://doi.org/10.1093/femsec/fix006.

Hugenholtz, Philip. 2002. "Exploring Prokaryotic Diversity in the Genomic Era." *Genome Biology* 3 (2): reviews0003.1. https://doi.org/10.1186/gb-2002-3-2-reviews0003.

Joint, Ian, Martin Mühling, and Joël Querellou. 2010. "Culturing Marine Bacteria – an Essential Prerequisite for Biodiscovery." *Microbial Biotechnology* 3 (5): 564–75. https://doi.org/10.1111/j.1751-7915.2010.00188.x.

Jung, Sokhee P., and Hojeong Kang. 2014. "Assessment of Microbial Diversity Bias Associated with Soil Heterogeneity and Sequencing Resolution in Pyrosequencing Analyses." *Journal of Microbiology* 52 (7): 574–80. https://doi.org/10.1007/s12275-014-3636-9.

Kämpfer, Peter. 2014. "Chapter 14 - Continuing Importance of the 'Phenotype' in the Genomic Era." In *Methods in Microbiology*, edited by Michael Goodfellow, Iain Sutcliffe, and Jongsik Chun, 41:307–20. New Approaches to Prokaryotic Systematics. Academic Press. https://doi.org/10.1016/bs.mim.2014.07.005.

Knicker, Heike. 2011. "Pyrogenic Organic Matter in Soil: Its Origin and Occurrence, Its Chemistry and Survival in Soil Environments." *Quaternary International*, Black Soils and Black Sediments -- Archives of Landscape Evolution, 243 (2): 251–63. https://doi.org/10.1016/j.quaint.2011.02.037.

Kuo, Hsiao-Che, Sun Hui, Jaeyoung Choi, Frederick O. Asiegbu, Jari P. T. Valkonen, and Yong-Hwan Lee. 2014. "Secret Lifestyles of *Neurospora Crassa*." *Scientific Reports* 4 (May):5135. https://doi.org/10.1038/srep05135.

Leadbetter, Jared R. 2003. "Cultivation of Recalcitrant Microbes: Cells Are Alive, Well and Revealing Their Secrets in the 21st Century Laboratory." *Current Opinion in Microbiology* 6 (3): 274–81. https://doi.org/10.1016/S1369-5274(03)00041-9.

Lilleskov, Erik A., Thomas W. Kuyper, Martin I. Bidartondo, and Erik A. Hobbie. 2019. "Atmospheric Nitrogen Deposition Impacts on the Structure and Function of Forest Mycorrhizal Communities: A Review." *Environmental Pollution* 246 (March):148–62. https://doi.org/10.1016/j.envpol.2018.11.074.

Malik, Khursheed A., and Dieter Claus. 1987. "Bacterial Culture Collections: Their Importance to Biotechnology and Microbiology." *Biotechnology and Genetic Engineering Reviews* 5 (1): 137–98. https://doi.org/10.1080/02648725.1987.10647837.

Malosso, Elaine, Ian S. Waite, Lorna English, David W. Hopkins, and Anthony G. O'Donnell. 2006. "Fungal Diversity in Maritime Antarctic Soils Determined Using a Combination of Culture Isolation, Molecular Fingerprinting and Cloning Techniques." *Polar Biology* 29 (7): 552–61. https://doi.org/10.1007/s00300-005-0088-z.

Martiny, Adam C. 2019. "High Proportions of Bacteria Are Culturable across Major Biomes." *The ISME Journal* 13 (8): 2125–28. https://doi.org/10.1038/s41396-019-0410-3.

Maynard, Daniel S., Mark A. Bradford, Kristofer R. Covey, Daniel Lindner, Jessie Glaeser, Douglas A. Talbert, Paul Joshua Tinker, Donald M. Walker, and Thomas W. Crowther. 2019. "Consistent Trade-Offs in Fungal Trait Expression across Broad Spatial Scales." *Nature Microbiology* 4 (5): 846–53. https://doi.org/10.1038/s41564-019-0361-5.

Michelotti, Lucas A., and Jessica R. Miesel. 2015. "Source Material and Concentration of Wildfire-Produced Pyrogenic Carbon Influence Post-Fire Soil Nutrient Dynamics." *Forests* 6 (4): 1325–42. https://doi.org/10.3390/f6041325.

Petersen, P. M. 1970. "Danish Fireplace Fungi-an Ecological Investigation on Fungi on Burns." *Dansk Botanisk Arkiv* 27 (3): 1–97.

Pierson, Derek N., Peter R. Robichaud, Charles C. Rhoades, and Robert E. Brown. 2019. "Soil Carbon and Nitrogen Eroded after Severe Wildfire and Erosion Mitigation Treatments." *International Journal of Wildland Fire* 28 (10): 814–21. https://doi.org/10.1071/WF18193.

