

THESIS

FIRE, FUNGI, FLORA, AND FLOW: POST-FIRE FUNGAL COMMUNITY
ASSEMBLAGES, VEGETATION ESTABLISHMENT, AND SOIL
HYDROPHOBICITY IN FORESTS OF THE SOUTHERN ROCKY
MOUNTAINS

Submitted by

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In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Summer 2023

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ABSTRACT

FIRE, FUNGI, FLORA, AND FLOW: POST-FIRE FUNGAL COMMUNITY ASSEMBLAGES, VEGETATION ESTABLISHMENT, AND SOIL HYDROPHOBICITY IN FORESTS OF THE SOUTHERN ROCKY MOUNTAINS

Wildfire is an important ecological driver of ecosystem dynamics in the southern Rocky Mountains at multiple landscape scales, guiding establishment of forest biota both aboveground and below. Size, frequency, and severity of wildfires in coniferous forests across the western United States is increasing at an unprecedented rate. Despite wildfire's significant but disparate influences on forest soils, post-fire research has often focused on aboveground vegetation in isolation from study of belowground soil characteristics and the fire ecology of soil biota. Fungi are vital to forest ecosystems for their functional roles, however, the effects of wildfire on forest-specific fungal communities and how those communities subsequently influence post-fire vegetation communities and soil environments has not been extensively researched in the region over the past several decades. This is a prominent knowledge gap, especially as fungi are highly variable across functional groups, space, and time even in unburned systems. Thus, to build on our understanding of contemporary fire ecology in forested ecosystems of the Southern Rockies, we investigate three wildfires that burned in the state of Colorado during the 2020 fire season and address three research objectives: 1) Examine the influence of forest type and fire severity on post-fire fungal community composition across soil depth and temporal gradients; 2) Determine the effects of post-fire fungal community diversity on forest understory plant diversity and abundance; and 3) Explore relationships between fungal assemblages and observed soil hydrophobicity in burned forested environments. We found that though fire severity and soil depth were the primary influences on quantified fungal diversity, the composition of fungal community assemblages was most heavily influenced by forest type: forests developed fungal communities compositionally unique to one another just two years after fire. Diversity of fungi showed significant influence on aboveground plant diversity and abundance, especially mutualistic fungi (ecto- and arbuscular mycorrhizae) and their respective plant hosts. Finally, significant relationships between fungal diversity and soil hydrophobicity were observed mediated by forest type, fire severity, soil depth, and year post-fire, though these patterns were difficult to surmise and the influence of other important variables may be at play. By more fully understanding how soil fungi interact with aboveground vegetation establishment and belowground soil conditions after wildfire, this research may help inform managers of pathways to better achieve desired post-fire outcomes by leveraging fungal relationships in soil remediation, site preparation, and conservation of post-fire forest ecosystems.

ACKNOWLEDGEMENTS

Firstly, I would like to acknowledge my advisor, Dr. Camille Stevens-Rumann, for her guidance supporting the navigation and completion of my dream research project over the last two years. I give the utmost appreciation to the Colorado Water Center, Colorado Forest Restoration Institute, and the BLM Joint Fire Science Program for contributing funding to research costs and tuition payments towards the completion of my Master of Science degree in Forest Sciences. This financial support helped to fund several incredible field and laboratory technicians, including Shannon Iral, Maddy Minard, and Grace Nykol. Many thanks to Kate Weimer, Jamie Woollet, Maria Vicini, Gabriela Szyniec, and Alison Goodrich for the additional technician and data collection support they provided.

My committee members and their respective research groups, including Dr. Chuck Rhoades with the USFS Rocky Mountain Research Station, Dr. Mike Wilkins with CSU's Soil and Crop Sciences Department, and Dr. Mike Remke formerly of the Mountain Studies Institute and Fort Lewis College, were instrumental in the preparation and thorough completion of this study and its deliverables. Additional collaborators include Gloria Edwards of the Southern Rockies Fire Science Network, who aided in obtaining funding for this project and will be vital in seeing it communicated to those outside academia who may find its contents useful, and Dr. Bo Stevens of the USDA Agricultural Science Service who instructed me on the skills necessary for bioinformatics and coding. I would also like to thank Alex Siggers of CSU's biology department for his additional statistical support in this regard.

Finally, I would like to thank my parents, Thea and Jarrod, who have supported me in more ways than I can count throughout the entirety of my life, and my partner, Amanda, without whom my experience over these last two years would have been filled with far less love, light, and laughter. Their affections have provided me with the strength and feeling of self-efficacy necessary to accomplish such a herculean task as this research has been. To all of these individuals, groups, family, and friends, this thesis bears the fruit of your labors of support and dedication.

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Chapter 1

Fire, Fungi, Flora, and Flow: Post-fire fungal community assemblages, vegetation establishment, and soil hydrophobicity in forests of the Southern Rocky Mountains

Background

Wildfires are an essential forest ecosystem process influencing soil conditions and guiding composition of organisms both aboveground and below. Area burned, frequency, and ecological impact of wildfire (hereafter fire severity) in coniferous forests across the western United States is increasing at an unprecedented rate (Parks and Abatzoglou, 2020; Dennison et al. 2014). Though wildfire impacts microbial communities at larger ecosystem scales via interacting effects on plant and soil characteristics (Adkins et al. 2020), post-fire research in recent decades has often focused on impacts of uncharacteristic fire behavior on aboveground vegetation in isolation from study of belowground soil characteristics and the ecology of important soil biota such as fungi. There remains a considerable knowledge gap in understanding post-fire drivers of succession and recovery in conifer-dominated ecosystems as soil fungi, though less directly visible, are no less indispensable for their role in long-term forest health at multiple interacting scales.

