



Pile burns as a proxy for high severity wildfire impacts on soil microbiomes

Julie A. Fowler^a, Amelia R. Nelson^a, Emily K. Bechtold^a, Raegan Paul^a,
Alexandra M. Wettengel^b, Michael A. McNorvell^c, Camille S. Stevens-Rumann^{c,d},
Timothy S. Fegel^e, Erik Anderson^f, Charles C. Rhoades^e, Michael J. Wilkins^{a,*}

^a Department of Soil and Crop Sciences, Colorado State University, 307 University Ave., Fort Collins, CO 80523, USA

^b Department of Biology, Colorado State University, 1878 Campus Delivery, Fort Collins, CO 80523, USA

^c Department of Forest and Rangeland Stewardship, Colorado State University, Fort Collins, CO 80521, USA

^d Colorado Forest Restoration Institute, Colorado State University, Fort Collins, CO 80521, USA

^e U.S. Forest Service, Rocky Mountain Research Station, 240 W Prospect Rd, Fort Collins, CO 80526, USA

^f Custer Gallatin National Forest, USDA Forest Service, P.O. Box 130 10 E Babcock Ave, Bozeman, MT 59715, USA

ARTICLE INFO

Handling Editor: N. Nunan

Keywords:

High severity wildfires
Pile burns
Pyrophilous microbes
Wildfire proxy
Mesocosm
Ectomycorrhizal fungi
Microbial traits
Forest disturbance
Soil microbiome

ABSTRACT

Wildfires in the western US are increasing in frequency, size, and severity. These disturbances alter soil microbiome structure and function, with greater fire severity leading to more pronounced impacts to bacterial, archaeal, and fungal communities. These changes have implications for the provisioning of microbially-mediated ecosystem services (e.g., carbon sequestration, clean water supplies) typically associated with forested watersheds. Challenges in sampling wildfire-impacted areas immediately post-burn have limited our assessment of short-term (i.e., days to weeks) changes in the soil microbiome and understanding of how microbial populations may influence post-fire biogeochemistry and ecosystem recovery. The identification of potential high severity wildfire proxies may help address some of these knowledge gaps. One potential proxy is pile burn scars, which are produced from a set of common techniques for fuel disposal and site preparation in conifer forests throughout the western US and beyond. We sampled depth-resolved layers from fire-impacted soil and combusted litter and woody materials in a series of recent pile burn scars near West Yellowstone, Montana and nearby unburned mineral soil controls to assess whether the pile burn scars exhibited microbial signatures characteristic of forest soils impacted by recent high severity wildfire. Changes in soil carbon and nitrogen chemistry and patterns of microbial alpha and beta diversity broadly aligned with those observed following wildfire, particularly the enrichment of so-called 'pyrophilous' taxa. Furthermore, many of the taxa enriched in burned soils likely encoded putative traits that benefit microorganisms colonizing these environments, such as the potential for fast growth or utilization of pyrogenic carbon substrates. We suggest that pile burn scars may represent a useful proxy along the experimental gradient from muffle furnace or pyrocosm studies to largescale prescribed burns in the field to advance understanding of the soil (and related layers, like ash) microbiome following high severity wildfires, particularly when coupled with experimental manipulation. Finally, we discuss existing research gaps that experimentally manipulated pile burns could be utilized to address.

1. Introduction

The forest soil microbiome (bacteria, archaea, fungi) drives

biogeochemical processes that support forest productivity and diversity including via important mycorrhizal relationships with roles in nitrogen (N) and phosphorus (P) provisioning to plants, belowground carbon (C)

Abbreviations: AMF, Arbuscular mycorrhizal fungi; ASV, Amplicon sequence variant; CSR, Competition-Stress tolerator-Ruderal; DOC, Dissolved organic carbon; EMF, Ectomycorrhizal fungi; LDA, Linear discriminant analysis; LEfSe, Linear discriminant analysis Effect Size; LWL, Lonesome Wood Lake; LWR, Lonesome Wood Ridge; NMDS, Non-metric multidimensional scaling; PyOM, Pyrogenic organic matter; USFS, United States Forest Service.

* Corresponding author.

E-mail addresses: julie.fowler@colostate.edu (J.A. Fowler), amelia.nelson@colostate.edu (A.R. Nelson), emily.bechtold@colostate.edu (E.K. Bechtold), raegan.paul@colostate.edu (R. Paul), alex.wettengel@colostate.edu (A.M. Wettengel), michael@mcnorvell.com (M.A. McNorvell), c.stevens-rumann@colostate.edu (C.S. Stevens-Rumann), timothy.fegel@usda.gov (T.S. Fegel), erik.anderson2@usda.gov (E. Anderson), charles.c.rhoades@usda.gov (C.C. Rhoades), mike.wilkins@colostate.edu (M.J. Wilkins).

<https://doi.org/10.1016/j.geoderma.2024.116982>

Received 26 February 2024; Received in revised form 22 July 2024; Accepted 24 July 2024

Available online 29 July 2024

0016-7061/© 2024 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

sequestration, and organic matter transformation and translocation (Baldrian, 2017). Wildfire influences both short- and long-term changes to soil microbiome structure and function through a combination of direct and indirect effects which are more pronounced in shallower soil depths and increase with greater fire severity (Bruns et al., 2020; Köster et al., 2021; Dove et al., 2022; Nelson et al., 2022; Caiafa et al., 2023). Direct impacts to soil microbiota include mortality from lethal heating and loss of habitat caused by disruption of soil aggregates, while indirect impacts relate to changes in soil chemistry (i.e., increased soil pH, changes in C and N pools) and vegetation (Köster et al., 2021). The increase in wildfire frequency, size, and severity observed across the western US (Dennison et al., 2014; Parks and Abatzoglou, 2020) has rippling impacts to the reestablishment and persistence of conifer vegetation, leaving these ecosystems open to delayed recovery or vegetation type conversion (Coop et al., 2020; Stevens-Rumann et al., 2022) potentially linked to microbially-mediated processes such as the loss of obligate ectomycorrhizal symbionts (Suz et al., 2021; Hewitt et al., 2023).

Despite the outsized importance of forest soil microbiomes in ecosystem service provisioning, few detailed studies have been conducted in the immediate days, weeks, and months post-moderate to high severity wildfire compared to 1+ years post-burn (Pulido-Chavez et al., 2021; Dove et al., 2022; Nelson et al., 2022; Caiafa et al., 2023; Pulido-Chavez et al., 2023). This is partially influenced by logistical challenges as sites may be under governmental land closures in addition to the hazards of falling trees and flooding or overland debris flows (Alexander et al., 2017). Pile burning exists as a possible experimental mesocosm, or proxy, to fill in the existing research gap of short-term impacts of high severity wildfire on forest soil microbiomes.

Pile burning is a common fuel disposal and site preparation practice typically used in conjunction with timber thinning and harvesting (Mott et al., 2021). This management activity has increased in practice in the forests of the Rocky Mountains and the larger Intermountain West, where disturbances like beetle outbreaks in lodgepole pine and other conifer forests have led to increased removal of dead timber and other woody materials (Jenkins et al., 2008; Donato et al., 2013; Rhoades & Fornwalt, 2015). These piled fuels are intended to combust completely at high temperatures and can smolder for days to weeks (U.S. Department of Agriculture, n.d.; Pastor et al., 2010; Busse et al., 2013). Numerous studies have been conducted on pile burn impacts to the underlying soil ecosystem, which include increased soil pH and bioavailable N and P, the loss of bacterial/archaeal and fungal biomass, and other changes that may negatively influence forest rehabilitation and recovery including the reduction of ectomycorrhizal fungi populations and viable seed banks through both heating and seed predation (Korb et al., 2004; Jiménez Esquilín et al., 2007; Rhoades et al., 2021). The extent of these impacts can be modulated by fuel type or size (Busse et al., 2013) and soil moisture (Busse et al., 2005; Busse et al., 2010; Massman, 2012), while also varying based on depth of measurement in the soil profile and position under the pile itself (center vs. edge; Busse et al., 2013), but not significantly by soil texture (Busse et al., 2010). These soil impacts can range from minimal to detectable for decades depending on these factors (Rhoades & Fornwalt, 2015; Rhoades et al., 2021), but are largely static (in one place on the land surface) with longer fire residence times than wildfires and deeper heat penetration into the soil profile (Massman et al., 2010; Rocky Mountain Research Station, 2015). Given the influence of site-specific factors like weather, climate, topography, and woody fuels on forest fire behavior generally (Dillon et al., 2011; Birch et al., 2015), it is likely that neighboring pile burn scars burned concurrently may have similar fire conditions and consequently similar impacts to the soil ecosystem.

While influenced by many of the same factors as pile burns, a singular wildfire is dynamic (with a moving fire front) (Massman et al., 2010) and results in heterogeneity of fire severities at micro and macro scales, with varying impacts to the soil ecosystem (Agbeshie et al., 2022). However, high severity wildfire impacted locations visually

resemble pile burn scars in their loss of litter or organic layers (Rhoades et al., 2015) and alterations to mineral soil color (Certini, 2005; Rhoades & Fornwalt, 2015). While there are few direct comparisons between pile burns and high severity wildfires on soil heating and resulting soil impacts, existing work reports overlapping soil/litter interface temperature ranges between pile burns and the upper reaches of wildfire temperatures (300–850+ °C; Santín & Doerr, 2016; Agbeshie et al., 2022) and both fire types can result in a loss of both symbiotic mycorrhizal fungi and vegetation seed banks and an increase in seed predation (Korb et al., 2004; Jiménez Esquilín et al., 2007; Nelson et al., 2022; Shi et al., 2022). Combined with the increasing prevalence of extreme wildfires (rare but disproportionately impactful or ‘ecological extremes’; Linley et al., 2022) that may increase overlap of wildfire and pile burn fire conditions (e.g., soil heating), this leads us to speculate that pile burn scars may be utilized as mesocosms for the study of high severity or extreme wildfire impacts on soil microbiomes with adequate replication (consistent fuel type and pile size; Rhoades et al., 2021) and experimental manipulation to address specific hypotheses. However, it has not yet been established whether pile burn scars generally contain the hallmarks of a high severity wildfire-impacted microbiome, such as the presence of known pyrophilous (i.e., ‘fire loving’) microbial taxa like the extensively studied fungi *Pyronema domesticum* (Bruns et al., 2020; Fischer et al., 2021; Steindorff et al., 2022).

This work is a proof-of-concept case study encouraging increased utilization of pile burns as research proxies or mesocosms representing a middle ground between small muffle furnace or pyrocosm studies (Bruns et al., 2020; Brucker et al., 2022; Myers-Pigg et al., 2024; VanderRoest et al., 2024) and largescale prescribed burns (Dove et al., 2021; Brucker et al., 2022) for the study of high severity wildfires on soil microbiomes. To this end, we quantitatively and qualitatively assessed whether a series of pile burn scars approximately 8 months post-burn near West Yellowstone, Montana exhibited the soil physicochemical-influenced microbial signatures (reduced alpha diversity, altered beta diversity, and taxonomic changes) that are characteristic of forest soils in the western US and beyond impacted by recent high severity wildfire. We hypothesized that the homogenizing impacts of high severity fire on soil microbiomes will generate consistent microbial signatures in our burn pile scars, despite pre-fire differences in location, soil types, soil chemistry, and pile burn fuel volume (and resulting scar size) that overlap with those reported from natural high severity wildfires (Dove et al., 2022; Nelson et al., 2022). Such signatures would likely include the enrichment of so-called cosmopolitan ‘pyrophilous’ bacterial and fungal taxa with putative beneficial traits for colonizing burned soil and overall similarities in community composition (Pulido-Chavez et al., 2021; Enright et al., 2022; Fox et al., 2022; Nelson et al., 2022; Caiafa et al., 2023; Fischer et al., 2023). Our results from this proof-of-concept study suggest that pile burns may complement existing research when combined with experimental manipulation (fuel type and size/amount, fire temperature, precipitation events, etc.) or time series sampling, enabling new insights into ecosystem change in the first days, weeks, and months following high severity wildfire. Finally, we examine existing research gaps in the wildfire literature to demonstrate research areas where pile burns may be a useful tool to disentangle the impacts of high severity wildfires on soil microbiomes.

2. Materials and methods

2.1. Field campaign

2.1.1. Sites description

This study was conducted on the Custer Gallatin National Forest near West Yellowstone, Montana, United States west of the boundary with Yellowstone National Park (Fig. A.1). The dominant vegetation in this area are conifers including lodgepole pine (*Pinus contorta*), Douglas fir (*Pseudotsuga menziesii*), and mixed Engelmann spruce (*Picea engelmannii*) and subalpine fir (*Abies lasiocarpa*) forests (Supplemental File 1). This

area ranges in elevation between 2002 and 3076 m a.s.l. Under the Köppen-Geiger climate system, this region is classified as subarctic with cool summers and year-round rainfall (Rubel & Kottek, 2010). Total annual precipitation averages 656 mm with a mean annual temperature of 3.8 °C for the years 1990 to 2019 (Zepner et al., 2021). A rainfall event occurred near the time of sampling, with sampled soils being saturated as result. Year-to-date precipitation (% of normal) for the area were 76% and 86% for all of 2019 and January to June 2020, respectively (Montana State Library, n.d).

Three sites were included in this study: Lonesome Wood Lake (LWL), Lonesome Wood Ridge (LWR), and Rendezvous (R). LWL sites were either mixed mesic conifer forests (lodgepole pine and Douglas fir) or mixed spruce/fir forests (Engelmann spruce and subalpine fir) with either Argic Cryoborolls, Typic Cryochrepts, or Typic Cryoboralfs and Argic Cryoborolls soils, LWR sites were all mixed mesic conifer forests (lodgepole pine and Douglas fir) with Mollic Cryoboralfs-Argic Cryoborolls association soils, and R sites were all lodgepole pine forests with Typic Cryochrepts soils (Soil Survey Staff, 2023). Additional location, vegetation, and soils information is available in Supplemental File 1.

2.1.2. Sites establishment

Thinning occurred in these sites located in commercial harvest units with the purpose of removing non-merchantable material and reduce excess fuel loading, with sampling for this study as a secondary goal. Resulting harvest materials, which were comprised of wood from the tree species at each site as detailed above and generally were smaller, non-merchantable size classes (mostly 1–6" diameter material, branches, tops, with some larger damaged/non-merchantable trees), were constructed into piles between 2016 and 2018 that reached 3–4 m tall on average. These piles were ignited using diesel fuel during October 2019 under cold and clear air conditions. These piles likely smoldered for months after ignition.

The resulting pile burn scars were between approximately 200 and 1000 m². Given that these piles were constructed and ignited for land management purposes with soil sampling for this study as a secondary priority, exact measurements of fuel volume were not taken. Using a shapefile of the resulting pile burn scars and formulas from Hardy (1996), we estimate the fuel volumes to range between 350 and 2000 m³. All calculations including estimations of pile lengths, widths, heights, and volumes are included in Supplemental File 1. Within each of the three sites, five pile burns scars had soils sampled from them.