Prakash, Om, Yogesh Shouche, Kamlesh Jangid, and Joel E. Kostka. 2013. "Microbial Cultivation and the Role of Microbial Resource Centers in the Omics Era." *Applied Microbiology and Biotechnology* 97 (1): 51–62. https://doi.org/10.1007/s00253-012-4533-y.

Preston, C. M., and M. W. I. Schmidt. 2006. "Black (Pyrogenic) Carbon in Boreal Forests: A Synthesis of Current Knowledge and Uncertainties." *Biogeosciences Discussions* 3 (1): 211–71.

Pulido-Chavez, M. Fabiola, James W. J. Randolph, Cassandra Zalman, Loralee Larios, Peter M. Homyak, and Sydney I. Glassman. 2023. "Rapid Bacterial and Fungal Successional Dynamics in First Year after Chaparral Wildfire." *Molecular Ecology* 32 (7): 1685–1707. https://doi.org/10.1111/mec.16835.

R Core Team. 2020. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/.

Reasoner, D J, and E E Geldreich. 1985. "A New Medium for the Enumeration and Subculture of Bacteria from Potable Water." *Applied and Environmental Microbiology* 49 (1): 1–7. https://doi.org/10.1128/aem.49.1.1-7.1985.

Reyes-César, Anaisell, Ángel E. Absalón, Francisco J. Fernández, Juan Manuel González, and Diana V. Cortés-Espinosa. 2014. "Biodegradation of a Mixture of PAHs by Non-Ligninolytic Fungal Strains Isolated from Crude Oil-Contaminated Soil." *World Journal of Microbiology and Biotechnology* 30 (3): 999–1009. https://doi.org/10.1007/s11274-013-1518-7.

Rubin, Benjamin E. R., Sean M. Gibbons, Suzanne Kennedy, Jarrad Hampton-Marcell, Sarah Owens, and Jack A. Gilbert. 2013. "Investigating the Impact of Storage Conditions on Microbial Community Composition in Soil Samples." *PLOS ONE* 8 (7): e70460. https://doi.org/10.1371/journal.pone.0070460.

Sánchez-Clemente, Rubén, María Isabel Igeño, Ana G. Población, María Isabel Guijo, Faustino Merchán, and Rafael Blasco. 2018. "Study of pH Changes in Media during Bacterial Growth of Several Environmental Strains." *Proceedings* 2 (20): 1297. https://doi.org/10.3390/proceedings2201297.

Seaver, Fred J., and Ernest D. Clark. 1910. "Studies in Pyrophilous Fungi: II. Changes Brought about by the Heating of Soils and Their Relation to the Growth of Pyronema and Other Fungi." *Mycologia* 2 (3): 109–24. https://doi.org/10.2307/3753620.

Sousa, Ana Margarida, Idalina Machado, Ana Nicolau, and Maria Olívia Pereira. 2013. "Improvements on Colony Morphology Identification towards Bacterial Profiling." *Journal of Microbiological Methods* 95 (3): 327–35. https://doi.org/10.1016/j.mimet.2013.09.020.

Stackebrandt, Erko, David Smith, Serge Casaregola, Giovanna Cristina Varese, Gerard Verkleij, Nelson Lima, and Paul Bridge. 2014. "Deposit of Microbial Strains in Public Service Collections as Part of the Publication Process to Underpin Good Practice in Science." *SpringerPlus* 3 (1): 208. https://doi.org/10.1186/2193-1801-3-208.

Stevenson, Bradley S., Stephanie A. Eichorst, John T. Wertz, Thomas M. Schmidt, and John A. Breznak. 2004. "New Strategies for Cultivation and Detection of Previously Uncultured Microbes." *Applied and Environmental Microbiology* 70 (8): 4748–55. https://doi.org/10.1128/AEM.70.8.4748-4755.2004.

Stoof, Cathelijne R., Jan G. Wesseling, and Coen J. Ritsema. 2010. "Effects of Fire and Ash on Soil Water Retention." *Geoderma* 159 (3): 276–85. https://doi.org/10.1016/j.geoderma.2010.08.002.

Swenson, Tami L., Ulas Karaoz, Joel M. Swenson, Benjamin P. Bowen, and Trent R. Northen. 2018. "Linking Soil Biology and Chemistry in Biological Soil Crust Using Isolate Exometabolomics." *Nature Communications* 9 (1): 19. https://doi.org/10.1038/s41467-017-02356-9.

Vieira, F. C. S., and E. Nahas. 2005. "Comparison of Microbial Numbers in Soils by Using Various Culture Media and Temperatures." *Microbiological Research* 160 (2): 197–202. https://doi.org/10.1016/j.micres.2005.01.004.