Fire severity significantly affects the degree to which forest fungal communities and their surrounding soil environments change. Fungi are sensitive to increases in soil temperature, with wildfire resulting directly in fungal mortality due to soil heating (Hart et al. 2005). Organic surficial soil horizons, where a majority of fungal species proliferate, are often completely destroyed in intense wildfire events (Joergensen and Wichern, 2008). Thus, wildfire generally results in temporary reductions in fungal community diversity and relative abundance compared to pre-fire levels (Holden et al. 2013; Dooley and Treseder, 2012). Soil organic matter

consumption by higher intensity fires decreases soil stability and water-holding capacity, and contributes to the creation of pyrolyzed hydrophobic compounds, soil water repellency, and erosion risk (Wondzell and King, 2003; Steindorff et al. 2021). High vegetative and fungal mortality in higher-severity fires also results in removal of soil-stabilizing plant root and fungal hyphae structures (Gyssels et al. 2005; Paul, 2014). In tandem, wildfires undermine soil structural and biological integrity in forest soil environments increasing erosion potential, expediting biologically-available resource outputs, and diminishing short-term recovery and long-term forest productivity (Hart et al. 2005; Atanassova et al. 2014; Certini, 2005; Smith et al. 2005; Mainwaring et al. 2013). With western US coniferous forests projected to experience an increase in frequency of uncharacteristically large and severe wildfires, forest management in post-fire environments will require better understanding of contemporary wildfire impacts on vegetation succession from multiple interacting perspectives, including that of plant-fungal-soil relationships, in order to minimize forest regeneration failures in post-fire ecosystems.

Fungi are essential pioneers in post-disturbance environments as they facilitate breakdown and plant-uptake of complex organic material. Decomposition driven by fungal communities provides key nutrients and resources for present and future forest vegetation cohorts, both indirectly through nutrient cycling and directly with plant roots through mycorrhizal facilitation (Paul, 2014). Organic matter decomposition and hyphal growth after fire contribute to soil aggregation, increasing water-holding capacity and reducing erosion potential post-fire (Filialuna and Cripps, 2021); laboratory studies indicate some species of fungi may even be responsible for the breakdown of pyrolyzed hydrophobic waxes and lipids in-situ, further demonstrating the role fungi play in reducing the likelihood of soil erosion after fire (Steindorff et al. 2021). Thus, in extremely inhospitable soil conditions resulting from

high-severity wildfires, fungal regeneration is likely linked to post-fire soil hydrophobicity dynamics and plant-nutrient availability, influencing post-fire vegetation composition in forest ecosystems (Claridge et al. 2009).

Functional group assignment is fundamental to determining fungal community response to fire in forested environments. Saprophytic fungi (saprophytes), the most abundant fungal functional group, source carbon directly from decomposition of dead wood and other organic matter sources (Volk, 2013). Saprophytes tend to dominate post-fire fungal community composition and relative abundance in dry and mesic forests alike, especially those species with particular aptitude for breaking down more recalcitrant carbon found within charred wood (Pulido-Chavez et al. 2021; Holden et al. 2016; Edman and Eriksson, 2016; Steindorff et al. 2022). Saprophytes may develop into ectomycorrhizal fungi (EMF), forming associations with vegetation roots in which plant-produced photosynthetic carbohydrates are exchanged for resources more easily acquired by fungal hyphae over wider distances and in smaller soil pore spaces. EMF species are significantly impacted by fire severity due to consumption of organic matter and association with plant species (i.e. shrubs and trees) that may take years to several decades to establish and dominate a post-fire site (Holden et al. 2013, Treseder et al. 2004). Arbuscular mycorrhizal fungi (AMF) rely almost exclusively on plant hosts for carbon sequestration and proliferation rather than through consumption of soil organic matter. Conversely to EMF, AMF often return to pre-fire levels of diversity and relative abundance in just a few years post-fire due to early post-fire presence of suitable pioneer plant hosts (i.e. graminoids and forbs) and a lack of reliance on organic matter accumulation in order to proliferate (Treseder et al. 2004). Research on fungal responses to forest fires has typically been limited by analysis within a single forest type. With such variable responses to wildfire across

both aboveground vegetation and belowground fungal functional groups, more fully understanding post-fire fungal dynamics, including analysis of functional subgroups and communities in disparate forest types, is critical to an expanded view of the ecological response of forested communities to wildfire.

The southern Rocky Mountains (hereafter the Southern Rockies) spanning from southern Wyoming to northern Arizona and New Mexico are comprised of multiple forest types influenced by elevation-driven climate gradients. Two such forest types of particular interest given their climatic, hydrologic, and historical fire regime contrasts are dry forests at lower-montane elevations (1,800-2,600 m) and mesic forests at subalpine elevations (3,000 to 3,600 m). Lower montane forests experience relatively high summer temperatures and low summer precipitation accumulation (Kaufmann et al. 2006). Typical tree indicator species including ponderosa pine (*Pinus ponderosa*) are drought-tolerant and adapted to frequent (7-50 years), low-to-moderate severity fires that burn a spatial mosaic of isolated individuals, denser clusters, and gaps occupied by herbaceous understory plants (2006). In contrast, subalpine forests experience lower average summer temperatures and more substantial average summer precipitation accumulation than lower montane forests and are home to codominant tree species Engelmann spruce (*Picea engelmannii*) and subalpine fir (*Abies lasiocarpa*) (Veblen et al. 1994). Fires in these more productive ecosystems are less frequent (300-600 years) than in lower-montane forests, but are typically higher in severity when fires do occur owing to greater stand (i.e. fuel) densities and connectivity (Romme and Knight, 1981).

Post-fire vegetation dynamics have been extensively studied in lower-montane and subalpine forests of the Southern Rockies over the past several decades, with clear evidence to support disparate compositional responses driven by forest-type variability (Kaufmann et al.