2.1.3. Soil sampling

One sample of three layers were collected from the center of each pile burn on June 15 and 16, 2020 for chemical and microbial analyses. Sparse or no vegetation was observed growing in the pile burn scars and, in cases where there was sparse vegetation, bulk soil was sampled away from plant roots. The three layers were sampled individually and included an ash layer, a charred residue layer consisting of partially combusted organic material (hereafter "charred layer"), and a mineral soil layer (0–5 cm depth) (five pile burn scars sampled per site with three layers sampled per burn scar across three sites). Layers were distinguished visually, where ash was white/grey and, the charred layer was deep black and contained visible charred partially intact organic material like pine needles, and the mineral soil was brown or otherwise lighter than the charred layer and lacked visible organic material. Additionally, seven samples of unburned mineral soil at a depth of 0–5 cm were sampled as controls outside of the pile burns in the remaining harvested areas neighboring each site. Control samples were taken at least 3 m away from the closest pile burn scar. The O horizon was removed and only mineral soil was collected from the unburned areas owing to no obvious unburned comparison for the ash and charred layers sampled from the pile burns. All samples were collected in duplicate for both chemical and microbial analyses. Samples collected for chemical analysis were stored in coolers on blue ice in the field before transport back to the laboratory at the U.S. Forest Service's Rocky

Mountain Research Station where they were stored at 4 °C. Samples for microbial analysis were collected with a trowel (sterilized between samples with 70% ethanol) into sterile Whirl-Pak® (Nasco, Fort Atkinson, WI, USA) bags and subsequently stored on dry ice in the field before transport back to the laboratory at Colorado State University where they were stored at –80 °C. A total of 60 samples for chemical analysis and 60 samples for microbial analysis were collected for this study.

2.2. Bulk soil physical and chemical analyses

A subset of mineral soil samples ($n = 9$) from all sites ($n = 3$ per site) underwent soil textural analysis through particle size analysis using a hydrometer resulting in soils that were sandy loams, sandy clay loams, and loams (Taylor Scientific, St. Louis, MO) (Fig. A.2) (Supplemental File 2). Given the limited impact of soil texture on soil heating (Busse et al., 2010) in conjunction with the aforementioned influence of site-specific factors like weather, climate, topography, etc. on fire behavior and impacts (Dillon et al., 2011; Birch et al., 2015) that are likely similar between our neighboring pile burns, we have hereafter reported samples from across our three sites together.

For each sample, a subsample was dried for 48 h at 60 °C, then ground and analyzed for total C and N (%) by dry combustion (LECO 1000 CHN analyzer, LECO Corporation, St. Joseph, MI, USA). Soil pH was assessed using a 1:1 soil: deionized (>18 mΩ) water slurry that underwent 1 h of agitation and measured using a temperature-corrected glass electrode (Hach Scientific, Loveland, CO). Water-extractable chemistry samples were created by air-drying 10 g of soil for 3 days then steeping in 50 mL of DI (>18 mΩ) at 70 °C for 18 h, shaken and filtered through 0.7 μm pore size glass fiber filters (Sparling, Vojvodic-Vukovic, & Schipper, 1998). Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) on water extractions were determined using a Shimadzu TOC-VCPN total organic carbon analyzer, with 2 M HCl addition before analysis to remove mineral C (Shimadzu Corporation Columbia, MD). Detection limits for DOC and TDN were 0.05 mg L⁻¹. Anion and cation concentrations were determined by ion chromatography with electrolytic suppression and conductivity detection (Thermo Fisher Scientific, Waltham, MA). Samples were analyzed within 72 h of collection. Detection limits were < 0.5 μeq L⁻¹ for K⁺, Na⁺, NH₄⁺, Cl⁻ and NO₃⁻, 1.0 μeq L⁻¹ for Ca²⁺ and SO₄²⁻, and 1.6 μeq L⁻¹ for Mg²⁺. Acid neutralizing capacity (ANC) was measured on unfiltered subsamples by Gran titration (Gran, 1952). Electrical conductance and pH on water extractable samples were analyzed automatically with PC Titrate sensors (Man-Tech Co., Guelph, ONT, CA). All chemistry data is available in Supplemental File 3.

2.3. DNA extraction, 16S rRNA gene and ITS amplicon sequencing

Genomic DNA was extracted from ash, charred layer, and both burned and unburned mineral soil samples using the Zymobiomics Quick-DNA fecal/soil microbe kits (Zymo Research) following manufacturer's protocol. There was difficulty extracting detectable DNA from ash samples, resulting in only 3 LWL and 3 LWR ash and no R samples that could be used for sequencing, resulting in a total of 43 samples utilized for DNA sequencing ($n = 6$ ash, $n = 15$ charred layer, $n = 15$ burned mineral soil, $n = 7$ unburned mineral soil). Soil bacterial and archaeal communities were amplified using the V4 region of the 16S rRNA gene using the primers 515F (5'-GTGYCAGCMGCCGCGGTAA-3') (Parada et al., 2016) and 806R (5'-GGACTACNVGGGTWTCTAAT-3') (Apprill et al., 2015). Soil fungal communities were amplified via the first internal transcribed spacer (ITS1) of the ribosomal DNA using the primers ITS1f (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2 (5'-GCTGCGTTCTTCATCGATGC-3') (White et al., 1990). Primer recommendations were from the Earth Microbiome Project (<https://earth-microbiome.org/>) (Thompson et al., 2017). All samples were sequenced on the Illumina MiSeq Platform with 251 bp paired-end sequencing chemistry at Microbial Community Sequencing Lab (University of

Colorado Boulder).

Resulting reads were processed using QIIME2 (release 2021.2) (Bolyen et al., 2019). Due to low quality, ITS reverse reads were discarded. Demultiplexed 16S and ITS samples were merged, filtered/denoised, and binned to infer amplicon sequence variants (ASVs) using DADA2 (Callahan et al., 2016). Following these steps, 16S rRNA gene read counts ranged from 16,363 to 52,077 and ITS amplicon sequencing read counts ranged from 11,446 to 71,330. Taxonomy was assigned to our resulting bacterial and archaeal ASVs using scikit-learn pre-trained SILVA classifiers (version 138; Quast et al., 2013; Bokulich et al., 2018; Robeson et al., 2021) and our resulting fungal ASVs using self-trained UNITE database classifiers (Nilsson et al., 2019; Kõljalg et al., 2020). Fungal sequences not assigned to the Kingdom Fungi and bacterial/archaeal sequences assigned to mitochondria or chloroplast by taxonomic assignment were discarded from ASV tables prior to downstream analysis. Fungal ASVs further had ecological guilds assigned to them using FUNGuild when guild assignments were classified as a single guild assignment at ‘highly probable’ or ‘probable’, per creator recommendations (Nguyen et al., 2016; Nelson et al., 2022). Resulting reads were deposited and are available at NCBI under BioProject PRJNA682830 (Supplemental File 4).

2.4. Statistical & microbial community analyses

2.4.1. Statistical & microbial community analyses for this study

To characterize how soil microbial communities differed by burn condition (burned vs. unburned) and the burned layers (ash, charred, and 0–5 cm mineral soil), statistical analyses were performed using R version 4.1.2 (R Core Team, 2021) with significance accepted at $p < 0.05$. All statistical codes are available at <https://github.com/julieafowler/Montana-Pile-Burns-Microbiome>. Samples from all sites were combined into one dataset given the aforementioned reasons in addition to the homogenizing impacts of high severity fire on soil microbiomes (Dove et al., 2022; Nelson et al., 2022). Differences in select soil chemistry variables (total carbon, total nitrogen, ammonium, nitrate, phosphate, pH) and alpha diversity (using measures of ASV richness and the Shannon index (H'), a combination of ASV richness and evenness) between distinct layers (both burned and unburned in all possible combinations) were assessed and tested using pairwise Wilcoxon signed-rank tests with a Bonferroni p -value adjustment for multiple tests using the function “stat_compare_means” in the package ggpubr (Kassambara, 2023) and the function “pairwise.wilcox.test” in the package stats (R Core Team, 2021) and between sites through Kruskal-Wallis tests using “stat_compare_means” and the function “kruskal.test” in the package stats. To test differences in bacterial/archaeal and fungal community composition between layers (burned and unburned), nonparametric permutational multivariate analysis of variance (PERMANOVA) were performed (Anderson, 2001) using Bray-Curtis dissimilarity matrices and the “adonis2” function in the vegan package (Okansen et al., 2022) and visualized using Non-Metric Multidimensional Scaling (NMDS). Soil chemistry variables, taxa relative abundance at the phyla level, and FUNGuild assignments were correlated with resulting ordination space using the “envfit” function in the vegan package with a Bonferroni p -value correction for multiple tests. To further identify potential pyrophilous taxa within our datasets, combined Linear discriminant analysis Effect Size (LEfSe) and Linear Discriminant Analysis (LDA) analyses in addition to a core microbiome analysis were performed. Combined LEfSe/LDA is a statistical method designed to find microbial taxa (bacterial/archaeal and fungal) with relative abundances that differ significantly between treatment groups of interest (LEfSe) coupled with estimations of effect size (LDA) where a larger effect size denotes a taxon important for distinguishing between the microbiomes of the treatment groups of interest (Chong et al., 2020). Combined LEfSe/LDA analyses were conducted using the MicrobiomeAnalyst 2.0 server (Lu et al., 2023) using the default parameters for data filtering and normalization to identify bacterial/archaeal and fungal phyla and ASVs discriminant

for one or more layers (burned or unburned) (Supplemental Files 8 and 9). Core microbiome analysis was conducted using the tidyverse package in R (Wickham et al., 2019). ASVs were determined to be a core community member if they were present in at least 50% of samples from a site/layer combination within all sites (Fig. A.3) (Supplemental Files 10 and 11). Finally, for bacterial communities 16S rRNA gene copy numbers were retrieved at the lowest (genus > phyla, for example) taxonomic assignment available using the ribosomal RNA operons database (rrnDB) (Stoddard et al., 2015) to assess the possible relationship between increased 16S rRNA gene copy number as a proxy for fast growth under post-fire soil conditions (Stoddard et al., 2015; Roller et al., 2016). None of our archaeal ASVs were present in the rrnDB database. All visualizations were produced using the package ggplot2 (Wickham, 2016) using aesthetics from the package ggthemr (Tobin, 2020), except for combined LEfSe/LDA visualizations (Figs. 5 and 7A) which were produced using MicrobiomeAnalyst 2.0 (Lu et al., 2023), and edited in Adobe Illustrator 2023 (v27.2).

2.4.2. Combined NMDS from multiple studies

We constructed a combined NMDS using the above methods for four studies on high severity fire impacts to shallow soils in western US coniferous forests: this study (pile burns) and Nelson et al. (2022), McNorvell (2023), and Caiafa et al. (2023) (all wildfires). These additional three studies together included samples from the Badger Creek (2018), Beaver Creek (2016), Calwood (2020), Cameron Peak (2020), Church’s Park (2010), Mullen (2020–2021), and Ryan (2018) wildfires. Details on sampling schemes including how long post-burn sampling occurred and location details including forest types, etc. can be found in each publication and in Table A.1, and some publications contain samples from the same wildfire, sometimes sampled in different years. A map of the boundaries of these wildfires is available in Fig. A.4 and was created using Google Earth Pro (version 7.3.6.9345, 64-bit) with shapefiles obtained from the file “InterAgencyFirePerimeterHistory All Years View” published by the National Interagency Fire Center on ArcGIS Online (Esri) and edited in Adobe Illustrator 2024 (v28.1). Raw reads were first obtained from publicly available BioProjects on NCBI (Nelson et al., 2022; Caiafa et al., 2023) or from collaborators (McNorvell, 2023; deposited and available at NCBI under BioProject PRJNA682830 with details in Supplemental File 5). ITS reverse reads were discarded and all remaining 16S and ITS samples were demultiplexed, merged, filtered/denoised, binned, and taxonomically classified according to above methods before feature tables were collapsed from the ASV level to the genus level and merged. Only samples that were from shallow mineral soil (0–5 cm) and high severity fires or reference unburned (control) sites were included in this dataset for downstream analyses and NMDS construction in R. PERMANOVA was performed on the influence of study, burn history (high severity burn or unburned control), presence/type of burn scar (pile burn scar vs. wildfire scar vs. no burn scar), and time since burn where applicable (8 months up to 11 years) within this dataset according to the above methods as well as testing for differences in beta dispersion between metadata categories using the function “betadisper” in the package vegan (Okansen et al., 2022) followed by an ANOVA test using the function “anova” and a post-hoc pairwise Tukey HSD test using the function “TukeyHSD” in the package stats (R Core Team, 2021) (Supplemental Files 6 and 7). These figures were constructed using the same methods as above.

3. Results and discussion

3.1. Soil chemistry in pile burn scars mirrors trends observed following severe wildfire

Highlighting the inherent heterogeneity in soil structure and chemistry, we observed significant differences in mineral soil chemistry across the three unburned control locations for variables including total C, total N, and nitrate ($p \leq 0.01$) (Supplemental File 3). Similarly, post-

combustion soil chemistry differed across sites, with the sampling of surface ash, charred organic material, and deeper mineral soil highlighting depth-resolved chemical variability. While the ash layer differed significantly between sites for the three above variables in addition to pH ($p \leq 0.05$), the charred layer exhibited significant differences between sites for total N, ammonium, and phosphate ($p \leq$

0.05). The burned mineral soil layer differed significantly between sites only in ammonium concentrations ($p = 0.039$).

Overall, nitrate, ammonium, phosphate, and pH were elevated in burned compared to unburned mineral soils ($p \leq 0.01$) (Fig. 1). Furthermore, depth-resolved soil analyses suggest that high severity burns may produce layers (ash, charred, and mineral soil in this case)

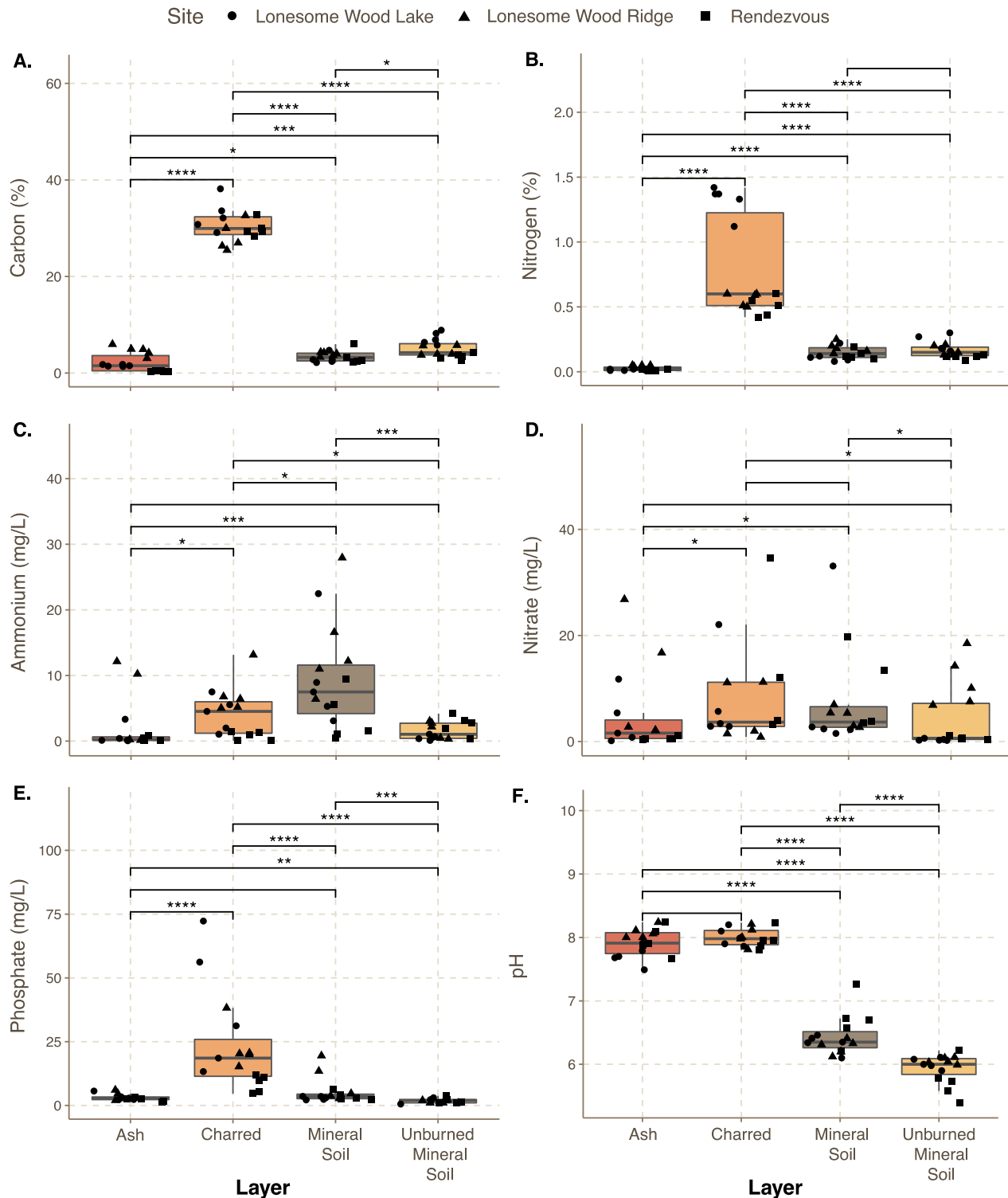


Fig. 1. Boxplots of soil total carbon (%) (A), total nitrogen (%) (B), ammonium (C), nitrate (D), phosphate (E), and pH (F) for the three burned layers (ash, charred, and the 0–5 cm mineral soil depth) and an unburned control (0–5 cm mineral soil). Point shapes represent the site of sampling (Lonesome Wood Lake, Lonesome Wood Ridge, or Rendezvous). Brackets represent Wilcoxon signed-rank tests with stars denoting significant differences. *: $p \leq 0.05$ **: $p \leq 0.01$ ***: $p \leq 0.001$ ****: $p \leq 0.0001$.

that are distinct microbial habitats, owing to chemical heterogeneity in the vertical soil profile (Honeyman et al., 2023). The highest values for total C and total N were detected in the charred layer, while inorganic N (ammonium and nitrate) concentrations were lowest in the ash layer. Although ammonium concentrations can initially be high in ash, the leaching of ammonium into deeper soil horizons or microbial nitrification may account for the depleted values detected here when comparing the ash and charred layer values ($p \leq 0.01$) (Smithwick et al., 2005).