Wan, Shiqiang, Dafeng Hui, and Yiqi Luo. 2001. "Fire Effects on Nitrogen Pools and Dynamics in Terrestrial Ecosystems: A Meta-Analysis." *Ecological Applications* 11 (5): 1349–65. https://doi.org/10.1890/1051-0761(2001)011[1349:FEONPA]2.0.CO;2.

Watrud, Lidia S., Kendall Martin, Kelly K. Donegan, Jeffrey K. Stone, and Clarace G. Coleman. 2006. "Comparison of Taxonomic, Colony Morphotype and PCR-RFLP Methods to

Characterize Microfungal Diversity." *Mycologia* 98 (3): 384–92. https://doi.org/10.1080/15572536.2006.11832673.

Whitman, Thea, Ellen Whitman, Jamie Woolet, Mike D. Flannigan, Dan K. Thompson, and Marc-André Parisien. 2019. "Soil Bacterial and Fungal Response to Wildfires in the Canadian Boreal Forest across a Burn Severity Gradient." *Soil Biology and Biochemistry* 138 (November):107571. https://doi.org/10.1016/j.soilbio.2019.107571.

Whitman, Thea, Jamie Woolet, Miranda Sikora, Dana B. Johnson, and Ellen Whitman. 2022. "Resilience in Soil Bacterial Communities of the Boreal Forest from One to Five Years after Wildfire across a Severity Gradient." *Soil Biology and Biochemistry* 172 (September):108755. https://doi.org/10.1016/j.soilbio.2022.108755.

Wickham, Hadley, Winston Chang, Lionel Henry, Thomas Lin Pedersen, Kohske Takahashi, Claus Wilke, Kara Woo, Hiroaki Yutani, and RStudio. 2019. "Ggplot2: Create Elegant Data Visualisations Using the Grammar of Graphics." https://CRAN.R-project.org/package=ggplot2.

Woolet, Jamie, and Thea Whitman. 2020. "Pyrogenic Organic Matter Effects on Soil Bacterial Community Composition." *Soil Biology and Biochemistry* 141 (February):107678. https://doi.org/10.1016/j.soilbio.2019.107678.

Yang, Seung-Jo, Ilnam Kang, and Jang-Cheon Cho. 2016. "Expansion of Cultured Bacterial Diversity by Large-Scale Dilution-to-Extinction Culturing from a Single Seawater Sample." *Microbial Ecology* 71 (1): 29–43. https://doi.org/10.1007/s00248-015-0695-3.

Zeba, Nayela, Timothy D. Berry, Monika S. Fischer, Matthew F. Traxler, and Thea Whitman. 2024. "Soil Carbon Mineralization and Microbial Community Dynamics in Response to Pyrogenic Organic Matter Addition." *Soil Biology and Biochemistry* 191 (April):109328. https://doi.org/10.1016/j.soilbio.2024.109328.

Figures:

Figure 1:

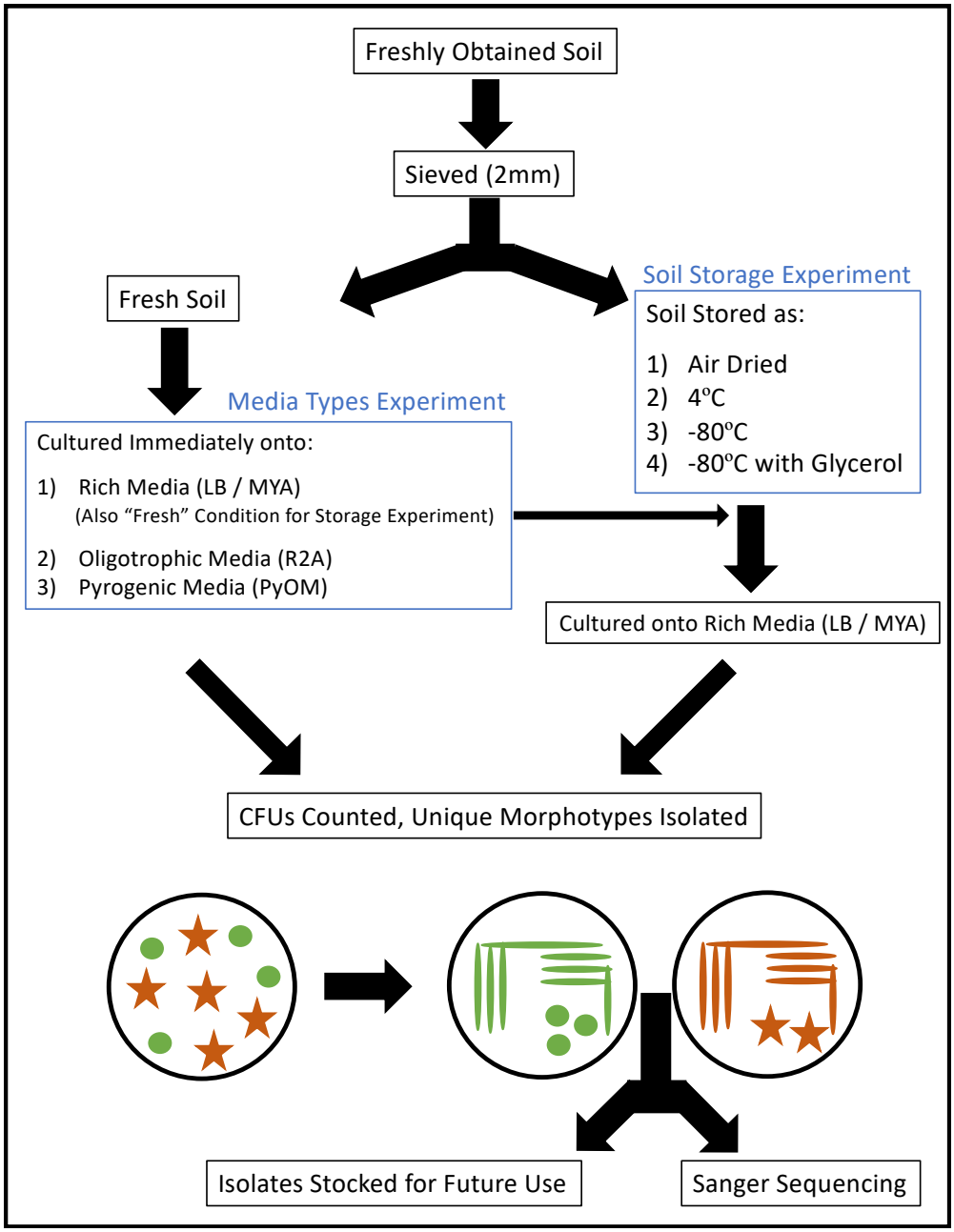

Figure 1: A conceptual diagram of the experimental design used in this experiment. "LB" is abbreviated for Lysogeny Broth, "MYA" is Malt Yeast Agar, "R2A" is Reasoner's 2 Agar, and "PyOM" is Pyrogenic Organic Matter. Note that the cultures obtained from fresh soil plated onto rich media also serve as 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 2:

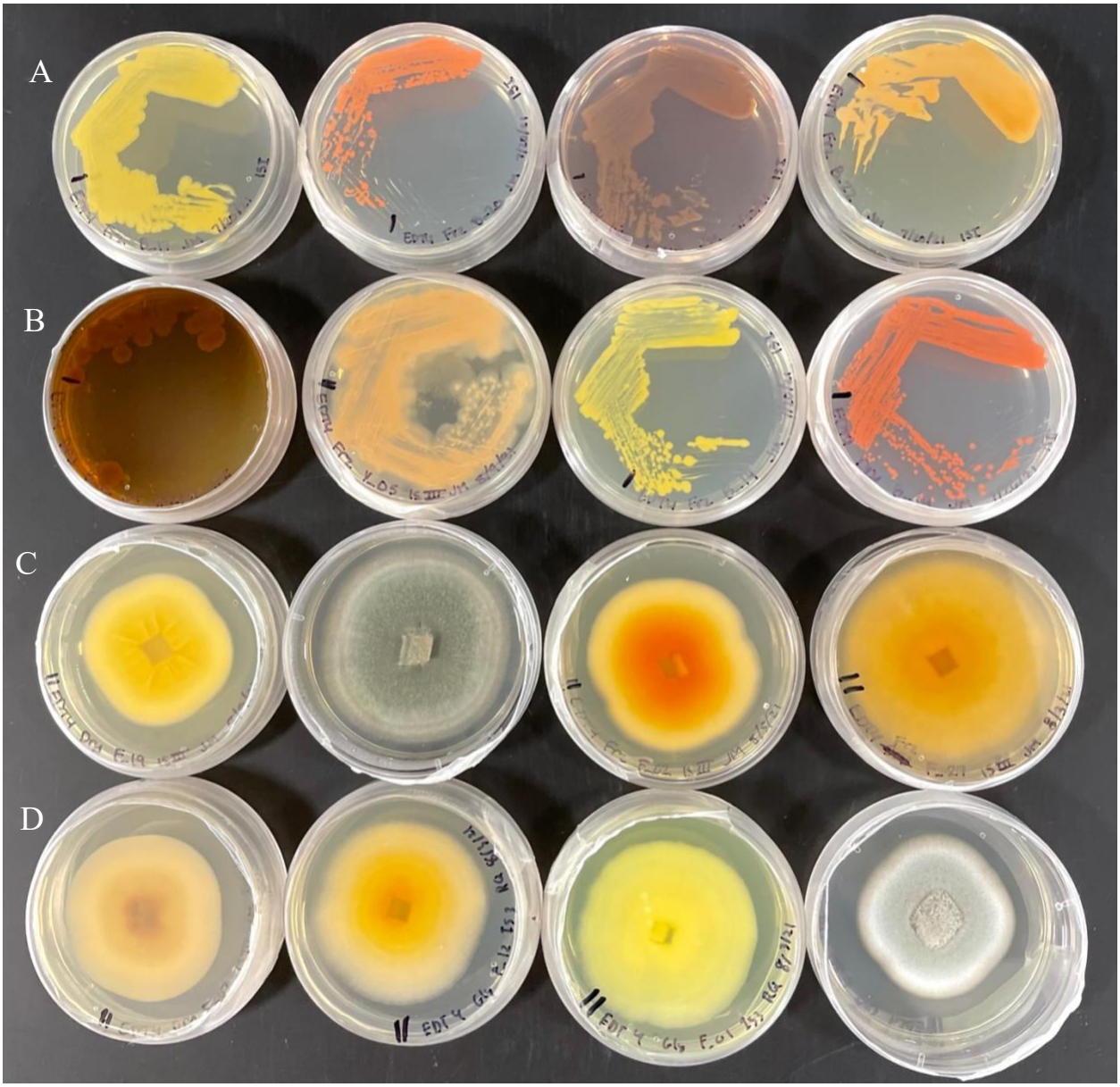

Figure 2: A snapshot of the morphological diversity of the cultures obtained from the El Dorado Fire across all soil storage and media type experiments. Identities were obtained via Sanger sequencing of the 16S or ITS regions for bacteria and fungi, respectively. Moving left to right across each row, 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:

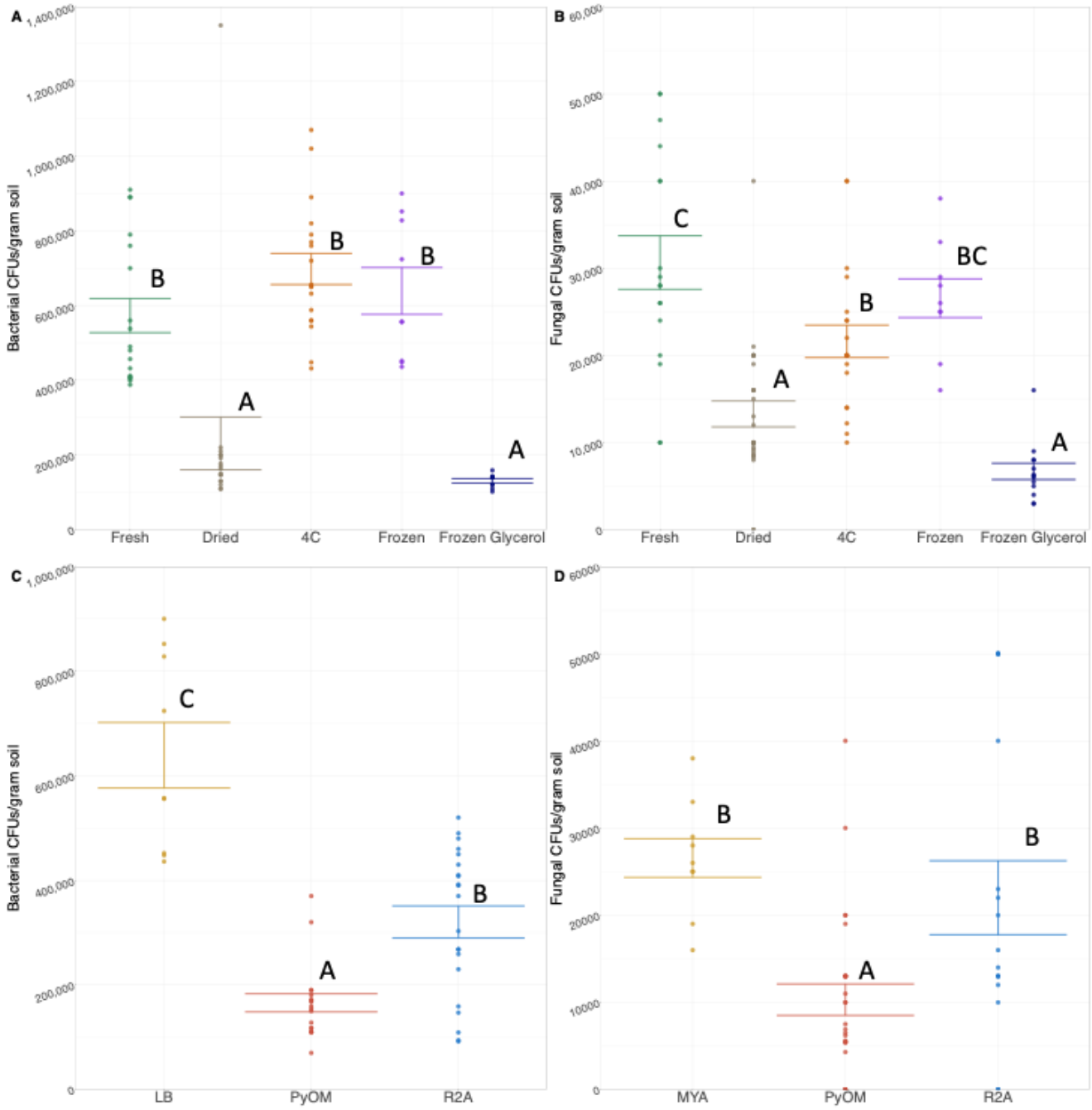

Figure 3: Calculated capturable CFUs per gram soil for bacteria among the soil storage experiment (A) and the media type experiment (C) and fungal CFUs per gram soil in the soil storage experiment (B) and the media type experiment (D). Significance was tested using ANOVA and Tukey Post-Hoc lettering has been applied. Points and bars are colored by treatment.

Figure 4:

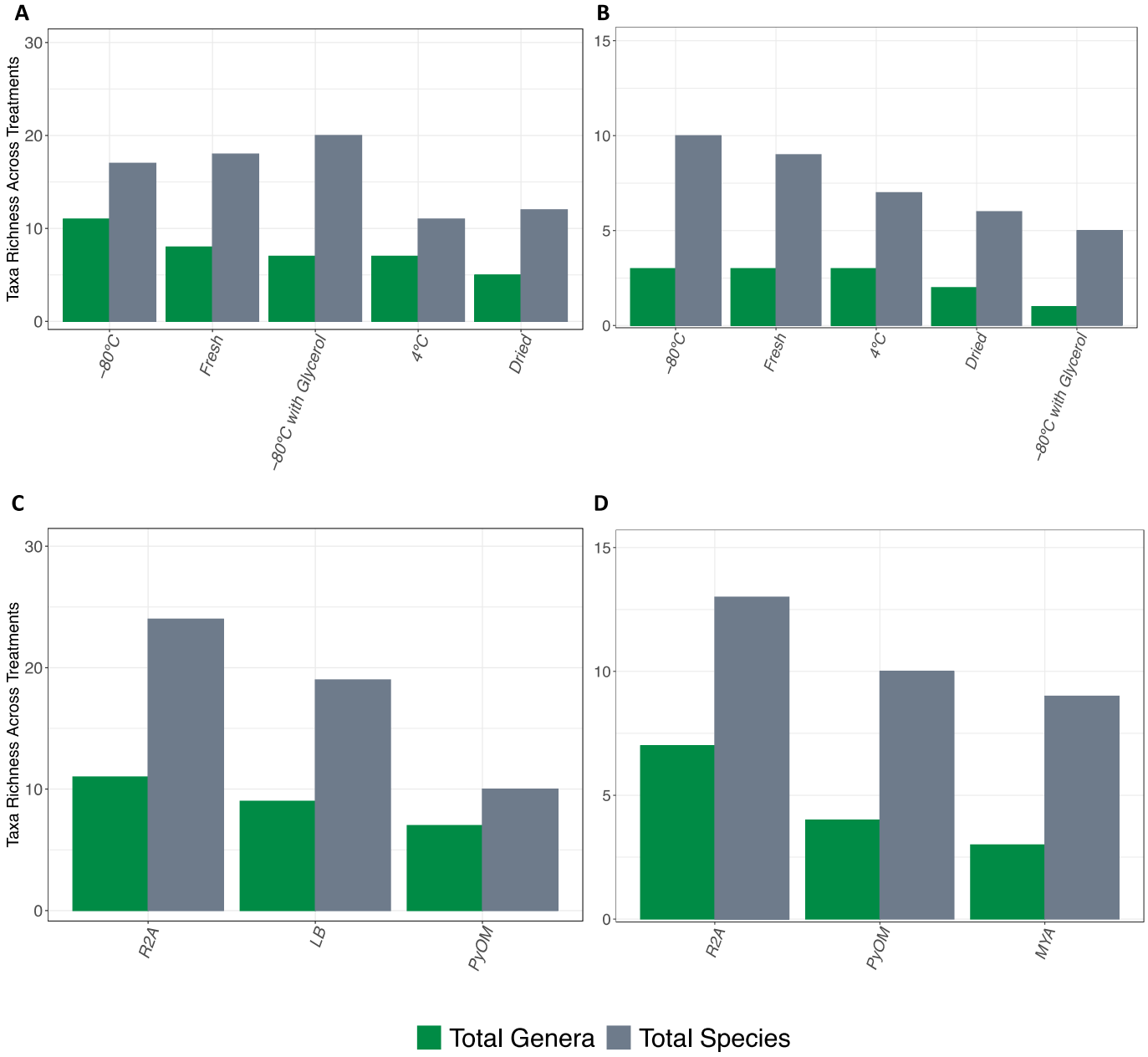

Figure 4: Summarized counts of pyrophilous bacterial and fungal genera (green) and species (grey) obtained from the soil storage experiment (A, Bacteria & B, Fungi) and from the media types experiments (C, Bacteria & D, Fungi). Treatments are ordered by highest amount of genera first, then species count second.

Figure 5:

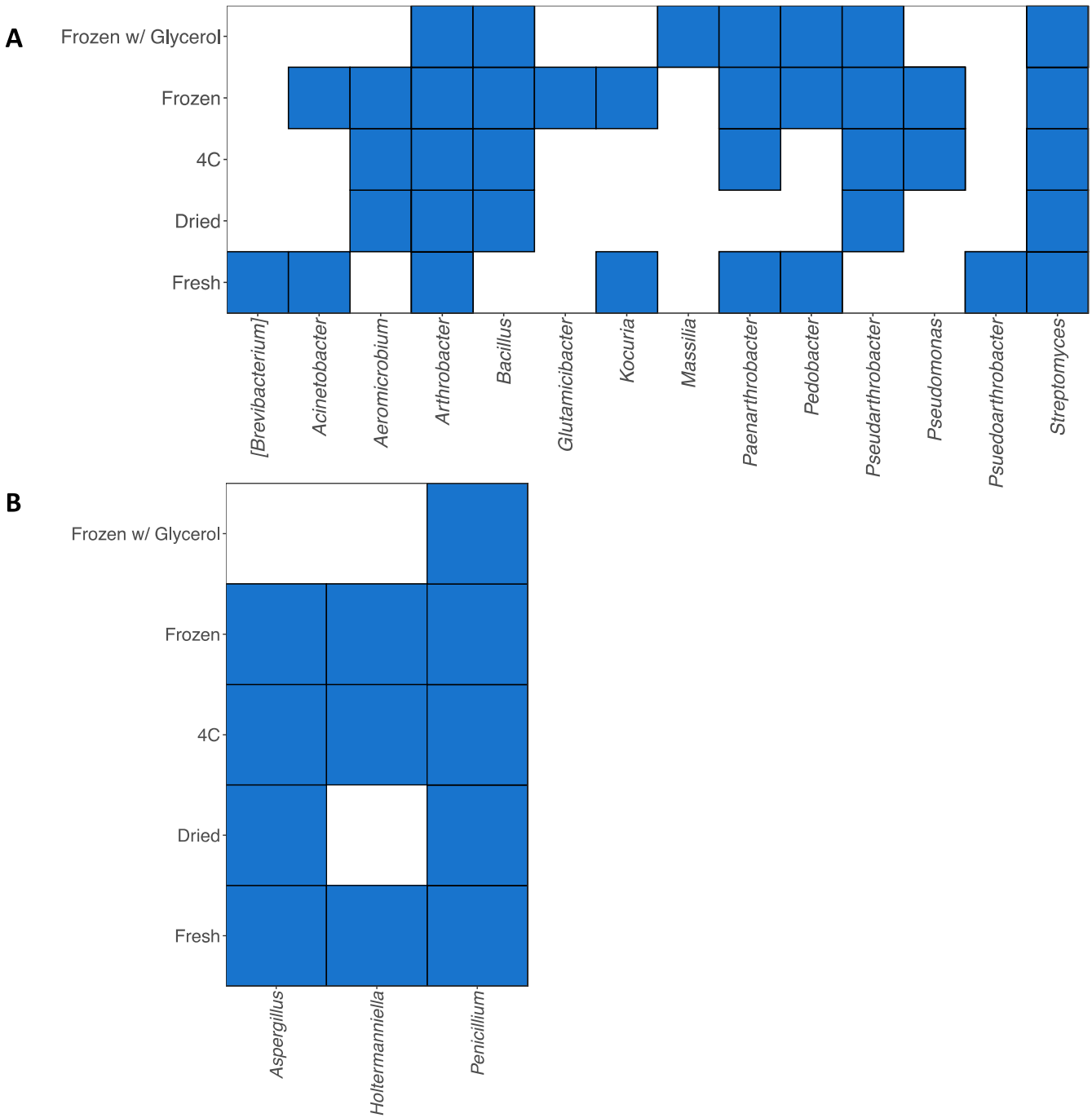

Figure 5: Heatmap-style visualization showing the diversity of pyrophilous bacterial (A) and fungal (B) genera across the soil storage experiment. A filled blue square indicates the presence of the genus on the x axis in the soil storage method indicated on the y axis.