2006; Veblen et al. 1994); however, research on fungal responses to fire in these forests is overall scarce. Hamman et al. (2007) analyzed the effects of fire severity on fungal community structure in lower montane forests fourteen months after the 2002 Hayman Fire in Colorado. Though burned stands showed significant decreases in overall fungal abundance as compared to their unburned counterparts, there was no significant difference in fungal species richness between stands burned at low and high-severity (Hamman et al. 2007). Additionally, AMF specifically were found to be most abundant in low-severity burn sites, while there were no significant differences in AMF abundance between high-severity sites and unburned controls (2007). Thirteen years after the 2000 Pumpkin Fire in northern Arizona, sporocarp surveys conducted by Owen et al. (2019) in ponderosa pine forests found that fungal density was lowest within and directly adjacent to high-severity burn patches, with stands in these patches also yielding a fungal community composition significantly different than other burn severity treatments. EMF density and species richness decreased with increasing burn severity, while saprophytic species maintained similar density and richness across burn severity classes. Studies exploring the fungal community composition of post-fire subalpine forest systems in the Southern Rockies are more rare. However, research in subalpine forests of Greater Yellowstone ecosystems in the Northern Rockies suggests that there is a strong correlation between post-fire surface vegetation cover type and fungal species composition (Smithwick et al. 2012). Further research on characterizing subalpine fungal communities and, further still, comparing lower-montane and subalpine forests and their respective responses to fire may provide essential local data on fungal community assemblages across the Southern Rockies after large wildfires. Additionally, it will aid in determining the influence of climate-driven hydrology and burn severity associated with forest type on post-fire fungal and plant community relationships—an important knowledge gap to

explore given the reliance of fungal proliferation on soil resource availability and interdependent feedbacks with plant communities.

To build on our understanding of post-fire landscape-scale interactions, we investigate the relationships between wildfire, forest type, fungal community structure, hydrologic behavior, and vegetation regeneration in coniferous forest ecosystems of the Southern Rockies. Through consideration and full utilization of large burn-scar and temporal gradients, we attempt to answer the following questions: 1) How do forest type, fire severity, and soil depth impact differences in post-fire fungal community composition, and how do these differences change over time? 2) How does post-fire fungal diversity correlate with observation of subsequent vegetation diversity and cover across forest type - fire severity strata? and 3) How is the relative abundance and diversity of specific fungal functional groups linked to the presence of soil hydrophobicity post-fire? Understanding the role of soil fungi in post-fire soil conditions and aboveground succession may help inform managers of site-specific pathways to better achieve desired post-fire outcomes, leveraging fungal relationships in soil remediation, site preparation, and conservation of post-fire environments in forest ecosystems.

Methods

In 2020, the Southern Rockies experienced one of the largest fire years in recorded history, with over 250,000 hectares (ha) of land burned in Colorado alone. Field data was collected in the Arapahoe-Roosevelt and Medicine Bow-Routt National Forests across three wildfires that burned during the 2020 wildfire season (Figure 1). The Calwood Fire burned through 243 ha dominated by lower-montane ponderosa pine forests. The Mullen Fire spanned 71,580 ha and burned primarily in high-elevation ecosystems, including subalpine forests. The

Cameron Peak Fire, the largest wildfire in Colorado's state recorded history, burned a total of 84,443 ha across an elevational gradient from lower-montane to subalpine forests.

For the summer months of June through September, lower-montane forests experience average temperatures of 13°C (USDA NWCC) and average precipitation accumulation of 244 cm (USDA NWCC). Soil types across all lower-montane study sites are complexes primarily comprised of Cypher family soils, with residual and alluvial deposits of igneous and metamorphic rock contributing to gravelly coarse sandy loam texture down to 25 cm within the soil profile (NRCS WSS). Subalpine forests encounter mean summer temperatures of 9°C (USDA NWCC) and summer precipitation accumulation of 399 cm (USDA NWCC). Ansel-Granite and Leighan family complexes dominate soil types in our subalpine forest sites, where rocky, sandy and silty loams create well-to-excessively drained soils of metamorphic rock and glacial till (USDA NWCC).

Site Selection and Plot Establishment

Data collection occurred one ("Year 1") and two ("Year 2") years post-fire (2021 and 2022) between the months of June and August using a nested sampling design stratified by forest type and burn severity. Sites were delineated based on elevation and dominant forest structure. Plot centers within each site were randomly established contingent on observed tree species composition (>80% basal area occupancy by ponderosa pine and Douglas-fir in lower-montane forests or Engelmann spruce and subalpine fir in subalpine forests). Plot centers were further parsed by burn severity: low-severity if <20% tree mortality by basal area; high-severity if there was 100% tree mortality and a distance of at least 100 m from plot edge to nearest live tree could be achieved to control for live edge effects. In the second year post-fire, unburned sites were

identified to establish control plots no closer than 100 m from the nearest burned edge, producing a baseline fungal community structure of comparison and controlling for the effects of smoke and ash deposition on subsequent fungal response.

Additionally, all plots were required to maintain a slope percentage angle no greater than 35 to maintain consistency in soil erosion potential across plots and reduce the likelihood of post-fire anthropogenic mulching risk. Soil sampling within each plot occurred in triplicate along 12.2 m radial transects in subcardinal directions (NE, SE, NW, and SW), with three sampling points per transect at distances of 3.0 m, 6.1 m, and 9.1 m. A total of 41 plots were established within a matrix of three fires, two forest types, two burn severities, and unburned control sites (Table 1).

Field Sample Design

Soil collection was performed at each sampling point for later laboratory-based fungal DNA and nutrient extraction and quantification. Sampling occurred at two depths to determine soil depth effects on fungal assemblages post-fire based on methods performed by Nelson et al. (2022): surficial soil from 0 to 5 cm and deep soil from 5 to 10 cm. After removing top litter layers at sampling points (if present), non-rhizospheric (i.e. dominantly occupied by plant roots) soil was collected using a stainless steel bulb corer and deposited into resealable plastic bags. Bulk soils were hand-sorted in the field to exclude larger pieces of soil debris (sticks, rocks, etc.). 1,968 soil samples (24 samples per plot per year) were collected and stored on dry ice in the field to limit microbial activity and preserve in-situ DNA conditions as completely as possible.