Phosphate followed a similar pattern, with significantly higher values in the charred layer compared to any other burned layer ($p \leq 0.001$). Soil pH was significantly higher in the ash and charred layers relative to samples from deeper horizons ($p \leq 0.0001$). These findings largely align with trends following wildfire across diverse ecosystems, which detail increases in soil alkalinity, bioavailable N and P, and altered total C and N (de la Rosa & Knicker, 2011; Ferrenberg et al., 2013; Fernández-García et al., 2019; Dove et al., 2022; Nelson et al., 2022).

3.2. Pile burning drives alpha and beta diversity changes in soils that mirror those from the natural wildfire literature

The direct impact of soil heating and the indirect alteration of soil physicochemical characteristics exerts a strong selective pressure on colonizing microorganisms (i.e., bacteria, archaea, and fungi) that results in decreased community diversity in the aftermath of wildfire (Pressler et al., 2019; Barreiro & Díaz-Raviña, 2021; Köster et al., 2021). Despite differing surface fuel characteristics between wildfires (O

horizon) and pile burns (bolewood, branches, and other foliage) and resulting fire attributes like residence times, we observed significantly lower alpha diversity values (ASV richness and Shannon's H') for bacterial/archaeal and fungal communities in our burned mineral soil compared to our unburned mineral soil, comparable to those reductions reported in the wildfire literature within the first weeks to 1+ years post-burn (Fig. 2) (Ferrenberg et al., 2013; Enright et al., 2022; Nelson et al., 2022; Caiafa et al., 2023; Pulido-Chavez et al., 2023). These alpha diversity metrics were also significantly lower in the ash and charred layers compared to the unburned mineral soil, except for the comparison of Shannon's H' between fungal communities of the ash and unburned mineral soil layers. Microbial diversity generally did not significantly differ between the different burned layers.

Beta diversity measurements revealed significant differences in bacterial/archaeal and fungal community composition between burned and unburned samples that correlated with changes in soil chemistry, including pH for the bacterial/archaeal communities (Fig. 3). Soil layer also exerted a significant influence on community composition (PERMANOVA, $p \leq 0.001$ and 0.0015 for bacterial/archaeal and fungal communities, respectively), supporting previous observations that deeper depths experience more muted heating effects during wildfire (Bruns et al., 2020; Nelson et al., 2022; Caiafa et al., 2023). Beyond soil pH, the integration of additional geochemical data into multivariate analyses (Fig. 3C and 3D) highlighted the influence of cations (Ca, Mg, and Na) and DOC concentrations in driving dissimilarity between communities in burned and unburned plots, with DOC likely serving as a pulse of energy for the microbes in the post-fire environment. These

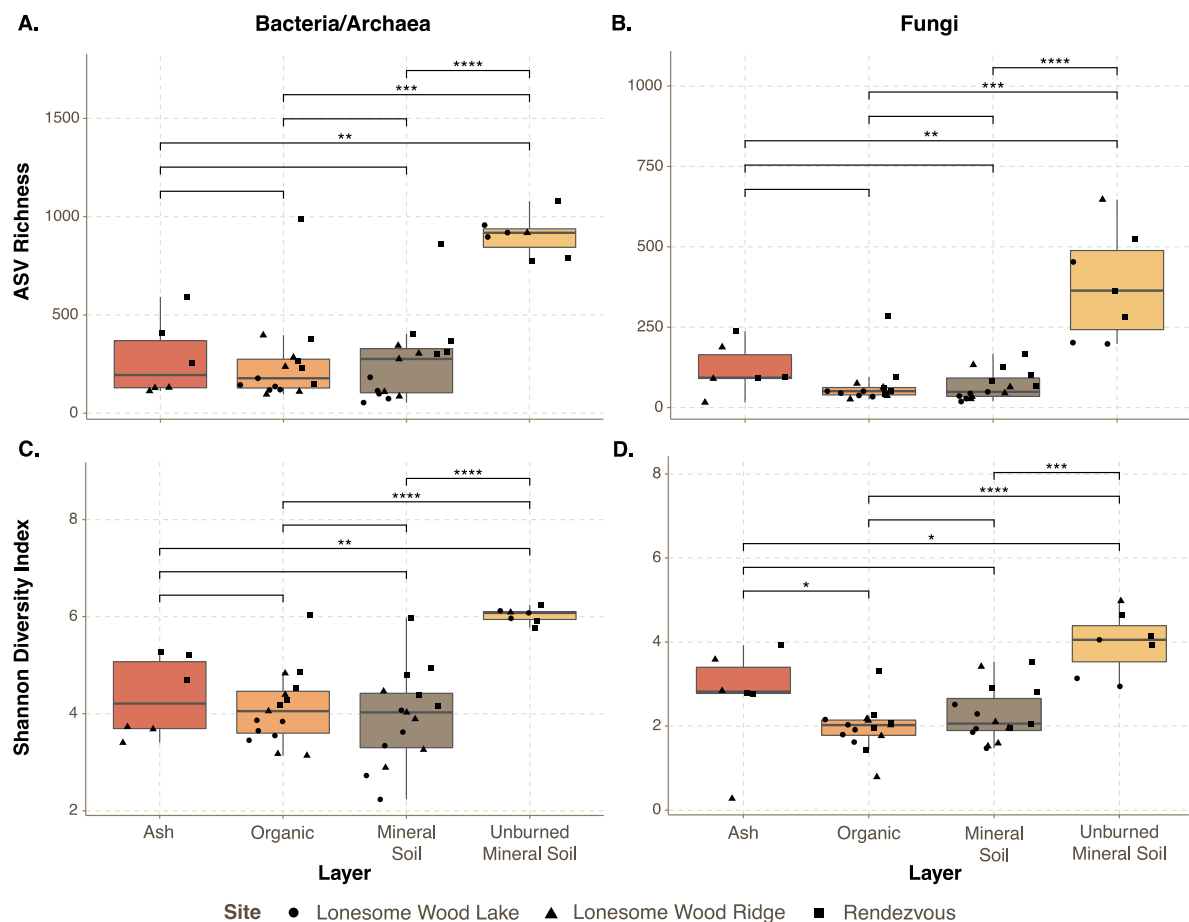


Fig. 2. Bacterial/archaeal (16S; A and C) and fungal (ITS; B and D) ASV richness (A and B) and Shannon's H' index (C and D), both measures of alpha diversity, for the three burned layers (ash, charred, and the 0–5 cm mineral soil depth) and an unburned control (0–5 cm mineral soil). Point shapes represent the site of sampling (Lonesome Wood Lake, Lonesome Wood Ridge, or Rendezvous). Brackets represent Wilcoxon signed-rank tests with stars denoting significant differences. *: $p \leq 0.05$ **: $p \leq 0.01$ ***: $p \leq 0.001$ ****: $p \leq 0.0001$.

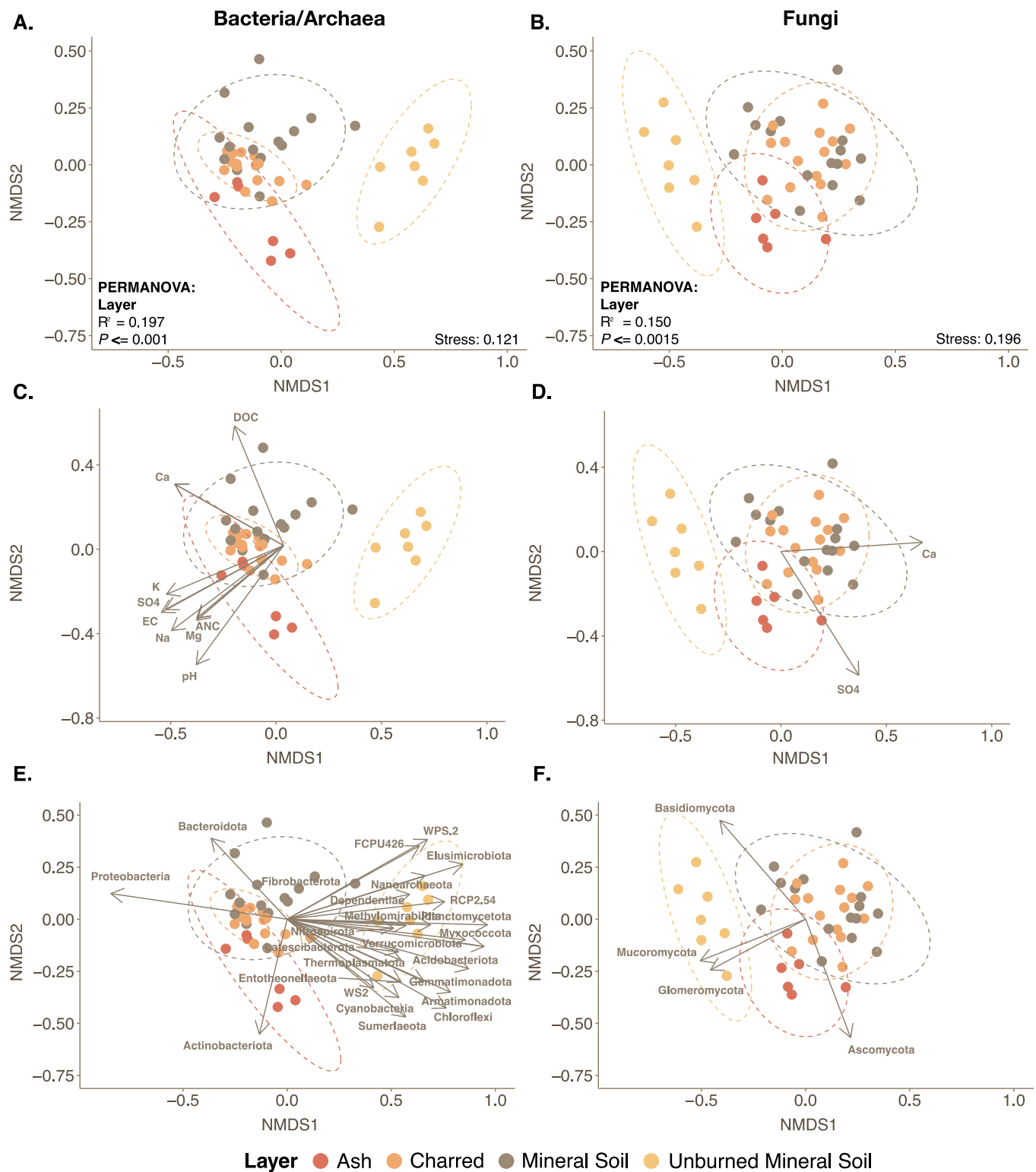


Fig. 3. Non-metric multidimensional scaling (NMDS) ordination plots using Bray-Curtis ASV community composition dissimilarities for bacterial/archaeal (16S; A, C, and E) and fungal (ITS; B, D, and F) taxa. Plots A and B include PERMANOVA tests on the influence of layer on microbial community composition. Plots C and D include overlaid environmental variable vectors while plots E and F include overlaid phyla-level taxa vectors from bacterial/archaeal (E) and fungal (F) community composition data (via *envfit*) denoting significant taxonomic drivers of community composition dissimilarity ($p < 0.05$).

results suggest that pile burning results in significant differences in microbial community composition due to the direct and indirect impacts of fire-induced alterations to soil chemistry compared to nearby unburned patches, resembling the impacts of natural wildfires to the soil ecosystem (Ferrenberg et al., 2013; Köster et al., 2021; Dove et al., 2022;

Nelson et al., 2022).

3.3. Pile burning generates soil microbial communities similar to those from natural wildfires

We compared data from this study with three other community

analyses performed following high severity wildfires in Colorado and Wyoming coniferous forests (Nelson et al., 2022, Caiafa et al., 2023, and McNorvell, 2023) to get a sense of how similar or different the soil microbiomes of these Montanan pile burn scars are to regionally