Figure 6:

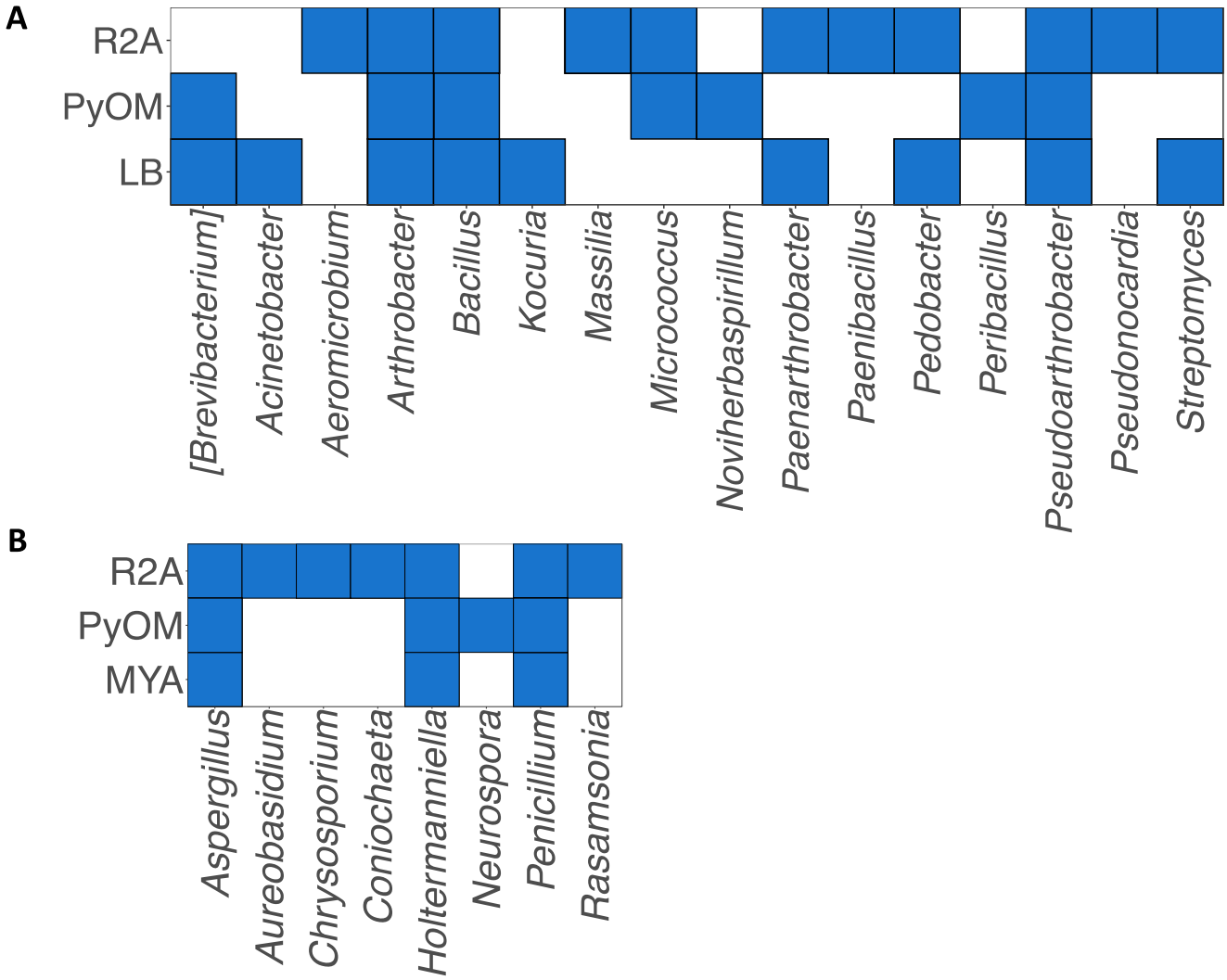

Figure 6: Heatmap-style visualization showing the diversity of pyrophilous bacterial (A) and fungal (B) genera across the media types experiment. A filled blue square indicates the presence of the genus on the x axis in the media indicated on the y axis.