Additional soil cores up to 10 cm were extracted using a profiled soil corer within 0.3 m of each soil collection point, subsequently performing water droplet penetration time (WDPT)

tests to measure soil hydrophobicity presence along the soil profile. Briefly, WDPT tests are applied by placing droplets of water at each centimeter of a soil core and timing the duration of complete water droplet infiltration into the soil; the longer a water droplet takes to infiltrate the soil, the more hydrophobic that depth of soil is considered to be. After preliminary testing, we delineated four hydrophobicity classes based on time-to-infiltration duration: low hydrophobicity (0.00 – 4.99 s), moderate hydrophobicity (5.00 – 9.99 s), high hydrophobicity (10.00 – 14.99 s), and extreme hydrophobicity (≥ 15.00 s). Percent of each hydrophobicity class was stratified by *surface* and *deep* soils. Soil saturation (water content) due to consistent precipitation in high elevations during the second year of field sampling was obstructive to data collection on soil hydrophobicity. Hydrologic measurements typically require unsaturated soil conditions to accurately assess the interaction between soil particles (both organic and mineral) and water, but between May and August of 2022 subalpine forests experienced abundant late-season snow melt and received 118% of the NRCS 1991-2020 average of precipitation (USDA NWCC). Thus, our ability to collect hydrologic data in subalpine plots was greatly constrained, and second-year analyses of soil hydrophobicity are focused on lower montane plots.

Inventory of understory plant community diversity and species richness was collected along each transect to quantify overall plant alpha-diversity and percent cover of functional vegetation groups. Line-point intercept was employed at 200 evenly spaced points to assess presence-absence of vegetation on all 8 transects in cardinal and sub cardinal directions every 0.3 m for 12.2 m. If present, all intersecting plants were identified to species and classified into functional groups of forb, graminoid, or shrub.

Laboratory Methods

After soil collection and preparation, genomic DNA was extracted and high-throughput polymerase-chain reaction (PCR) sequencing run using Internal Transcribed Spacer (ITS) ribosomal RNA gene primers to amplify, barcode, and quantify fungal DNA for post-fire relative abundance. Bulk soil samples were temporarily stored at -20°C until being composited at the plot and soil depth levels for analyses, then subsequently moved to a -50°C freezer for long-term storage until soil extraction. To composite at plot and depth levels, bulk soil samples were subsampled for each point and depth at 0.5 g in triplicate, resulting in a 1.5 g contribution of soil from each sample and approximately 18 total g of soil per plot-depth composite. DNA was then extracted from soil composites using Quick-DNA Fecal/Soil Microbe Microprep kits per manufacturer's instructions (Zymo Research Corporation, 2022). Finally, DNA concentrations of extracts were quantified for quality assurance using a Qubit DNA fluorometer (ThermoFisher Scientific Inc. 2023), plated, and subsequently sequenced via PCR on the Illumina MiSeq platform at the University of Colorado, Boulder.

Bioinformatics for fungal operational taxonomic units (OTUs) attained from PCR sequencing were processed using Qiime 2 (Bolyen et al. 2019). Raw sequences were paired as forward and reverse reads with their respective assigned barcodes, and subsequently demultiplexed using DADA2 for downstream statistical analyses. Sequence returns were identified at the species taxonomic level using the UNITE+INSDC v. 8.2 fungal ITS database (Kõljalg et al. 2013) and further parsed into functional guilds for comparative functional group analysis using FUNGuild (Nguyen et al. 2016).

Statistical Methods

To assess the effects of fire severity, forest type, soil depth, and year post-fire on differences in fungal community composition, fungal diversity was first quantified using a weighted unique fraction (hereafter Weighted UniFrac) dissimilarity matrix (Lozupone and Knight, 2005) for pairwise comparisons of forest type-fire severity strata within each year of sampling. Weighted UniFrac dissimilarity matrices were used to represent biological beta-diversity by clustering fungal species by phylogenetic relationships and statistically exploring evolutionary relatedness between forest type-severity strata using Permutational Multivariate Analysis of Variance (PERMANOVA) pairwise comparisons at 999 permutations. Unlike other statistical tests, PERMANOVA assumes only exchangeability, or that permutable units such as our plot-depth composites can be reordered under an unrejected null hypothesis without changing distribution (Anderson, 2017). To compare species richness of post-fire fungal community composition across forest types, fire severities, depths, and years post-fire, Shannon's Diversity Index (SDI) was quantified for fungal functional groups of interest using the "vegan" package in R (Shannon, 1948; Oksanen et al. 2019).

To explore relationships between fungal diversity and hydrophobicity, we created linear mixed-effects models examining alpha-diversity relationships between presence of each hydrophobicity class and fungal SDI stratified by functional group and analyzed using an Analysis of Variance (ANOVA) where residuals and random effect coefficients were independent and relatively normally distributed (Schielzeth et al. 2020). The same method was employed to analyze relationships between each fungal functional group and plant functional groups of forbs, graminoids, and shrubs. All linear models were created and analyzed using ANOVA pairwise comparisons operated by the "car" package in R (Fox and Weisberg, 2019).

Results

PCR sequencing successfully replicated and barcoded a total of 4,131,262 unique ITS sequences: 1,977,999 and 2,153,263 sequence returns for the first and second year of post-fire soil sampling, respectively. After filtering to a sequencing depth of 14,000 reads per sample and selecting functional guild classifications with confidence of Probable and Highly Probable, a total of 2,623,246 sequences and 7,638 OTUs were analyzed from identified species of saprotrophic, ectomycorrhizal, and arbuscular mycorrhizal fungal species (Table 2).

Fungal sequence counts were grouped and summed by treatment, and mean relative abundance was calculated for taxa in each treatment group (Table 3). In fire-affected lower-montane forests, the most dominant fungi were saprophytes in the genera *Geminibasidium* (whose dominance is unique to lower-montane forests), *Calypetrozoma*, *Leucosporidium*, *Warcupia*, and *Mortierella* genera (Figure 2). Though the dominant genus in deep soils was *Calypetrozoma* in both years of sampling, *Geminibasidium* was particularly prevalent in year 1 surface soils burned at high-severity, and maintained compositional dominance in low-severity deep soils between year 1 and year 2 - though its overall relative abundance was reduced. All but one of these taxa were dominant in post-fire conditions specifically; only *Mortierella* dominated in fire-affected and control plots alike.