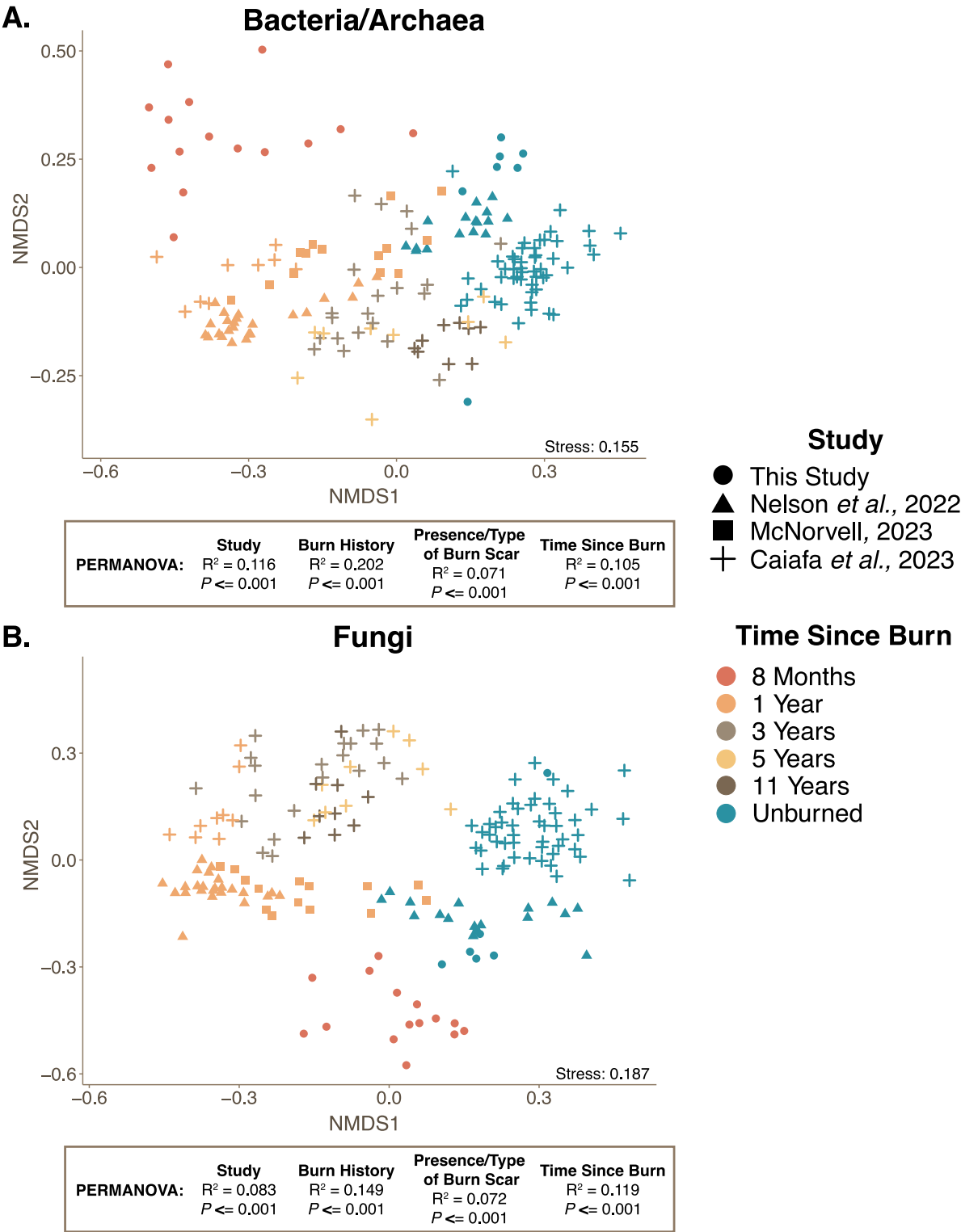


Fig. 4. Non-metric multidimensional scaling (NMDS) ordination plots using Bray-Curtis community composition dissimilarities at the genus level for bacterial/archaeal (16S; A) and fungal (ITS; B) taxa from four studies: this study (pile burns) and Nelson et al. (2022), Caiafa et al. (2023), and McNorvell (2023) (all wildfires). Samples are from the 0–5 cm mineral soil depth and represent either unburned control sites or sites impacted by high severity fire (either wildfire or pile burn derived). Both plots include PERMANOVA tests on the influence of study, burn history (high severity burn or unburned control), presence/type of burn scar (pile burn scar vs. wildfire scar vs. no burn scar), and time since burn (8 months up to 11 years) on microbial community composition.

disparate field samples. We found that soil microbiomes in pile burn scars are structured by many of the same factors as those impacted by high severity wildfire but clustered separately in ordination space (Table A.1, Fig. 4). Multivariate analyses revealed that the heterogeneity inherent in soils from different locations (Vos et al., 2013; Baldrian, 2017; Fierer, 2017; Philippot et al., 2023) and time since burn (Pérez-Valera et al., 2020; Dove et al., 2022; Caiafa et al., 2023) structure both bacterial/archaeal and fungal communities at the same level of significance as the presence/type of burn scar (pile burn scar vs. wildfire scar vs. no burn scar) ($p \leq 0.001$ for all). Bacterial/archaeal beta dispersion was significantly higher for the pile burn samples (8 months post-burn) in this study compared to all 1-year post-wildfire samples in the comparison studies (Fig. A.5). These include Nelson et al. (2022) for the Badger Creek and Ryan wildfires (both $p < 0.000001$), McNorvell (2023) for the Calwood, Cameron Peak, and Mullen wildfires (all $p < 0.05$), and Caiafa et al. (2023) for the Mullen wildfire ($p = 0.000015$) (Supplemental File 6). No significant differences in bacterial/archaeal beta dispersion values between the 1-year post-wildfire samples were observed. These findings align with previous studies that report greater stochasticity in bacterial community assembly with increased fire severity (Qin et al., 2022; Pinto et al., 2023), reflecting the observation that pile burns reach the upper limits of known human-induced fire severities and residence times which may contribute to the separation in ordination space of the pile burn scar samples (Santín & Doerr, 2016).

Fungal communities were structured similarly, with differences along NMDS1 representing burned and unburned populations and differences along NMDS2 representing time since burn within the burned samples and/or by study (Fig. 4). Concordant with bacterial/archaeal trends, differences in initial unburned communities varied by study location, likely owing to the heterogeneity of soil ecosystems (Vos et al., 2013; Baldrian, 2017; Fierer, 2017; Philippot et al., 2023). Unlike the bacterial/archaeal communities however, differences in fungal beta dispersion were less pronounced between the pile burn and 1-year post-burn wildfire samples (Fig. A.5, Supplemental File 7), suggesting greater resilience in fungal communities exposed to the increased fire severity stress associated with pile burning.

Together, these results suggest that the soil microbial communities in pile burn scars are structured by many of the same factors as those following high severity wildfire, namely study location (Vos et al., 2013; Baldrian, 2017; Fierer, 2017; Philippot et al., 2023), time since burn (Pérez-Valera et al., 2020; Dove et al., 2022; Caiafa et al., 2023), and likely the stochastic impacts of reduced population sizes that enhance founder effects during recolonization (Kim et al., 2013). The differences observed are likely partially attributable to initial alpha diversity and differing dispersal dynamics associated with the distance to unburned “fire refugia” between pile burn scars and high severity wildfire scars (Fig. 2) (Peay et al., 2012; Applestein et al., 2021; Barbour et al., 2023). Indeed, perturbation-driven stochastic microbial community shifts that lead to transitions from stable to unstable community states have been reported in a range of other systems, including human and animal microbiomes (Zaneveld et al., 2017).

While the pile burn scar soil microbiomes clearly clustered away from those of the wildfire scars, it is difficult to elucidate the extent to which inherent differences between pile burns and wildfires (e.g., residence time, etc.) lead to this separation versus attributes like time since fire, given the rapid changes in the soil microbiome in the first year post-burn (Pulido-Chavez et al., 2023) and distance between samples. Future work comparing pile burn and wildfire scar soil microbiomes that better control attributes like time since fire and fire location should help to resolve these challenges. However, the presence/type of burn scar structuring microbial community composition at the same significance level as ‘time since burn’ and ‘study’ supports the idea that pile burn scars can serve as a useful proxy for studying impacts of high severity or extreme wildfires on soil microbiomes at the community level. Building upon these community-level studies, analyses of community membership reveal the presence of cosmopolitan pyrophilous bio-indicator taxa

within burn pile scars.

3.4. Microbial community compositional changes following pile burning favor fire-tolerant taxa

The period of rapid microbial colonization post-wildfire results in taxa-specific changes to both soil bacterial/archaeal and fungal communities, with pyrophilous (i.e., ‘fire loving’) microorganisms frequently selected for and enriched up to five years post-burn (Caiafa et al., 2023). We observed increased relative abundances of known pyrophilous bacterial phyla *Firmicutes* and *Actinobacteria* in addition to *Bacteroidetes*, which exhibits varied post-fire responses in the wildfire literature, in our burned pile burn scar layers (ash, charred, and mineral soil) compared to the unburned mineral soil samples (Fig. A.6) (Ferrenberg et al., 2013; Weber et al., 2014; Whitman et al., 2019; Enright et al., 2022; Nelson et al., 2022; Caiafa et al., 2023; Fernández-González et al., 2023). Burning also resulted in decreases in the relative abundance of bacteria and archaea associated with nine different phyla, including *Acidobacteria*, *Planctomycetes*, *Verrucomicrobiota*, and *Gemmatimonadota*, between the unburned mineral soil layer and each of the three burned layers, again consistent with the phyla reported to be fire-sensitive in the wildfire literature (Whitman et al., 2019; Enright et al., 2022; Nelson et al., 2022; Fernández-González et al., 2023). The integration of phyla-level taxonomic information into multivariate ordinations further highlighted how specific microbial groups contributed to dissimilarity between sample types (Fig. 3E and 3F). While members of *Proteobacteria* and *Actinobacteria* were responsible for the separation of microbial communities between burned and unburned samples, fire-responding taxa within the *Actinobacteria* (e.g., ASVs affiliated with *Frankiales*, *Paeniglutamicibacter*, and *Cryobacterium*) further differentiated communities in ash from all other sample types as revealed by combined LefSe/LDA analyses (Fig. 5), a statistical method designed to find microbial taxa with relative abundances that differ significantly between treatment groups of interest (LefSe) coupled with estimations of effect size (LDA) where a larger effect size denotes a taxa important for distinguishing between the microbiomes of the treatment groups of interest (here, burned vs. unburned soil) (Chong et al., 2020).

Highlighting the frequent disconnect between consistent phyla-level shifts and the responses of individual microbial taxa to the pulse disturbance of fire (Philippot et al., 2010; Stone et al., 2023), combined LefSe/LDA analyses revealed three *Proteobacteria* ASVs (family *Xanthobacteraceae*, genus *Burkholderia*-*Caballeronia*-*Paraburkholderia*, and genus *TRA3-20*) discriminant for unburned mineral soil samples compared to the other burned layers, two of which were additionally core members of this layer (Figs. 5 and A.6, Supplemental File 8) (Neu et al., 2021), where an ASV was deemed a core community member if that ASV was present in at least 50% of samples from a site/layer combination within all sites. This suggests that despite broad phyla-level trends, specific ASVs within the *Proteobacteria* are highly discriminant towards the unburned sites compared to the burned layers.

Further, we observed large increases in the relative abundances of putative pyrophilous taxa affiliated with *Arthrobacter* (*Actinobacteria*), *Massilia* (*Proteobacteria*), and *Paenibacillus* (*Firmicutes*) between unburned and burned samples (Fig. A.7), mirroring trends reported in other post-fire studies (Weber et al., 2014; Enright et al., 2022; Fischer et al., 2023; Pulido-Chavez et al., 2023). In total, we identified 58 bacterial ASVs discriminant between the burned and unburned layers within the dataset, the majority of which occurred at higher relative abundances in one or more of the three burned layers relative to the unburned mineral soil layer (Segata et al., 2011) (Fig. 5). Of these, 45 ASVs could be identified down to the genus level and included the aforementioned *Paenibacillus* and *Massilia* ASVs (Pulido-Chavez et al., 2023).

Fire-associated shifts in soil fungal communities from *Basidiomycota* to *Ascomycota*-dominated have been previously reported (Fox et al., 2022) and we observed this shift to *Ascomycota*-dominance between

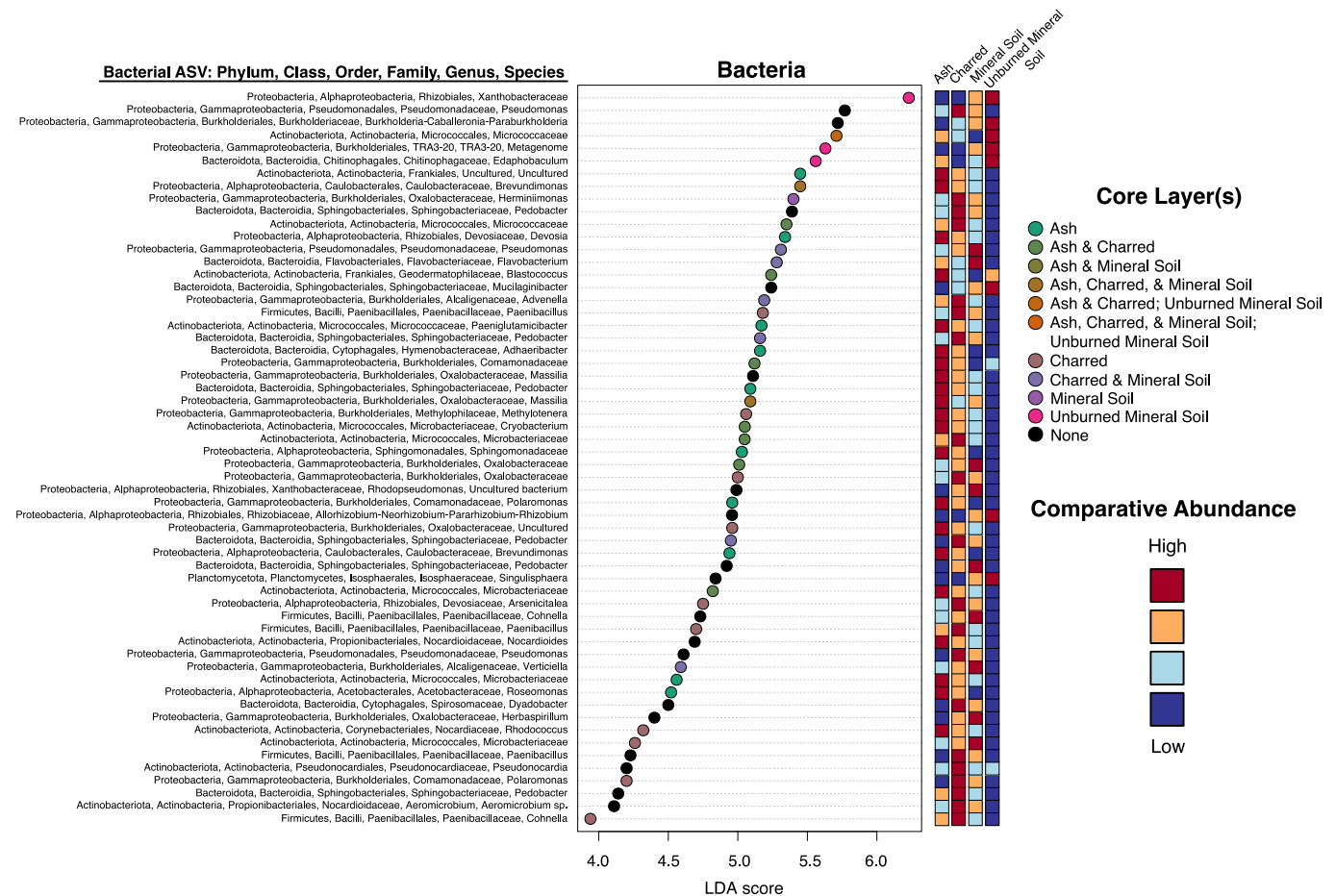


Fig. 5. Results of a Kruskal-Wallis rank sum test followed by combined Linear discriminant analysis Effect Size (LEfSe) and Linear Discriminant Analysis (LDA) analysis for biomarker discovery at the bacterial (no archaea were found to be discriminant by this analysis) (16S) ASV level between the burned (ash, charred, and the 0–5 cm mineral soil depth) layers and an unburned control (0–5 cm mineral soil) layer with a significance cutoff of $p < 0.05$ after FDR adjustment and a log LDA score = 2.0. Scale colors represent comparative abundance, where red represents high relative abundance compared to the other soil layers for that ASV, followed by orange, light blue, and with dark blue representing low relative abundance compared to the other soil layers for that ASV. ASV codes have been replaced with taxonomic assignment from the SILVA database (Quast et al., 2013). Points have been colored according to membership within core bacterial microbiome community in our dataset, where ASVs were determined to be a core community member if they were present in at least 50% of samples from a site/layer combination within all sites. A single ASV may be a core member of more than one soil layer, as represented by the 11 colors in this figure, including both burned and unburned layers. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

unburned and burned soils in this study (Fig. A.6B). This finding was further supported by multivariate analyses (Fig. 3F) and combined LEfSe/LDA analyses at the phyla level, with *Basidiomycota* and *Ascomycota* revealed as the only discriminant fungal phyla between these layers (Fig. A.6D, Supplemental File 9).

Three genera within the *Ascomycota* are theorized as potential “cosmopolitan” fungal taxa by Fox & Sikes et al. (2022): *Pyronema* (*Pyronemataceae*), *Anthracobia* (*Pyronemataceae*) and *Morchella* (*Morchellaceae*). While we detected no ASVs affiliated with *Pyronema* and no increase in the relative abundance of *Morchella* in our analyses, we identified a large increase in the relative abundance of the genus *Anthracobia* largely attributable to one known pyrophilous core ASV (*Anthracobia macrocystis*; ~ 13%) in ash samples (Fig. 6, Fig. A.7) (Fox & Sikes et al., 2022; Porter et al., 2023). Together, this ASV and another core ASV identified as a pyrophilous species known to only fruit post-fire, *Tricharina praecox*, accounted for nearly 30% of all fungal ASVs within the ash samples (Van Vooren et al., 2017; Bruns et al., 2020; Raudabaugh et al., 2020; Steindorff et al., 2022) (Fig. 6). Future work should examine the role of microbial community assembly post-fire in the ash layer specifically, assessing whether the differences observed throughout this work between the ash layer and other post-fire layers (charred organic matter, mineral soil) are due to dispersal from the

surrounding intact vegetation or other mechanisms.

Highlighting the niche differentiation between the distinct burned layers, the pyrophilous members of the genus *Penicillium* (Pulido-Chavez et al., 2021) exhibited the greatest relative abundance increases in the deeper charred and mineral soil layers, ranging from <1% relative abundance in unburned mineral soil samples to >8% in burned mineral soils. Combined LEfSe/LDA analyses identified 13 fungal ASVs discriminant between the different layers, 10 of which could be taxonomically resolved at the genus level, including a *Penicillium* ASV with high relative abundance in burned charred and mineral soil layers relative to the ash layer and unburned mineral soil layer (Fig. 7A).

High severity fires are frequently associated with a loss of ectomycorrhizal (EMF) (Nelson et al., 2022) and arbuscular mycorrhizal (AMF) fungi and increases in the relative proportion of saprophytic fungi (Pulido-Chavez et al., 2023). Pile burning resulted in a reduction in fungal guild diversity, with a complete loss of litter saprotroph, lichen parasite, and orchid mycorrhizal ASVs from unburned to burned soils (Fig. 7B). Ericoid mycorrhizal relative abundance additionally decreased from an average of ~0.2% to between 0.001% and 0.03% in the burned layers. We further observed a >99% reduction in relative abundance for both EMF and AMF (although there are limitations to utilizing ITS amplicon sequencing for AMF; Delavaux et al., 2022)

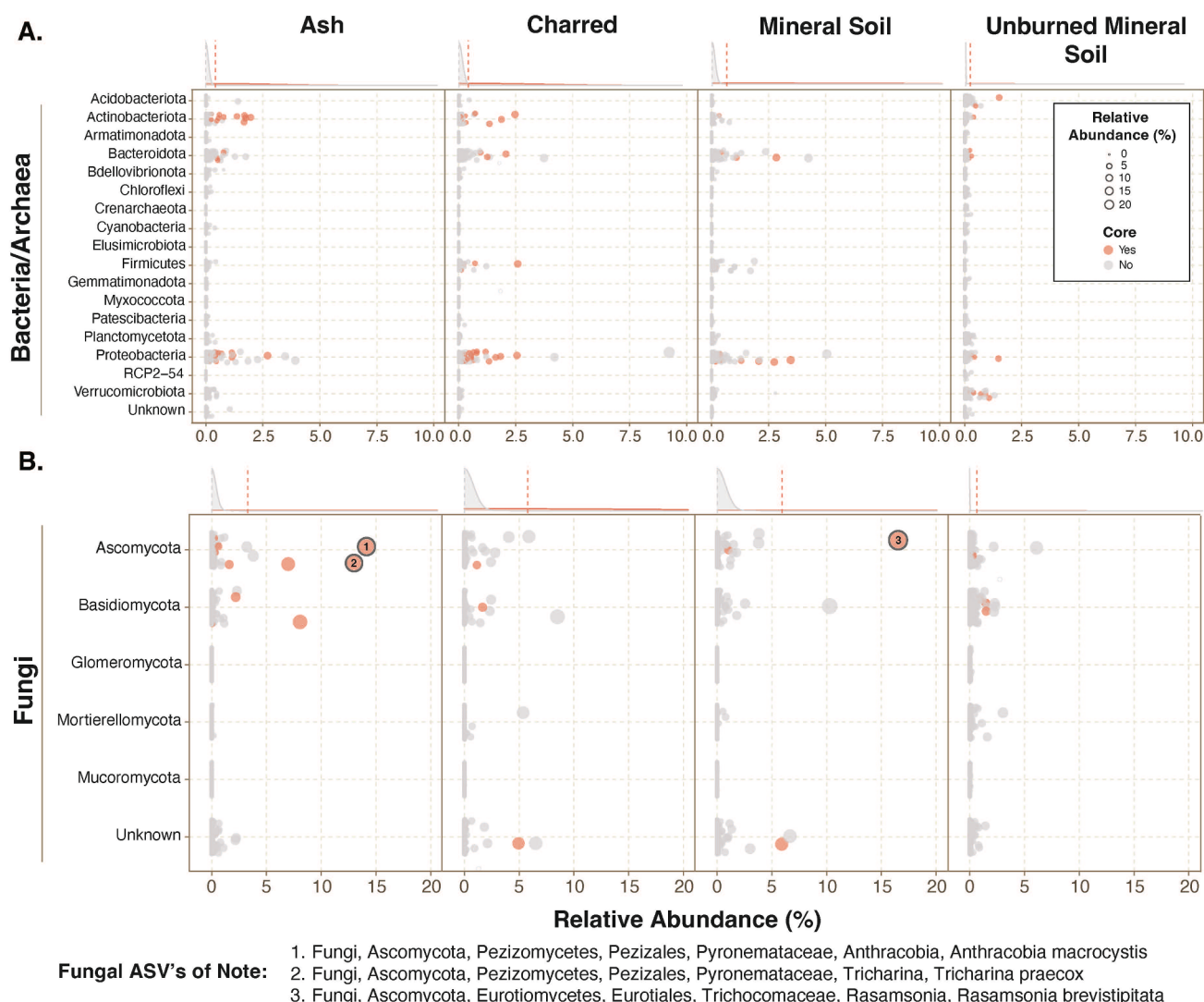


Fig. 6. Dotplots showing individual amplicon sequence variants (ASVs) (as circles) within each layer (ash, charred, burned mineral soil, or unburned mineral soil) and the relationship between ASV relative abundance (%) and taxonomic assignment at the phyla level for both bacteria/archaea (A) and fungi (B). Circle size corresponds to the relative abundance of the ASV. Circle color (orange or grey) corresponds to ASV core membership, where ASVs were determined to be a core community member if they were present in at least 50% of samples from a site/layer combination. Density distributions (above each dotplot) show the distribution of ASVs across the range of relative abundances and the dotted lines display the average relative abundance for core microbes within each layer (orange) and all other ASVs within that layer (grey). Three fungal ASVs of note are marked with a black outline and the numbers “1”, “2”, and “3” with their respective taxonomic assignments detailed at the bottom of the figure. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

between unburned and burned soils, regardless of layer, supported by a >99% decrease in the relative abundance of taxa affiliated with the *Glomeromycota* phyla of AMF between the unburned mineral soil layer and all burned layers (Fig. A.6). Other (“undefined”) saprotroph relative abundance increased with burning (Fig. 7B and 7C).

Studies have reported three ectomycorrhizal fungi (crucial to conifer establishment and growth in the forests of western North America) genera believed to favor post-fire soil environments: *Wilcoxina*, *Rhizopogon*, and *Pustularia* (Glassman et al., 2016; Fox & Sikes et al., 2022). We observed near complete or complete loss of these three genera, aside from a slight increase in the relative abundance of *Pustularia* from unburned to burned conditions in mineral soil samples (Fig. A.7). *Wilcoxina* relative abundance decreased from >5% to almost zero with burning, although one species, *Wilcoxina rehmii*, was core to the ash layer. These differences may stem from the coupled disturbance of thinning and harvesting prior to pile burning. While limited, some pre-burn host mortality may delay vegetation recovery in the burn scars due

to EMF loss, in addition to high temperature-induced degradation of remaining EMF (Rhoades et al., 2021; Caiafa et al., 2023; Pulido-Chavez et al., 2023). The integration of time since host death into pile burn studies may help to elucidate the impacts of other coupled disturbances in western conifer forests such as mountain pine beetle (*Dendroctonus ponderosae*) outbreaks followed by wildfire (Caiafa et al., 2023).

3.5. Core microbiome members likely encode beneficial traits for the post-fire soil ecosystem, including increased average 16S copy number suggesting faster growth rate

Like wildfire impacted soil ecosystems, we observed that pile burn scars select for microbes that display beneficial traits for colonization and post-fire survival (Dove et al., 2022; Nelson et al., 2022; Nelson et al., 2024). Per Grime’s Competition-Stress tolerator-Ruderal (CSR) succession theory, traits such as production of thermotolerant structures, the ability to utilize pyrogenic organic matter, and fast growth

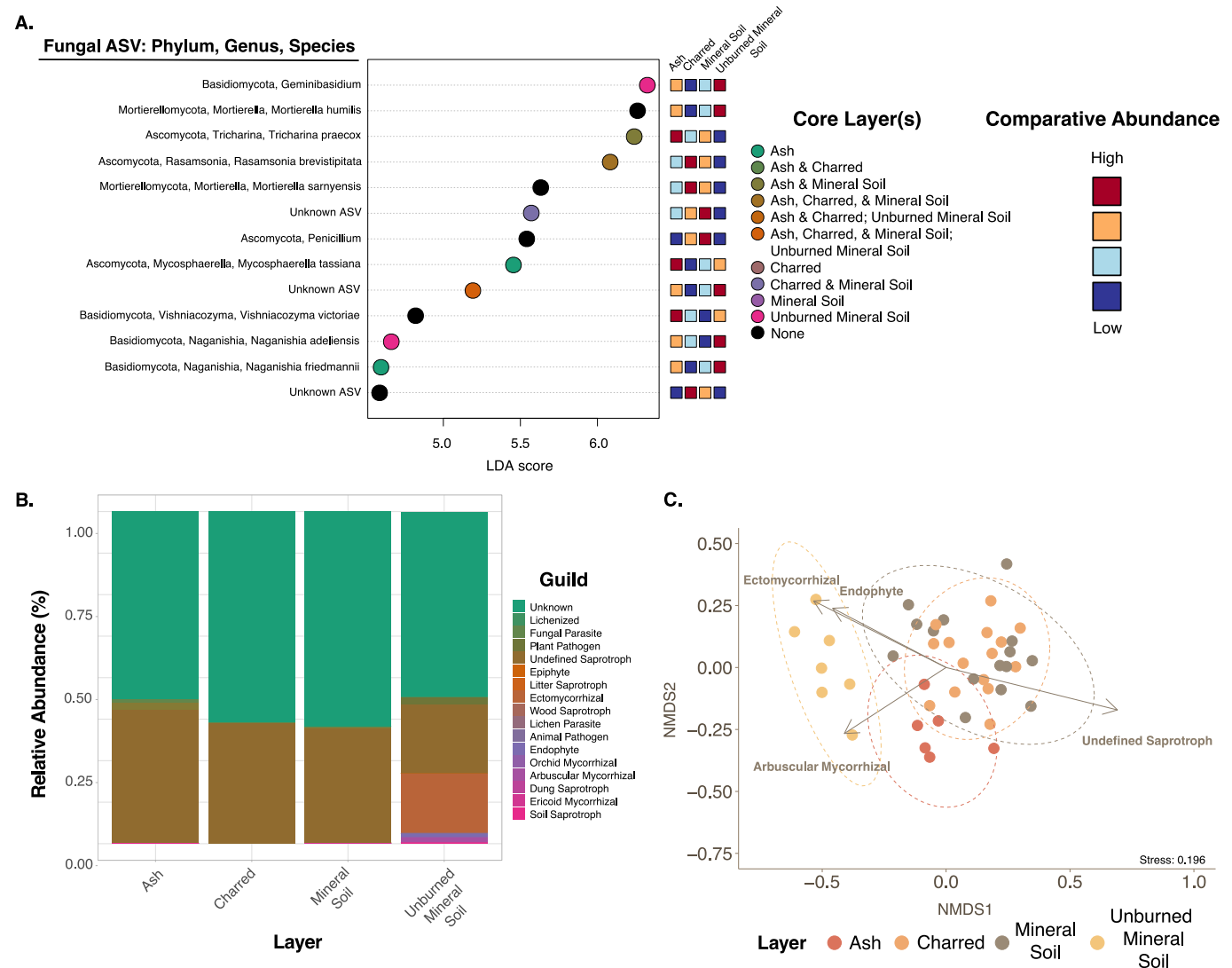


Fig. 7. (A) Results of a Kruskal-Wallis rank sum test followed by combined Linear discriminant analysis Effect Size (LEfSe) and Linear Discriminant Analysis (LDA) analysis for biomarker discovery at the fungal (ITS) ASV level between the burned (ash, charred, and the 0–5 cm mineral soil depth) and an unburned control (0–5 cm mineral soil) layers with a significance cutoff of $p < 0.05$ after FDR adjustment and a log LDA score = 2.0. Scale colors represent comparative abundance, where red represents high relative abundance compared to the other soil layers for that ASV, followed by orange, light blue, and with dark blue representing low relative abundance compared to the other soil layers for that ASV. ASV codes have been replaced with taxonomic assignment from the UNITE database (Nilsson et al., 2019). Points have been colored according to membership within a core fungal microbiome community in our dataset, where ASVs were determined to be a core community member if they were present in at least 50% of samples from a site/layer combination within all sites. A single ASV may be a core member of more than one soil layer, as represented by the 11 colors in this figure, including both burned and unburned layers. (B) Barchart showing relative abundances expressed as a percentage within the three burned layers (ash, charred, and the 0–5 cm mineral soil depth) and an unburned control (0–5 cm mineral soil) averaged across all sites for fungal functional guilds as reported by FUNGuild (Nguyen et al., 2016). (C) Non-metric multidimensional scaling (NMDS) ordination plot using Bray-Curtis ASV community composition dissimilarities for fungal taxa. The plot includes overlaid fungal function guild vectors from FUNGuild denoting significant drivers of community composition dissimilarity in relative abundance of each fungal guild within each sample between groups ($p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

rates trade off against one another (Pulido-Chavez et al., 2021; Enright et al., 2022). While acknowledging the limitations of inferring microbial physiology and metabolism from marker gene data, here we link our results with prior studies of traits associated with common pyrophilous microorganisms. Within this framework, the pyrophilous genera observed in this study likely leverage each of the lifestyles described within the CSR theory. Enriched *Paenibacillus* represents the Stress Tolerant (S) mode of ecological trade-off, with the ability to form thermotolerant structures including endospores (Pulido-Chavez et al., 2023). *Massilia* represents the Competitive (C) and Ruderal (R) modes of ecological trade-off, with the ability to grow quickly and exploit complex post-fire resources (Enright et al., 2022; Pulido-Chavez et al.,

2023). *Noviherbaspirillum* utilizes C and R strategies but may also degrade complex pyrogenic organic matter (PyOM) (Pulido-Chavez et al., 2023). Within the fungal communities, *Penicillium* utilizes R modes of ecological trade-off, with fast growth and production of asexual reproductive spores (Pulido-Chavez et al., 2021; Enright et al., 2022), while *Pholiota highlandensis* has been noted as a potential utilizer of PyOM or other post-fire resources (Enright et al., 2022).