Burned subalpine sites were dominated by saprophytes *Calypetrozoma*, *Leucosporidium*, and *Mortierella*, ectomycorrhizae of *Piloderma*, and plant endophytes of genus *Leptodontidium* (Figure 3). *Leucosporidium* was the most prevalent genus in burned surface soils after one year post-fire (Table 3). Dominant relative abundance transitioned then to *Mortierella* in low-severity plots and *Calypetrozoma* at high-severity.

Forest Type and Fire Severity Drive Fungal Beta- and Alpha-Diversity

PERMANOVA analyses of weighted UniFrac dissimilarity found significant differences in fungal community composition between most forest type-fire severity treatment groups stratified by soil depth in both years of sampling (Table 4). Fungal community beta-diversity by forest type-fire severity strata is ordinated and visualized using a Principal Coordinate Analysis (PCoA) for both years post-fire (Figure 4). Forest type was the strongest contributor to differences in fungal composition between treatment groups in Year 1, accounting for 59.64% of significant inter-group variation. Subalpine plots had more than twice the inter-group variation in fungal diversity of lower montane plots between fire severities ($\Sigma psF_{SA-Y1} = 14.04$, $\Sigma psF_{LM-Y1} = 6.93$) and the greatest overall difference in fungal composition was found between forest types in surface soils burned at high-severity ($psF_{HS-S-Y1} = 10.02$, q-value = 0.003). Year 2 showed even greater inter-group variation in fungal community composition driven by forest type (69.66%), with species dissimilarity increasing between forest types in both low- and high-severity plots ($\Sigma psF_{LS-Y2} = 14.81$, $\Sigma psF_{HS-Y2} = 24.13$). Fungal communities of surface soils burned at high-severity increased in dissimilarity between forest types ($psF_{HS-S-Y2} = 14.35$, q-value = 0.005), while depth soils became the most dissimilar between forest types burned at low-severity ($psF_{LS-D-Y2} = 8.46$). Fire severity as a secondary driver of significant inter-group variation between treatment groups was particularly visible in subalpine forests (Figure 5). By Year 2, inter-group fungal variation increased between fire severities ($\Sigma psF_{SA-Y2} = 16.96$). Meanwhile, lower montane plots ceased to show significant difference in fungal community composition driven by fire severity by Year 2 at any soil depth.

Soil depth showed significant impact on variation in fungal beta-diversity in specific forest type-fire severity strata (Figure 6). Fungal beta-diversity between soil depths was

significantly different only in subalpine sites burned at high-severity in Year 1 ($psF_{SA-HS-Y1} = 2.32$, $q\text{-value} = 0.021$). Year 2 showed an increase in this dissimilarity ($psF_{SA-HS-Y2} = 2.40$, $q\text{-value} = 0.031$), with the addition of lower montane forests burned at low-severity also showing significantly different fungal composition between soil depths ($psF_{LM-LS-Y2} = 1.98$, $q\text{-value} = 0.043$).

Differences in fungal community composition between unburned control plots and fire-affected plots collected in Year 2 was again mediated by forest type (Table 5). Unburned fungal diversity was significantly different from both low- and high-severity plots in lower montane forests ($\Sigma psF_{LM-UN-LS} = 8.91$, $\Sigma psF_{LM-UN-HS} = 13.53$), especially in surface soils ($psF_{LM-UN-LS-S} = 5.38$, $q\text{-value} = 0.007$; $psF_{LM-UN-HS-S} = 8.2$, $q\text{-value} = 0.014$). Subalpine plots burned at high-severity showed the greatest inter-group variation from unburned controls ($\Sigma psF_{SA-UN-HS} = 25.27$). However, when burned at low-severity, subalpine plots showed no significant differences in community composition from unburned controls in surface or depth soils. Finally, no significant inter-group variation was observed when comparing fungal composition of unburned lower montane and subalpine control plots.

The effects of forest type and fire severity between soil depths and years post-fire were explored to determine interactions with fungal alpha-diversity stratified by functional group. For fungi broadly, fire severity had the most significant effect on fungal diversity (Figure 7) with SDI peaking in low-severity burns, followed by unburned and high-severity plots ($p = 0.009$). Fungal SDI at specific soil depths was significantly different across fire severities, with highest fungal diversity observed in low-severity surface soils, followed by unburned depth, low-severity depth, high-severity depth, high-severity surface, and finally unburned surface ($p = 0.013$). Fire severity and soil depth interactions significantly influenced saprophyte alpha-diversity specifically, where

deep soils had the highest SDI in unburned plots, and diversity of surface soils remained highest when burned at low-severity ($p = 0.009$). However, no significant difference in overall fungal or saprophytic alpha-diversity was observed between forest types.

Fire severity was a powerful predictor of observed alpha-diversity for both mycorrhizal guilds, with a clear inverse correlation between fire severity and observed EMF and AMF SDI ($p < 0.001$, $p < 0.005$). When considering forest type, inverse correlation between fire severity and EMF diversity held for both forests ($p = 0.023$)(Figure 8). Differences in mycorrhizal diversity were also apparent along soil profiles, with depth soils yielding higher EMF and AMF diversity than surface soils ($p < 0.001$, $p = 0.006$). EMF diversity did not fluctuate between years post-fire, but AMF diversity did drop significantly between Year 1 and Year 2 post-fire ($p < 0.001$). Overall, forest type was the most significant driver of AMF diversity (Figure 9), with greater SDI observed in lower montane than subalpine plots ($p < 0.001$).

Fungal Diversity Influences Observed Plant Diversity and Percent Cover

Post-fire fungal alpha-diversity had nuanced effects on understory vegetation alpha-diversity and percent cover across plant functional groups. Though SDI of plant diversity was significantly different between years post-fire alone ($p < 0.001$), the interaction of overall fungal diversity and year post-fire was a pronounced influence on understory diversity, where increases in fungal SDI showed greater plant SDI overall in Year 2 - though correlation was more strongly positive in Year 1 ($p = 0.019$). Fungal diversity showed significantly different effects on percent forb cover between years post-fire. In Year 1, fungal SDI was positively correlated with percent cover of forbs, while in Year 2, though there was greater forb coverage overall, the correlation with fungal SDI was negative ($p = 0.037$). Fungal diversity had variable impacts on

shrub cover between forest types. In lower montane forests, fungal SDI was negatively correlated with shrub coverage, while in subalpine forests the two were positively associated ($p = 0.031$). Effects on shrub coverage intensifies when fungal SDI and forest type interact with fire severity, with strongest relationships uncovered for unburned plots in both forest types (negative correlation) and low-severity subalpine sites (positive correlation) ($p = 0.021$). Saprophytic alpha-diversity showed no significant effects on plant diversity or percent shrub cover. However, the effect of saprophytic SDI on graminoid and forb cover was significantly different between years sampled ($p = 0.009$ and $p = 0.033$, respectively). Graminoid and forb cover increased in tandem with saprophytic SDI in Year 1, but this trend was inverted (though not as strongly correlated) in Year 2.

Plant functional group cover was significantly influenced by mycorrhizal alpha-diversity across treatment gradients. Forb cover was significantly affected by EMF diversity when interacting with fire severity and year post-fire ($p = 0.041$), being greater for similar levels of EMF SDI at both severities in Year 2 and at low-severity in both years of sampling compared to high-severity. EMF diversity had the most powerful direct influence on shrub percent cover being strongly positively correlated ($p < 0.001$), a pattern that held in unburned and low-severity plots when EMF SDI interacted with fire severity ($p = 0.005$). Forest type also interacted with fire severity to show significant effects on the relationship between EMF SDI and shrub cover, with lower montane forests showing positive correlation only in unburned sites, and subalpine forests showing a strongly negative correlation in unburned plots and positive correlation at low-severity ($p = 0.009$).

AMF had significantly different levels of influence on plant alpha-diversity between forest types. Though both were positively correlated, greater plant SDI was observed in lower

montane forests at similar levels of AMF SDI ($p = 0.021$). Graminoid, forb, and shrub cover were all significantly influenced by AMF richness and evenness, with positive correlation for graminoids and forbs, and negative correlation for shrubs ($p < 0.001$; $p < 0.001$; $p = 0.041$). AMF SDI interactions with forest type and year post-fire had significant effects on graminoid cover (Figure 10). Graminoid coverage at a given AMF SDI was greater in lower montane than subalpine forests ($p = 0.005$). Year 2 showed a stronger positive correlation between AMF SDI and graminoid coverage than Year 1 ($p = 0.033$). Percent forb cover was also influenced by the interaction with year post-fire, showing greater forb coverage at similar AMF SDI and a stronger positive correlation in Year 2 than in Year 1 ($p = 0.041$). Shrub cover was affected by interactions between AMF SDI and forest type, with shrub cover at similar levels of AMF SDI greater in subalpine forests ($p = 0.037$).

Fungal Diversity Affects Presence of Soil Hydrophobicity

Soil hydrophobicity was significantly affected by forest type, fire severity, soil depth, and year post-fire (Figure 11). Percentage of observed extremely hydrophobic soil was greater in subalpine plots than lower-montane ($p < 0.001$) and surface than deep soils ($p < 0.001$), was positively correlated with increasing fire severity ($p = 0.02$), and negatively correlated with year post-fire ($p < 0.001$). Fungal alpha-diversity overall had limited effects on the presence of all soil hydrophobicity classes across forest types and fire severities. Fungal SDI was significantly varied in its influence on percent highly hydrophobic soil between years post-fire and their interaction with forest type and soil depth. While Year 1 presence of high hydrophobicity showed a weak positive correlation with fungal SDI, a more strongly negative correlation was observed in Year 2 post-fire ($p = 0.005$). Overall fungal diversity correlation with year-post was largely

driven by observations in lower montane forests and surface soils, as subalpine plots showed the inverse relationship in Year 1 and Year 2 soils ($p = 0.043$), and positive correlation appeared in depth soils in both years of sampling ($p = 0.037$).

Saprophytes specifically were correlated with presence of high soil hydrophobicity when interacting with year post-fire ($p = 0.019$). While Year 1 post-fire showed a weaker positive correlation, Year 2 showed a more strongly negative correlation between saprophytic SDI and percent highly hydrophobic soil. Significant influence on presence of extremely hydrophobic soil was observed based on interactions between saprophytic SDI, forest type, and year post-fire ($p = 0.040$).

EMF had more obvious influences over percent severity of soil hydrophobicity. When interacting with forest type and soil depth, EMF alpha-diversity had a significant relationship with percent low soil hydrophobicity showing negative correlation in lower montane forests and more strongly positive correlation in subalpine forests ($p = 0.002$), with greater percentage low hydrophobicity in depth soils versus surface soils - though both were positively correlated with EMF SDI ($p = 0.047$). Percent high soil hydrophobicity was directly affected by EMF SDI ($p = 0.040$), but this relationship differed between forest types and soil depths (Figure 12). Highly hydrophobic soils were positively correlated with EMF SDI in lower montane plots, but negatively correlated in subalpine ($p < 0.001$). Surface soils saw greater presence of highly hydrophobic soil at similar levels of EMF SDI versus depth soils ($p = 0.017$). Extreme soil hydrophobicity presence had significantly different relationships with EMF diversity when interacting with forest type, being positively correlated in lower montane and more strongly negatively correlated in subalpine forests ($p = 0.010$).

Discussion

The diversity of soil biological communities post-fire is essential to supporting forest recovery processes. Fungal diversity supports diversity and productivity of aboveground vegetation through nutrient cycling, resource facilitation, and soil stabilization (van der Heijden et al. 1998; Filialuna and Cripps, 2021). However, the degree to which fungal diversity changes across disparate forest systems exposed to similar disturbance agents is not well understood. In our study, differences in forest environments were significant in mediating soil fungal community response to wildfire gradients in the Southern Rockies. Though no significant difference was observed in overall fungal alpha-diversity, elevation-driven forest types developed post-fire fungal communities compositionally unique to one another. Fungal composition differences in lower-montane, ponderosa-pine dominated forests were stimulated by fire, and our study confirms that fungal communities exposed to fire homogenize compositionally regardless of severity (Hamman et al. 2007). In contrast, subalpine environments dominated by spruce and fir forests recorded greater heterogeneity in phylogenetic relatedness of post-fire fungal species between fire severities, requiring high-magnitude fires in order to significantly change fungal community composition from unburned forests.