Prior studies have observed a relationship between higher 16S rRNA gene copy numbers and faster growth rates (Roller et al., 2016), enabling us to focus on the potential for fast growth as a mechanism to quickly colonize environmental niches post-fire per CSR theory within our dataset, with the caveat that relative abundance and 16S rRNA gene

copy number may be confounded within this analysis (Větrovský & Baldrian, 2013; Louca et al., 2018). We observed significant relationships between ASV relative abundance and 16S rRNA gene copy number within the burned layers that differed from the unburned mineral soil layer (Fig. 8). ASV relative abundances within the ash, charred, and burned mineral soil layers were positively correlated with 16S rRNA gene copy number, in direct contrast to trends observed in unburned mineral soils. Additionally, ASVs with higher-than-average 16S rRNA gene copy numbers and relative abundances were frequently present as members of the core microbiome within the same burned layer. These results suggest that up to 8 months post-burn, microbes that prioritize fast growth over C use efficiency are favored. This is likely due to abundant C substrates in burned soils, derived from fire-induced nutrient-rich necromass (Bruns et al., 2020) and energetically-favorable labile pools of PyOM (Bostick et al., 2021; Zhang et al., 2023). This alteration of the soil ecosystem post-burning likely selects for microbes that can grow quickly and take advantage of these resources.

4. Limitations

We observed chemical and microbial responses to fire within burn pile scars that resemble those observed in soils disturbed by high severity wildfire, including when combined in the same dataset. Given that this sampling represents a singular timepoint after management-focused pile burns, we likely missed the earliest successional pyrophilous taxa following fire, potentially explaining the lack of *Pyronema*, including *Pyronema domesticum*, and other common pyrophilous genera in our dataset (Pulido-Chavez et al., 2023). Temporal sampling of burn pile scars initiated immediately following burning should better characterize the rapid succession that likely occurs within the soil microbiome following wildfire.

5. Management implications and accessible research gaps

Pile burn scars may represent a study system for future explorations of ecologically rare ‘extreme’ wildfires (Santín & Doerr, 2016; Linley et al., 2022) on soil microbiomes, resulting in increased mechanistic understanding of the impacts of these disturbances under varying intensities (Smith, 2011). As wildfires increase in frequency, size, and

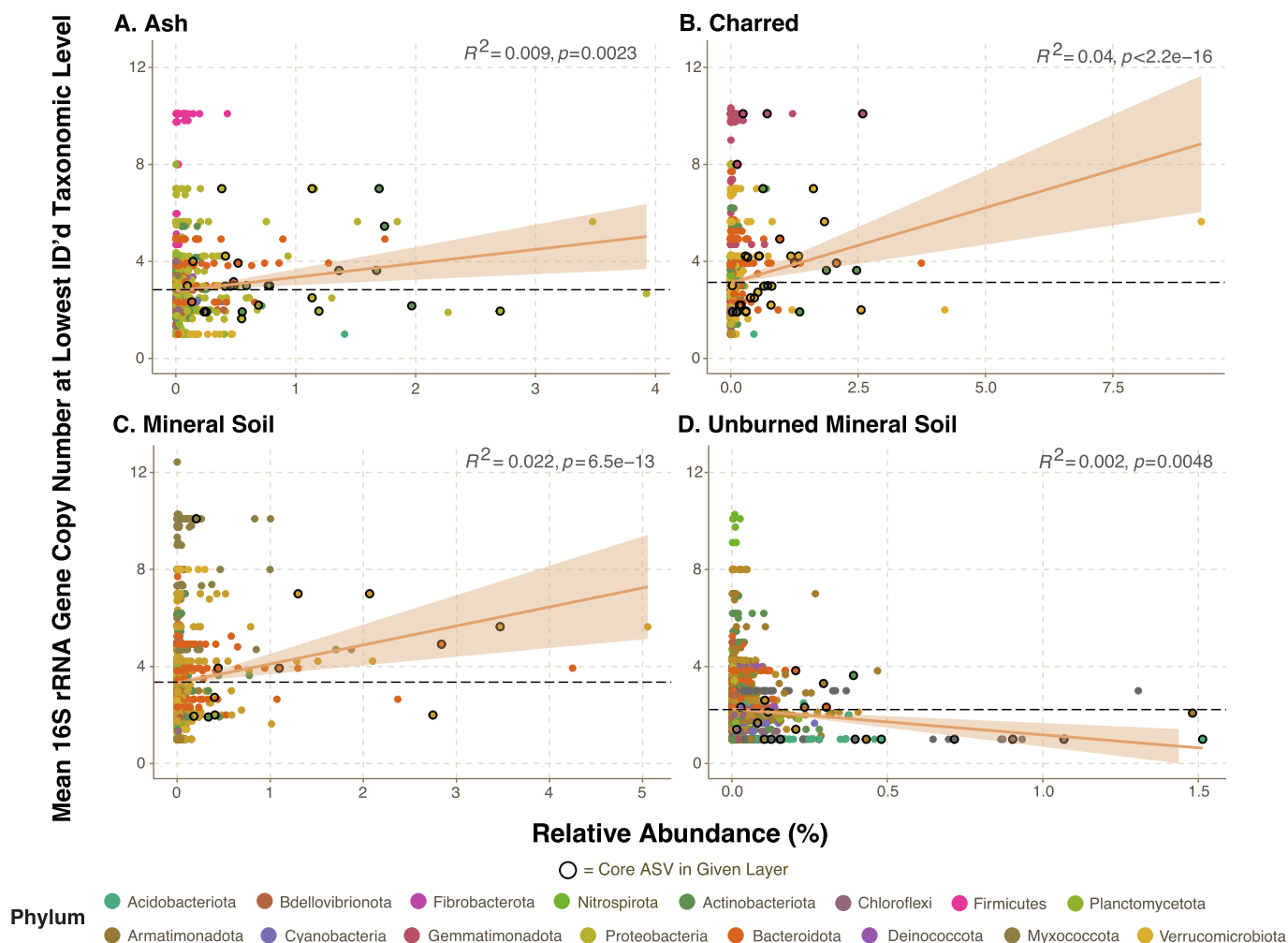


Fig. 8. Scatterplots representing the relationship between relative abundance of each ASV and the mean 16S rRNA gene copy number at the lowest available taxonomic assignment as reported in the rrnDB database (<https://rrnodb.umms.med.umich.edu>) for the ash (A), charred (B), burned mineral soil (C), and unburned mineral soil (D) layers. Each point represents an individual ASV with color corresponding to bacterial (no archaea in this dataset were present in the rrnDB database) phylum and those core to that layer outlined in black, where ASVs were determined to be a core community member if they were present in at least 50% of samples from a site/layer combination. Orange line is a fitted linear regression line with the 95% confidence interval in light orange. The horizontal dashed line represents the average 16S rRNA gene copy number for each layer. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

severity across the globe there is a need to examine “worst case scenarios” and potential mechanisms for studying fire effects while controlling myriad variables present in natural environments (Dennison et al., 2014; Parks and Abatzoglou, 2020). With the fostering of governmental and academic partnerships to utilize the tens of thousands of pile burns that take place each year, these mesocosms represent a wealth of data on a temporal scale that is otherwise difficult to study with natural wildfires alone.

Future work coupling management through pile burning with robust experimental manipulation, replication, and the use of time series sampling will enable researchers to fill existing gaps in our understanding of high severity wildfire impacts on forest soils. Some noted research gaps that these methods may be able to address include assessing the survival of mycorrhizal fungi spores and/or propagules with implications for vegetation recovery (Hewitt et al., 2023), the influence of time since burn on microbial community recovery (time series studies), and the impact of repeated fires (same or varying intensity; Certini et al., 2021), under varying temperatures, amounts of fuel, volumes of water to simulate rainfall events, and other forms of experimental manipulation. Additionally, pile burns may serve as a useful experimental setup to test the impacts of wildfire restoration, such as through inoculation with fungi (Korb et al., 2004; Hewitt et al., 2023) or mulching, including the implications of varying mulch cover and depth that can result from largescale (and costly) helimulching operations on soil microbiomes and the ecosystem as a whole (Dodson & Peterson, 2010; Fernández et al., 2016; Girona-García et al., 2021; Espinosa et al., 2023; Girona-García et al., 2023). Pile burn scars represent an excellent “open air” (Myers-Pigg et al., 2024) mesocosm, or intermediary between the well-controlled setting of a lab and realism of the field with intact surrounding vegetation and potential for microbial dispersal into the scars, for the study of severe wildfire disturbances in a rapidly changing world.

6. Conclusions

Pile burning is an established forest management technique that will likely increase in application as wildfire risk mitigation efforts grow across the western US (Schoennagel et al., 2017; Hoover, 2020); indeed, the Medicine Bow–Routt National Forest of Colorado and Wyoming conducted approximately 26,000 pile burns during the winter of 2022–2023 alone (National Wildfire Coordinating Group, 2023). With this in mind, we quantitatively and qualitatively assessed whether recent pile burn scars near West Yellowstone, Montana exhibited the soil chemical and microbiological signatures of high severity wildfire in the western USA while also comparing the overall trends in community composition to those of related high severity wildfire soil microbiome studies. Pile burning influenced bulk chemistry, patterns of microbial alpha and beta diversity (within and between studies) including the enrichment of ‘pyrophilous’ taxa at the phyla, genus, and species level, and the loss of fungal guilds that mimicked similar observations from high severity wildfires. Further, many of the enriched ‘pyrophilous’ taxa likely utilized a series of beneficial traits for colonization and survival in these ecosystems. These findings were despite the extensive heterogeneity associated with soil samples at both the micro- and macro-scale and additional geographic variables such as climate. Our findings in this proof-of-concept study support the assertion that pile burns can be utilized as high severity wildfire proxies given the conserved chemical and microbial patterns described in this study. Pile burns fill a needed gap along the experimental gradient from muffle furnace or small pyrocosm studies to largescale prescribed burns in wildfire research. We encourage their use with detailed experimental manipulation to begin to fill in the research gaps in the early post-wildfire soil microbiome literature.

CRedit authorship contribution statement

Julie A. Fowler: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Amelia R. Nelson:** Writing – review & editing, Methodology, Formal analysis. **Emily K. Bechtold:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Raegan Paul:** Writing – review & editing, Formal analysis. **Alexandra M. Wettengel:** Writing – review & editing, Investigation. **Michael A. McNorvell:** Writing – review & editing, Investigation. **Camille S. Stevens-Rumann:** Writing – review & editing, Supervision, Resources. **Timothy S. Fegel:** Writing – review & editing, Investigation. **Erik Anderson:** Writing – review & editing, Resources. **Charles C. Rhoades:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Michael J. Wilkins:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Details on code availability and DNA sequence archiving are available in the manuscript.

Acknowledgements

This work was supported through a USDA NIFA award [2021-67019-34608] to M.J.W. and C.C.R., an NSF award [DEB-2114868] to M.J.W., and an award through the DOE BER Genomic Science Program [DE-SC0023127] to M.J.W.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.geoderma.2024.116982>.

References

- Agbeshie, A.A., Abugre, S., Atta-Darkwa, T., Awuah, R., 2022. A review of the effects of forest fire on soil properties. *J. For. Res.* 33 (5), 1419–1441. <https://doi.org/10.1007/s11676-022-01475-4>.
- Alexander, M.E., Mutch, R.W., Davis, K.M., Bucks, C.M., 2017. Wildland fires: Dangers and survival. In: Auerbach, P.S. (Ed.), *Auerbach's Wilderness Medicine*, 1. Elsevier, Philadelphia, PA, pp. 276–318.
- Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecol.* 26, 32–46. <https://doi.org/10.1111/j.1442-9993.2001.01070.x>.
- Applestein, C., Simler-Williamson, A.B., Germino, M.J., 2021. Weather and distance to fire refugia limit landscape-level occurrence of fungal disease in an exotic annual grass. *J. Ecol.* 109 (5), 2247–2260. <https://doi.org/10.1111/1365-2745.13638>.
- Apprill, A., McNally, S., Parsons, R., Weber, L., 2015. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquat. Microb. Ecol.* 75 (2), 129–137. <https://doi.org/10.3354/ame01753>.
- Baldrian, P., 2017. Forest microbiome: diversity, complexity and dynamics. *FEMS Microbiol. Rev.* 109–130. <https://doi.org/10.1093/femsre/fuw040>.
- Barbour, K.M., Weihe, C., Walters, K.E., Martiny, J.B.H., 2023. Testing the contribution of dispersal to microbial succession following a wildfire. *mSystems* 8 (5), e00579–e00623. <https://doi.org/10.1128/mSystems.00579-23>.
- Barreiro, A., Díaz-Raviña, M., 2021. Fire impacts on soil microorganisms: Mass, activity, and diversity. *Curr. Opin. Environ. Sci. Health* 22 (100264). <https://doi.org/10.1016/j.coesh.2021.100264>.
- Birch, D.S., Morgan, P., Kolden, C.A., Abatzoglou, J.T., Dillon, G.K., Hudak, A.T., Smith, A.M.S., 2015. Vegetation, topography and daily weather influenced burn severity in central Idaho and western Montana forests. *Ecosphere* 6 (1), 1–23. <https://doi.org/10.1890/ES14-00213.1>.
- Bokulich, N.A., Kaehler, B.D., Rideout, J.R., Dillon, M., Bolyen, E., Knight, R., Huttley, G.A., Gregory Caporaso, J., 2018. Optimizing taxonomic classification of marker-gene

- amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome* 6 (1), 90. <https://doi.org/10.1186/s40168-018-0470-z>.
- Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.M., Chase, J., Caporaso, J.G., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* 37 (8), 852–857. <https://doi.org/10.1038/s41587-019-0209-9>.
- Bostick, K.W., Zimmerman, A.R., Goranov, A.I., Mitra, S., Hatcher, P.G., Wozniak, A.S., 2021. Biolability of Fresh and Photodegraded Pyrogenic Dissolved Organic Matter From Laboratory-Prepared Chars. *J. Geophys. Res. Biogeo.* 126 (5) <https://doi.org/10.1029/2020JG005981>.
- Brucker, C.P., Livneh, B., Minear, J.T., Rosario-Ortiz, F.L., 2022. A review of simulation experiment techniques used to analyze wildfire effects on water quality and supply. *Environ. Sci. Processes Impacts* 24 (8), 1110–1132. <https://doi.org/10.1039/D2EM00045H>.
- Bruns, T.D., Chung, J.A., Carver, A.A., Glassman, S.I., 2020. A simple pyrocosm for studying soil microbial response to fire reveals a rapid, massive response by *Pyronema* species. *PLoS One* 15 (3). <https://doi.org/10.1371/journal.pone.0222691>.
- Busse, M.D., Hubbert, K.R., Fiddler, G.O., Shestak, C.J., Powers, R.F., 2005. Lethal soil temperatures during burning of masticated forest residues. *Int. J. Wildland Fire* 14 (3), 267. <https://doi.org/10.1071/WF04062>.
- Busse, M.D., Shestak, C.J., Hubbert, K.R., Knapp, E.E., 2010. Soil Physical Properties Regulate Lethal Heating during Burning of Woody Residues. *Soil Sci. Soc. Am. J.* 74 (3), 947–955. <https://doi.org/10.2136/sssaj2009.0322>.
- Busse, M.D., Shestak, C.J., Hubbert, K.R., 2013. Soil heating during burning of forest slash piles and wood piles. *Int. J. Wildland Fire* 22 (6), 786. <https://doi.org/10.1071/WF12179>.
- Caiafa, M.V., Nelson, A.R., Borch, T., Roth, H.K., Fegler, T.S., Rhoades, C.C., Wilkins, M.J., Glassman, S.I., 2023. Distinct fungal and bacterial responses to fire severity and soil depth across a ten-year wildfire chronosequence in beetle-killed lodgepole pine forests. *For. Ecol. Manage.* 544, 121160 <https://doi.org/10.1016/j.foreco.2023.121160>.
- Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* 13 (7), 581–583. <https://doi.org/10.1038/nmeth.3869>.
- Certini, G., 2005. Effects of fire on properties of forest soils: A review. *Oecologia* 143 (1), 1–10. <https://doi.org/10.1007/s00442-004-1788-8>.
- Certini, G., Moya, D., Lucas-Borja, M.E., Mastrodonato, G., 2021. The impact of fire on soil-dwelling biota: A review. *For. Ecol. Manage.* 488, 118989 <https://doi.org/10.1016/j.foreco.2021.118989>.
- Chong, J., Liu, P., Zhou, G., Xia, J., 2020. Using MicrobiomeAnalyst for comprehensive statistical, functional, and meta-analysis of microbiome data. *Nat. Protoc.* 15 (3), 799–821. <https://doi.org/10.1038/s41596-019-0264-1>.
- Coop, J.D., Parks, S.A., Stevens-Rumann, C.S., Crausbay, S.D., Higuera, P.E., Hurteau, M.D., Tepley, A., Whitman, E., Assal, T., Collins, B.M., Davis, K.T., Dobrowski, S., Falk, D.A., Fornwalt, P.J., Fulé, P.Z., Harvey, B.J., Kane, V.R., Littlefield, C.E., Margolis, E.Q., Rodman, K.C., 2020. Wildfire-Driven Forest Conversion in Western North American Landscapes. *Bioscience* 70 (8), 659–673. <https://doi.org/10.1093/biosci/biaa061>.
- de la Rosa, J.M., Knicker, H., 2011. Bioavailability of N released from N-rich pyrogenic organic matter: An incubation study. *Soil Biol. Biochem.* 43 (12), 2368–2373. <https://doi.org/10.1016/j.soilbio.2011.08.008>.
- Delavaux, C.S., Ramos, R.J., Sturmer, S.L., Bever, J.D., 2022. Environmental identification of arbuscular mycorrhizal fungi using the LSU rDNA gene region: An expanded database and improved pipeline. *Mycorrhiza* 32 (2), 145–153. <https://doi.org/10.1007/s00572-022-01068-3>.
- Dennison, P.E., Brewer, S.C., Arnold, J.D., Moritz, M.A., 2014. Large wildfire trends in the western United States, 1984–2011. *Geophys. Res. Lett.* 41 (8), 2928–2933. <https://doi.org/10.1002/2014GL059576>.
- Dillon, G.K., Holden, Z.A., Morgan, P., Crimmins, M.A., Heyerdahl, E.K., Luce, C.H., 2011. Both topography and climate affected forest and woodland burn severity in two regions of the western US, 1984 to 2006. *Ecosphere* 2 (12). <https://doi.org/10.1890/ES11-00271.1>.
- Dodson, E.K., Peterson, D.W., 2010. Mulching effects on vegetation recovery following high severity wildfire in north-central Washington State, USA. *For. Ecol. Manage.* 260 (10), 1816–1823. <https://doi.org/10.1016/j.foreco.2010.08.026>.
- Donato, D.C., Simard, M., Romme, W.H., Harvey, B.J., Turner, M.G., 2013. Evaluating post-outbreak management effects on future fuel profiles and stand structure in bark beetle-impacted forests of Greater Yellowstone. *For. Ecol. Manage.* 303, 160–174. <https://doi.org/10.1016/j.foreco.2013.04.022>.
- Dove, N.C., Klingeman, D.M., Carrell, A.A., Cregger, M.A., Schadt, C.W., 2021. Fire alters plant microbiome assembly patterns: Integrating the plant and soil microbial response to disturbance. *New Phytol.* 230 (6), 2433–2446. <https://doi.org/10.1111/nph.17248>.
- Dove, N.C., Tag, N., Hart, S.C., 2022. Ecological and genomic responses of soil microbiomes to high-severity wildfire: Linking community assembly to functional potential. *ISME J.* 16 (7), 1853–1863. <https://doi.org/10.1038/s41396-022-01232-9>.
- Enright, D.J., Frangioso, K.M., Isobe, K., Rizzo, D.M., Glassman, S.I., 2022. Mega-fire in redwood tanoak forest reduces bacterial and fungal richness and selects for pyrophilous taxa that are phylogenetically conserved. *Mol. Ecol.* 31 (8), 2475–2493. <https://doi.org/10.1111/mec.16399>.
- Espinosa, J., Dejene, T., Fernández, C., Madrigal, J., Aponte, C., Martín-Pinto, P., 2023. Does helimulching after severe wildfire affect soil fungal diversity and community composition in a Mediterranean ecosystem? *Sci. Total Environ.* 892, 164752 <https://doi.org/10.1016/j.scitotenv.2023.164752>.
- Fernández, C., Vega, J.A., Fontúrbel, T., 2016. Reducing post-fire soil erosion from the air: Performance of heli-mulching in a mountainous area on the coast of NW Spain. *Catena* 147, 489–495. <https://doi.org/10.1016/j.catena.2016.08.005>.
- Fernández-García, V., Miesel, J., Baeza, M.J., Marcos, E., Calvo, L., 2019. Wildfire effects on soil properties in fire-prone pine ecosystems: Indicators of burn severity legacy over the medium term after fire. *Appl. Soil Ecol.* 135, 147–156. <https://doi.org/10.1016/j.apsoil.2018.12.002>.
- Fernández-González, A.J., Lasa, A.V., Cobo-Díaz, J.F., Villadas, P.J., Pérez-Luque, A.J., García-Rodríguez, F.M., Tringe, S.G., Fernández-López, M., 2023. Long-Term Persistence of Three Microbial Wildfire Biomarkers in Forest Soils. *Forests* 14 (7), 1383. <https://doi.org/10.3390/f14071383>.
- Ferrenberg, S., O'Neill, S.P., Knelman, J.E., Todd, B., Duggan, S., Bradley, D., Robinson, T., Schmidt, S.K., Townsend, A.R., Williams, M.W., Cleveland, C.C., Melbourne, B.A., Jiang, L., Nemergut, D.R., 2013. Changes in assembly processes in soil bacterial communities following a wildfire disturbance. *ISME J.* 7 (6), 1102–1111. <https://doi.org/10.1038/ismej.2013.11>.
- Fierer, N., 2017. Embracing the unknown: Disentangling the complexities of the soil microbiome. *Nat. Rev. Microbiol.* 15 (10), 579–590. <https://doi.org/10.1038/nrmicro.2017.87>.
- Fischer, M.S., Stark, F.G., Berry, T.D., Zeba, N., Whitman, T., Traxler, M.F., 2021. Pyrolyzed substrates induce aromatic compound metabolism in the post-fire fungus, *pyronema domesticum*. *Front. Microbiol.* 12 <https://doi.org/10.3389/fmicb.2021.729289>.
- Fischer, M.S., Patel, N.J., De Lorimier, P.J., Traxler, M.F., 2023. Prescribed fire selects for a pyrophilous soil sub-community in a northern California mixed conifer forest. *Environ. Microbiol.* 25 (11), 2498–2515. <https://doi.org/10.1111/1462-2920.16475>.
- Fox, S., Sikes, B.A., Brown, S.P., Cripps, C.L., Glassman, S.I., Hughes, K., Semenova-Nelsen, T., Jumpponen, A., 2022. Fire as a driver of fungal diversity—A synthesis of current knowledge. *Mycologia* 1–27. <https://doi.org/10.1080/00275514.2021.2024422>.
- Girona-García, A., Vieira, D.C.S., Silva, J., Fernández, C., Robichaud, P.R., Keizer, J.J., 2021. Effectiveness of post-fire soil erosion mitigation treatments: a systematic review and meta-analysis. *Earth Sci. Rev.* 217, 103611 <https://doi.org/10.1016/j.earscirev.2021.103611>.
- Girona-García, A., Cretella, C., Fernández, C., Robichaud, P.R., Vieira, D.C.S., Keizer, J.J., 2023. How much does it cost to mitigate soil erosion after wildfires? *J. Environ. Manage.* 334, 117478 <https://doi.org/10.1016/j.jenvman.2023.117478>.
- Glassman, S.I., Levine, C.R., DiRocco, A.M., Battles, J.J., Bruns, T.D., 2016. Ectomycorrhizal fungal spore bank recovery after a severe forest fire: Some like it hot. *ISME J.* 10 (5), 1228–1239. <https://doi.org/10.1038/ismej.2015.182>.
- Gran, G., 1952. Determination of the Equivalence Point in Potentiometric Titrations. *Part II Analyst* 77, 661–671.
- Hardy, C.C., 1996. Guidelines for estimating volume, biomass, and smoke production for piled slash. *Gen. Tech. Rep. PNW-GTR-364*. Pacific Northwest Research Station, Portland, OR.
- Hewitt, R.E., Day, N.J., DeVan, M.R., Taylor, D.L., 2023. Wildfire impacts on root-associated fungi and predicted plant-soil feedbacks in the boreal forest: Research progress and recommendations. *Funct. Ecol.* 37 (8), 2110–2125. <https://doi.org/10.1111/1365-2435.14205>.
- Honeyman, A.S., Merl, T., Spear, J.R., Koren, K., 2023. Optode-based chemical imaging of laboratory burned soil reveals millimeter-scale heterogeneous biogeochemical responses. *Environ. Res.* 224, 115469 <https://doi.org/10.1016/j.envres.2023.115469>.
- Hoover, K., 2020. Federal Wildfire Management: Ten-Year Funding Trends and Issues (FY2011-FY2020). Congressional Research Service. <https://crsreports.congress.gov/product/pdf/R/R46583>.
- Jenkins, M.J., Hebertson, E., Page, W., Jorgensen, C.A., 2008. Bark beetles, fuels, fires and implications for forest management in the Intermountain West. *For. Ecol. Manage.* 254 (1), 16–34. <https://doi.org/10.1016/j.foreco.2007.09.045>.
- Jiménez Esquilín, A.E., Stromberger, M.E., Massman, W.J., Frank, J.M., Shepperd, W.D., 2007. Microbial community structure and activity in a Colorado Rocky Mountain forest soil scarred by slash pile burning. *Soil Biol. Biochem.* 39 (5), 1111–1120. <https://doi.org/10.1016/j.soilbio.2006.12.020>.
- Kassambara, A., 2023. ggpubr: 'ggplot2' Based Publication Ready Plots. R Package Version (6). <https://rpkgs.datanovia.com/ggpubr/>.
- Kim, M., Heo, E., Kang, H., Adams, J., 2013. Changes in Soil Bacterial Community Structure with Increasing Disturbance Frequency. *Microb. Ecol.* 66 (1), 171–181. <https://doi.org/10.1007/s00248-013-0237-9>.
- Köljal, U., Nilsson, H.R., Schigel, D., Tederöo, L., Larsson, K.-H., May, T.W., Taylor, A.F.S., Jeppesen, T.S., Frølev, T.G., Lindahl, B.D., Pöldmaa, K., Saar, I., Suja, A., Savchenko, A., Yatsiuk, I., Adojaan, K., Ivanov, F., Piirmann, T., Pöhönen, R., Abarenkov, K., 2020. The Taxon Hypothesis Paradigm—On the Unambiguous Detection and Communication of Taxa. *Microorganisms* 8 (12), 1910. <https://doi.org/10.3390/microorganisms8121910>.
- Korb, J.E., Johnson, N.C., Covington, W.W., 2004. Slash Pile Burning Effects on Soil Biotic and Chemical Properties and Plant Establishment: Recommendations for Amelioration. *Restor. Ecol.* 12 (1), 52–62. <https://doi.org/10.1111/j.1061-2971.2004.00304.x>.
- Köster, K., Aaltonen, H., Berninger, F., Heinonsalo, J., Köster, E., Ribeiro-Kumara, C., Sun, H., Tederöo, L., Zhou, X., Purnaman, J., 2021. Impacts of wildfire on soil microbiome in Boreal environments. *Curr. Opin. Environ. Sci. Health* 22, 100258. <https://doi.org/10.1016/j.coesh.2021.100258>.