Several ecological theories may pertain to this phenomenon for fungi. Historic patterns of magnitude and return interval influence organism adaptation to specific forest fire regimes (McLauchlan et al. 2020). Thus, post-fire fungal species composition in disparate forest types may respond more strongly to fire severities historically experienced within a particular forest system (in this case, low-to-moderate intensity fire in lower-montane and high-severity fire in subalpine). Alternatively, fungal composition across fire severities may be greatly influenced by burn-adjacent sources of inoculum. Spatial arrangement of fire-severity gradients has been

shown to influence fungal composition (Owen et al. 2019) and thus may influence regeneration based on relative proximity of inoculum sources. Literature suggests that soil fungal diversity richness and composition is most strongly correlated with pre-fire sources of spore-bank inocula (Glassman et al. 2015), and this is certainly supported with the dominance of *Mortierella* and *Piloderma* in both unburned and burned forests. However, our results more generally indicate that unique fungal genera, such as *Calyptozyma* and *Leucosporidium*, may come to dominate burned environments across forest types regardless of their potential pre-fire relative abundance. Differing nutrient availability between forest types may also contribute to forest type-fire severity relationships. Total carbon, total nitrogen, and total phosphorus have been shown to greatly influence fungal regeneration composition. Pulido-Chavez et al. (2021) observed that nearly a third of saprophytic community variation post-fire was driven by total carbon levels, while total nitrogen and total phosphorus were significant predictors of EMF species richness. Future research would benefit not only from explicit spatial analysis of burn patch size and arrangement as a possible effect on differences in fungal community composition between fire severities, but exploration of soil total nutrient content in order to ascertain its effects on fungal regeneration, especially across functional groups and species that may utilize unique post-fire microsite nutrient depositions such as charred carbon (Steindorff et al. 2022).

Interestingly, we did not observe any significant differences in fungal community composition in unburned soils between forest types. This is surprising, given how strongly inter-group variability was driven by forest-type differences between burned forests in our study. However, it is likely that not enough data on unburned control plots was collected to produce statistically significant results in quantified variation, as the number of unburned plots ($n = 7$) was less than half the number of burned plots in either severity class. If considering unburned

control plots to establish a fungal community baseline in different forests, care should be taken to ensure future work involves adequate density of control plots in each forest type of interest.

Our research demonstrates that the magnitude with which fungal diversity is impacted by fire severity in lower-montane and subalpine forests predictably differs by fungal functional group. Saprophytic alpha-diversity was stimulated by wildfire across forest types, but this effect was mostly limited to surface soils. Mycorrhizae responded negatively to increasing fire severity across forest types, especially within surface soils. Greater mycorrhizal diversity at depth may indicate that fire is stimulating the expansion of these fungal communities, while also conserving a majority of prefire species as they are not as strongly subject to heat death at these soil depths. Reductions in EMF diversity post-fire is particularly salient, and has been associated with slow forest recovery in other pine-dominated forest ecosystems (Smith et al. 2005, Nunez et al. 2009). Given signals of increased potential for complete land conversion to non-forest in the western US and loss of important forest ecosystem services in the coming decades (Halofsky et al, 2020; Stevens-Rumann et al. 2017; Coop et al. 2020; Rocca et al. 2014), a deficit in regeneration of fungal tree mutualists (largely EMF) may indeed exacerbate potential for tree regeneration failure and require anthropogenic intervention such as soil inoculation. AMF specifically are also the only fungal functional group studied to show a significant temporal response to fire regardless of forest type or fire severity. Longer-term sampling in future research may show AMF alpha-diversity continue to increase rapidly as shown in Treseder et al. (2004). Our results showed that forbs and graminoids indeed were greater as AMF diversity increased, and a similar correlation emerged between EMF and shrub cover. Given the limited nutrient and water resource availability characteristic of post-fire forests, it follows that we observed significant relationships between fungal mutualists to their respective potential plant host groups, as soil

conditions mediated by fire severity may necessitate the mutualism between the two biological groups in order to survive and proliferate.

Data on mycorrhizal groups in particular may be skewed due to omission of additional functional groups analyzed and efficacy of primers used for fungal sequencing. The relative dearth of AMF in subalpine forests may be due to niche overlap with ericoid mycorrhizae, an additional mutualistic fungal functional group that associates with ericaceous plant species (Cazares et al. 2005) that often populate spruce-fir understories. ITS primers also display higher levels of variation when replicating glomeromycota (AMF) compared to dikarya (saprophytes and EMF), and thus specific primers for genes such as AML1-AML2 are more effective at replicating AMF as their relationship glomeromycota is more phylogenetically understood (Lee et al. 2008).

Considering that study of relationships between post-fire fungal community diversity and *in-situ* soil hydrophobicity is relatively novel, the potential for fungal community composition to impact observed soil hydrophobicity is difficult to surmise at the functional group level. We hypothesized that presence of carbon-mineralizing fungal functional groups (i.e. saprophytes and EMF) would be more clearly associated with low-to-moderate soil hydrophobicity, and thus may be leveraged in post-fire soil remediation to reduce the potential for erosion. Saprophytic diversity tended to reduce the presence of high hydrophobicity in high-severity burns, but this relationship was inverted in low-severity sites. Similarly, EMF diversity reduced the presence of high and extreme hydrophobicity in subalpine forests, but again this correlation reverses itself in lower-montane sites. Though significant correlations between fungal diversity and soil hydrophobicity were calculated, there may be some tertiary or quaternary confounding variable influencing fungal community diversity and observed soil hydrophobicity that cannot be

determined from this study. Thus, it is difficult to make definitive statements about the drivers of hydrophobicity from broader fungal groups alone and what implications they may have for pragmatic soil remediation. Future work should seek to isolate specific post-fire fungal genera such as those identified in Steindorff et al. (2021) and test their correlation with particular classes of soil hydrophobicity. This information may then be leveraged in future inoculation in order to limit the residence time of high-to-extreme hydrophobic compounds in post-fire forest soils using particular fungal species.