- Linley, G.D., Jolly, C.J., Doherty, T.S., Geary, W.L., Armenteras, D., Belcher, C.M., Bliege Bird, R., Duane, A., Fletcher, M., Giorgis, M.A., Haslem, A., Jones, G.M., Kelly, L.T., Lee, C.K.F., Nolan, R.H., Parr, C.L., Pausas, J.G., Price, J.N., Regos, A., Poulter, B., 2022. What do you mean, 'megafire'? *Glob. Ecol. Biogeogr.* 31 (10), 1906–1922. <https://doi.org/10.1111/geb.13499>.
- Louca, S., Doebeli, M., Parfrey, L.W., 2018. Correcting for 16S rRNA gene copy numbers in microbiome surveys remains an unsolved problem. *Microbiome* 6 (1), 41. <https://doi.org/10.1186/s40168-018-0420-9>.
- Lu, Y., Zhou, G., Ewald, J., Pang, Z., Shiri, T., Xia, J., 2023. MicrobiomeAnalyst 2.0: Comprehensive statistical, functional and integrative analysis of microbiome data. *Nucleic Acids Res.* gkad407 <https://doi.org/10.1093/nar/gkad407>.
- Massman, W.J., 2012. Modeling soil heating and moisture transport under extreme conditions: Forest fires and slash pile burns. *Water Resour. Res.* 48 (10) <https://doi.org/10.1029/2011WR011710>, 2011WR011710.
- Massman, W.J., Frank, J.M., Mooney, S.J., 2010. Advancing Investigation and Physical Modeling of First-Order Fire Effects on Soils. *Fire Ecology* 6 (1), 36–54. <https://doi.org/10.4996/fireecology.0601036>.
- McNorvell, M., 2023. (thesis). Fire, fungi, flora, and flow: post-fire fungal community assemblages, vegetation establishment, and soil hydrophobicity in forests of the southern Rocky Mountains. Mountain Scholar. Retrieved January 22, 2024, from <https://mountainscholar.org/items/7a22319b-2ae3-45dc-bc00-cfeb27fb36d>.
- Montana State Library, n.d.. Statewide maps - precipitation water year to date (% of normal). Precipitation Water Year to Date. https://mslservices.mt.gov/geography/c_information/maps/watersupply/statewide/StatewidePrecipitationWY2D.aspx.
- Mott, C.M., Hofstetter, R.W., Antoninka, A.J., 2021. Post-harvest slash burning in coniferous forests in North America: A review of ecological impacts. *For. Ecol. Manage.* 493, 119251 <https://doi.org/10.1016/j.foreco.2021.119251>.
- Myers-Pigg, A.N., Grieger, S., Roebuck, J.A., Barnes, M.E., Bladon, K.D., Bailey, J.D., Barton, R., Chu, R.K., Graham, E.B., Homolka, K.K., Kew, W., Lipton, A.S., Scheibe, T., Toyoda, J.G., Wagner, S., 2024. Experimental Open Air Burning of Vegetation Enhances Organic Matter Chemical Heterogeneity Compared to Laboratory Burns. *Environ. Sci. Tech.* 58 (22), 9679–9688. <https://doi.org/10.1021/acs.est.3c10826>.
- National Wildfire Coordinating Group, 2023, February 23. Arapaho & Roosevelt National Forests Pile Burning. Coarf Arapaho Roosevelt National Forests Pile Burning Information. Retrieved February 27, 2023, from <https://inciweb.nwgc.gov/incident-information/coarf-arapaho-roosevelt-national-forests-pile-burning>.
- Nelson, A.R., Narrowe, A.B., Rhoades, C.C., Fegol, T.S., Daly, R.A., Roth, H.K., Chu, R.K., Amundson, K.K., Young, R.B., Steindorff, A.S., Mondo, S.J., Grigoriev, I.V., Salamov, A., Borch, T., Wilkins, M.J., 2022. Wildfire-dependent changes in soil microbiome diversity and function. *Nat. Microbiol.* 7 (9), 1419–1430. <https://doi.org/10.1038/s41564-022-01203-y>.
- Nelson, A.R., Fegol, T.S., Danczak, R.E., Caiata, M.V., Roth, H.K., Dunn, O.I., Turvold, C. A., Borch, T., Glassman, S.I., Barnes, R.T., Rhoades, C.C., Wilkins, M.J., 2024. Soil microbiome feedbacks during disturbance-driven forest ecosystem conversion. *ISME J.* 18 (1), wrae047. <https://doi.org/10.1093/ismej/wrae047>.
- Neu, A.T., Allen, E.E., Roy, K., 2021. Defining and quantifying the core microbiome: Challenges and prospects. *Proc. Natl. Acad. Sci.* 118 (51) <https://doi.org/10.1073/pnas.2104429118> e2104429118.
- Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., Schilling, J.S., Kennedy, P.G., 2016. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecol.* 20, 241–248. <https://doi.org/10.1016/j.funeco.2015.06.006>.
- Nilsson, R.H., Larsson, K.-H., Taylor, A.F.S., Bengtsson-Palme, J., Jeppesen, T.S., Schigel, D., Kennedy, P., Picard, K., Glöckner, F.O., Tedersoo, L., Saar, I., Köljalg, U., Abarenkov, K., 2019. The UNITE database for molecular identification of fungi: Handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Res.* 47 (D1), D259–D264. <https://doi.org/10.1093/nar/gky1022>.
- Okansen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Wagner, H., 2022. *vegan: Community Ecology Package. R Package Version 2 (5-7)*, 2020.
- Parada, A.E., Needham, D.M., Fuhrman, J.A., 2016. Every base matters: Assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples: Primers for marine microbiome studies. *Environ. Microbiol.* 18 (5), 1403–1414. <https://doi.org/10.1111/1462-2920.13023>.
- Parks, S.A., Abatzoglou, J.T., 2020. Warmer and Drier Fire Seasons Contribute to Increases in Area Burned at High Severity in Western US Forests From 1985 to 2017. *Geophys. Res. Lett.* 47 (22) <https://doi.org/10.1029/2020GL089858>.
- Pastor, E., Pérez, Y., Agueda, A., Miralles, M., Planas, E., 2010. Pile burning of cutting debris in stands of hazel (*Corylus avellana*): An experimental study of smouldering combustion towards the validation of a burning protocol. *Fire Saf. J.* 45 (1), 69–81. <https://doi.org/10.1016/j.firesaf.2009.10.003>.
- Peay, K.G., Schubert, M.G., Nguyen, N.H., Bruns, T.D., 2012. Measuring ectomycorrhizal fungal dispersal: Macroecological patterns driven by microscopic propagules. *Mol. Ecol.* 21 (16), 4122–4136. <https://doi.org/10.1111/j.1365-294X.2012.05666.x>.
- Pérez-Valera, E., Verdú, M., Navarro-Cano, J.A., Goberna, M., 2020. Soil microbiome drives the recovery of ecosystem functions after fire. *Soil Biol. Biochem.* 149, 107948 <https://doi.org/10.1016/j.soilbio.2020.107948>.
- Philippot, L., Andersson, S.G.E., Battin, T.J., Prosser, J.I., Schimel, J.P., Whitman, W.B., Hallin, S., 2010. The ecological coherence of high bacterial taxonomic ranks. *Nat. Rev. Microbiol.* 8 (7), 523–529. <https://doi.org/10.1038/nrmicro2367>.
- Philippot, L., Chenu, C., Kappler, A., Rillig, M.C., Fierer, N., 2023. The interplay between microbial communities and soil properties. *Nat. Rev. Microbiol.* <https://doi.org/10.1038/s41579-023-00980-5>.
- Pinto, R., Ansola, G., Calvo, L., Sáenz De Miera, L.E., 2023. High resilience of soil bacterial communities to large wildfires with an important stochastic component. *Sci. Total Environ.* 899, 165719 <https://doi.org/10.1016/j.scitotenv.2023.165719>.
- Porter, T.M., Smenderovac, E., Morris, D., Venier, L., 2023. All boreal forest successional stages needed to maintain the full suite of soil biodiversity, community composition, and function following wildfire. *Sci. Rep.* 13 (1), 7978. <https://doi.org/10.1038/s41598-023-30732-7>.
- Pressler, Y., Moore, J.C., Cotrufo, M.F., 2019. Belowground community responses to fire: Meta-analysis reveals contrasting responses of soil microorganisms and mesofauna. *Oikos* 128 (3), 309–327. <https://doi.org/10.1111/oik.05738>.
- Pulido-Chavez, M.F., Alvarado, E.C., DeLuca, T.H., Edmonds, R.L., Glassman, S.I., 2021. High-severity wildfire reduces richness and alters composition of ectomycorrhizal fungi in low-severity adapted ponderosa pine forests. *For. Ecol. Manage.* 485, 118923 <https://doi.org/10.1016/j.foreco.2021.118923>.
- Pulido-Chavez, M.F., Randolph, J.W.J., Zalman, C., Larios, L., Homyak, P.M., Glassman, S.I., 2023. Rapid bacterial and fungal successional dynamics in first year after chaparral wildfire. *Mol. Ecol. mec.* 16835 <https://doi.org/10.1111/mec.16835>.
- Qin, Q., Wang, Y., Qiu, C., Zheng, D., Liu, Y., 2022. Wildfire drives the transition from deterministic- to stochastic-dominated community assembly of abundant bacterial in forest soils. *Catena* 215, 106290. <https://doi.org/10.1016/j.catena.2022.106290>.
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarla, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Res.* 41 (D1), 590–596. <https://doi.org/10.1093/nar/gks1219>.
- R Core Team, 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Raudabaugh, D.B., Matheny, P.B., Hughes, K.W., Iturriaga, T., Sargent, M., Miller, A.N., 2020. Where are they hiding? Testing the body snatchers hypothesis in pyrophilous fungi. *Fungal Ecol.* 43, 100870 <https://doi.org/10.1016/j.funeco.2019.100870>.
- Rhoades, C.C., Fegol, T.S., Zaman, T., Fornwalt, P.J., Miller, S.P., 2021. Are soil changes responsible for persistent slash pile burn scars in lodgepole pine forests? *For. Ecol. Manage.* 490, 119090 <https://doi.org/10.1016/j.foreco.2021.119090>.
- Rhoades, C.C., Fornwalt, P.J., 2015. Pile burning creates a fifty-year legacy of openings in regenerating lodgepole pine forests in Colorado. *For. Ecol. Manage.* 336, 203–209. <https://doi.org/10.1016/j.foreco.2014.10.011>.
- Rhoades, C.C., Fornwalt, P.J., Paschke, M.W., Shanklin, A., Jonas, J.L., 2015. Recovery of small pile burn scars in conifer forests of the Colorado Front Range. *For. Ecol. Manage.* 347, 180–187. <https://doi.org/10.1016/j.foreco.2015.03.026>.
- Robeson, M.S., O'Rourke, D.R., Kaehler, B.D., Ziemski, M., Dillon, M.R., Foster, J.T., Bokulich, N.A., 2021. RESCRIPt: Reproducible sequence taxonomy reference database management. *PLoS Comput. Biol.* 17 (11), e1009581.
- Rocky Mountain Research Station, Rhoades, C., Schnackenberg, L., Fornwalt, P., Schroder, E., 2015. 15 Slash from the past: Rehabilitating pile burn scars 1–9. Fort Collins, CO; U.S. Dept. of Agriculture, Forest Service, Rocky Mountain Research Station.
- Roller, B.R.K., Stoddard, S.F., Schmidt, T.M., 2016. Exploiting rRNA operon copy number to investigate bacterial reproductive strategies. *Nat. Microbiol.* 1 (11), 16160. <https://doi.org/10.1038/nmicrobiol.2016.160>.
- Rubel, F., Kottek, M., 2010. Observed and projected climate shifts 1901–2100 depicted by world maps of the Köppen-Geiger climate classification. *Meteorol. Z.* 19 (2), 135–141. <https://doi.org/10.1127/0941-2948/2010/0430>.
- Santín, C., Doerr, S.H., 2016. Fire effects on soils: The human dimension. *Philos. Trans. R. Soc.* B 371 (1696), 20150171. <https://doi.org/10.1098/rstb.2015.0171>.
- Schoennagel, T., Balch, J.K., Brenkert-Smith, H., Dennison, P.E., Harvey, B.J., Krawchuk, M.A., Mietkiewicz, N., Morgan, P., Moritz, M.A., Rasker, R., Turner, M.G., Whitlock, C., 2017. Adapt to more wildfire in western North American forests as climate changes. *Proc. Natl. Acad. Sci.* 114 (18), 4582–4590. <https://doi.org/10.1073/pnas.1617464114>.
- Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., Huttenhower, C., 2011. Metagenomic biomarker discovery and explanation. *Genome Biol.* 12 (6) <https://doi.org/10.1186/gb-2011-12-6-r60>.
- Shi, Y.-F., Shi, S.-H., Jiang, Y.-S., Liu, J., 2022. A global synthesis of fire effects on soil seed banks. *Global Ecol. Conserv.* 36, e02132.
- Smith, M.D., 2011. An ecological perspective on extreme climatic events: A synthetic definition and framework to guide future research: Defining extreme climate events. *J. Ecol.* 99 (3), 656–663. <https://doi.org/10.1111/j.1365-2745.2011.01798.x>.
- Smithwick, E.A.H., Turner, M.G., Mack, M.C., Chapin, F.S., 2005. Postfire Soil N Cycling in Northern Conifer Forests Affected by Severe, Stand-Replacing Wildfires. *Ecosystems* 8 (2), 163–181. <https://doi.org/10.1007/s10021-004-0097-8>.
- Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Web Soil Survey. Available online at <http://websoilsurvey.nrcs.usda.gov/> accessed [June/14/2023].
- Steindorff, A.S., Seong, K., Carver, A., Calhoun, S., Fischer, M.S., Stillman, K., Liu, H., Drula, E., Henrissat, B., Simpson, H.J., Schilling, J.S., Lipzen, A., He, G., Yan, M., Andreopoulos, B., Pangilinan, J., LaButti, K., Ng, V., Traxler, M., Grigoriev, I.V., 2022. Diversity of genomic adaptations to the post-fire environment in Pezizales fungi points to crosstalk between charcoal tolerance and sexual development. *New Phytol.* 236 (3), 1154–1167. <https://doi.org/10.1111/nph.18407>.
- Stevens-Rumann, C.S., Prichard, S.J., Whitman, E., Parisien, M.-A., Meddens, A.J.H., 2022. Considering regeneration failure in the context of changing climate and disturbance regimes in western North America. *Can. J. For. Res.* 52 (10), 1281–1302. <https://doi.org/10.1139/cjfr-2022-0054>.
- Stoddard, S.F., Smith, B.J., Hein, R., Roller, B.R.K., Schmidt, T.M., 2015. rrnDB: Improved tools for interpreting rRNA gene abundance in bacteria and archaea and a new foundation for future development. *Nucleic Acids Res.* 43 (D1), D593–D598. <https://doi.org/10.1093/nar/gku1201>.

- Stone, B.W.G., Dijkstra, P., Finley, B.K., Fitzpatrick, R., Foley, M.M., Hayer, M., Hofmockel, K.S., Koch, B.J., Li, J., Liu, X.J.A., Martinez, A., Mau, R.L., Marks, J., Monsaint-Queeney, V., Morrissey, E.M., Propster, J., Pett-Ridge, J., Purcell, A.M., Schwartz, E., Hungate, B.A., 2023. Life history strategies among soil bacteria—Dichotomy for few, continuum for many. *ISME J.* <https://doi.org/10.1038/s41396-022-01354-0>.
- Suz, L.M., Bidartondo, M.I., Van Der Linde, S., Kuyper, T.W., 2021. Ectomycorrhizas and tipping points in forest ecosystems. *New Phytol.* 231 (5), 1700–1707. <https://doi.org/10.1111/nph.17547>.
- Thompson, L.R., Sanders, J.G., McDonald, D., Amir, A., Ladau, J., Locey, K.J., Prill, R.J., Tripathi, A., Gibbons, S.M., Ackermann, G., Navas-Molina, J.A., Janssen, S., Kopylova, E., Vázquez-Baeza, Y., González, A., Morton, J.T., Mirarab, S., Zech Xu, Z., Jiang, L., Zhao, H., 2017. A communal catalogue reveals Earth's multiscale microbial diversity. *Nature* 551 (7681), 457–463. <https://doi.org/10.1038/nature24621>.
- Tobin, C., 2020. ggthemr: Themes for 'ggplot2'. R Package Version 1 (1).
- U.S. Department of Agriculture, n.d. Pile Burning. Forest Service National Website. https://www.fs.usda.gov/detail/arp/landmanagement/resourcemanagement/?cid=fsm_91_058291.
- Van Vooren, N., Lindemann, U., Healy, R., 2017. Emendation of the genus *Tricharina* (Pezizales) based on phylogenetic, morphological and ecological data. *Ascomycete. org* 9, 101–123.
- VanderRoest, J.P., Fowler, J.A., Rhoades, C.C., Roth, H.K., Broeckling, C.D., Fegol, T.S., McKenna, A.M., Bechtold, E.K., Boot, C.M., Wilkins, M.J., Borch, T., 2024. Fire Impacts on the Soil Metabolome and Organic Matter Biodegradability. *Environ. Sci. Technol.* [acs.est.3c09797](https://doi.org/10.1021/acs.est.3c09797). <https://doi.org/10.1021/acs.est.3c09797>.
- Větrovský, T., Baldrian, P., 2013. The Variability of the 16S rRNA Gene in Bacterial Genomes and Its Consequences for Bacterial Community Analyses. *PLoS One* 8 (2), e57923.
- Vos, M., Wolf, A.B., Jennings, S.J., Kowalchuk, G.A., 2013. Micro-scale determinants of bacterial diversity in soil. *FEMS Microbiol. Rev.* 37 (6), 936–954. <https://doi.org/10.1111/1574-6976.12023>.
- Weber, C.F., Lockhart, J.S., Charaska, E., Aho, K., Lohse, K.A., 2014. Bacterial composition of soils in ponderosa pine and mixed conifer forests exposed to different wildfire burn severity. *Soil Biol. Biochem.* 69, 242–250. <https://doi.org/10.1016/j.soilbio.2013.11.010>.
- White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols: a Guide to Methods and Applications*. Academic Press, New York, pp. 315–322.
- Whitman, T., Whitman, E., Woolet, J., Flannigan, M.D., Thompson, D.K., Parisien, M.-A., 2019. Soil bacterial and fungal response to wildfires in the Canadian boreal forest across a burn severity gradient. *Soil Biol. Biochem.* 138, 107571. <https://doi.org/10.1016/j.soilbio.2019.107571>.
- Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T., Miller, E., Bache, S., Müller, K., Ooms, J., Robinson, D., Seidel, D., Spinu, V., Yutani, H., 2019. Welcome to the tidyverse. *J. Open Sour. Software* 4 (43), 1686.
- Wickham, H., 2016. *Ggplot2: Elegant graphics for data analysis* (2nd ed.) [PDF]. Springer International Publishing.
- Zaneveld, J.R., McMinds, R., Vega Thurber, R., 2017. Stress and stability: Applying the Anna Karenina principle to animal microbiomes. *Nat. Microbiol.* 2 (9), 17121. <https://doi.org/10.1038/nmicrobiol.2017.121>.
- Zepner, L., Karrasch, P., Wiemann, F., Bernard, L., 2021. ClimateCharts.net – an interactive climate analysis web platform. *Int. J. Digital Earth* 14 (3), 338–356. <https://doi.org/10.1080/17538947.2020.1829112>.
- Zhang, Q., Wang, Y., Guan, P., Zhang, P., Mo, X., Yin, G., Qu, B., Xu, S., He, C., Shi, Q., Zhang, G., Dittmar, T., Wang, J., 2023. Temperature Thresholds of Pyrogenic Dissolved Organic Matter in Heating Experiments Simulating Forest Fires. *Environ. Sci. Tech.* 57 (45), 17291–17301. <https://doi.org/10.1021/acs.est.3c05265>.