There are further key limitations to consider pertaining to the methodology performed within the scope of this study. Community and species spatial autocorrelation of environmental variables occurs at relatively small spatial scales. Lilleskov et al. (2004) found that spatial autocorrelation may influence fungal community composition in samples observed 3 to 17 m from one another across forest types, while Smithwick et al. (2012) found this number ranged from 1.5 to 10.5 m in subalpine forests specifically. Microsite factors like topography and soil nutrient content also tend to influence trends in fungal diversity in forest ecosystems (Lilleskov et al. 2004). Therefore, though soil collection conducted for this research occurred within an acceptable range of 6.1 m along each of our transects, compositing samples at the plot level for downstream PCR sequencing may have omitted important intra-plot information on fungal assemblage contrasts that is more spatially explicit than can be observed from our testing. Future work should seek to increase sampling density within smaller spatial ranges and consider further microsite characteristics in order to accurately spatially assess post-fire fungal composition.

Though wildfire generally results in temporary reductions in fungal community diversity compared to pre-fire levels (Holden et al. 2013; Dooley and Treseder, 2012), these changes are often caused by the mortality and removal of rarer rather than dominant pre-fire fungal species

(Glassman et al. 2016; Brown et al. 2016). Further analysis of fungal species present within forest type-fire severity strata is necessary in order to deduce whether that pattern holds true for the data we collected as well. Stand age may be influential in the community composition of forest fungal populations by influencing the diversity and abundance of certain fungal functional groups. Indeed, Hart et al. (2005) found that mycorrhizal fungal abundance of ponderosa pine forests was positively correlated with stand age, peaking in stand ages of 60-70 years old. Thus, longitudinal studies into the effects of fire on forest fungal communities should ideally include consideration of stand age. Unburned control plots were difficult to establish as unburned stands directly adjacent to burn patches often did not fulfill forest-type parameters required for plot establishment. Though some analysis uncovered statistically significant associations with unburned sites, with such variable densities established across fires and forest types it is difficult to say with certainty whether a clear fungal community baseline was established to compare the effects of burned versus unburned conditions on fungal assemblages.

Conclusion

Wildfires are increasing in size, frequency, and severity in coniferous forests such as those found in the Southern Rockies. We are faced with a multitude of issues as fire regime changes continue to take effect, including poor soil nutrient availability, plant regeneration failures, and increased erosion and landscape destabilization, all of which are influenced in some part by the state of soil fungal communities. Novel approaches to whole-community ecology then are required to answer pertinent questions regarding the impacts of fire on forest ecosystems in the modern-day. Here, we have bridged microbiology, botany, and soil hydrology in order to

ascertain relationships between fungal assemblages, understory vegetation, and soil hydrophobicity after fire in the Southern Rockies.

Our study indicates clear variation in the response of fungal communities to fire in disparate forest types in terms of diversity and composition, which subsequently influences both understory plant regeneration and fire-driven soil hydrophobicity. In response to Question 1, we found that while post-fire fungal alpha-diversity is primarily driven by the severity of fire experienced - and to some degree the location of the community throughout the soil profile - composition of the fungal community is determined largely by elevation- and climate-driven forest-type gradients. In answer to Question 2, relationships between fungal and understory plant communities is most obviously expressed in the positive correlation between diversity of mutualistic mycorrhizal functional groups and the abundance of available related plant hosts (shrubs in the case of EMF and graminoids and forbs in the case of AMF). Finally, to address Question 3, though correlational patterns emerged from our data, our conclusions are rather that ecological relationships between fungal communities and soil hydrophobicity cannot be clearly determined simply from analysis at the functional group level.

With the relative lack of post-fire microbiological research occurring in post-fire forest ecosystems, we only just begin to answer questions about the complex ecological interactions that consider soil fungal communities, and more research is needed. Relationships with fungi may extend regeneration benefits to plant species of interest in forest conservation, be it tree species for the maintenance of forest ecosystem services or understory species with important ecological or cultural value. The results of this study highlight the importance of forest type and long-term soil health monitoring in guiding post-fire forest management, as elevation-driven forest type and yearly post-fire increments are clearly capable of presenting biologically

significant differences in relationships between below- and aboveground organisms which may ultimately affect regeneration and conservation success.

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Appendix

Tables

Table 1. Distribution of plots established and sampled one and two years post-fire. Plots stratified by forest type and fire severity. Forest type classified by dominant tree species (>80% by basal area). Fire severity classified by observed tree mortality.

| Forest Type | Unburned | Low-severity (<20%) | High-severity (>80%) | Total (N) |
|--------------------------------|----------|---------------------|----------------------|-----------|
| Lower Montane (ponderosa pine) | 4 | 8 | 9 | 21 |
| Subalpine (spruce-fir) | 3 | 8 | 9 | 20 |
| Total (N) | 7 | 16 | 18 | 41 |

Table 2: Distribution of fungal sequencing reads and identified OTUs resulting from PCR replication of ITS primers across fungal functional groups. Sequences filtered to sequencing depth of 14,000 reads using Qiime 2 (Bolyen et al. 2019) and to “Probable” and “Highly Probable” species identities reported by Funguild (Nguyen et al. 2016).

| Functional Group | sequence reads | OTUs |
|------------------|----------------|-------|
| Saprophytes | 1,968,750 | 4,975 |
| EMF | 601,931 | 1,662 |
| AMF | 3,466 | 183 |
| Total | 2,623,246 | 7,638 |

Table 3: Dominant taxa in terms of relative abundance for each treatment group. Relative abundance quantified as proportion (percent) of sequence reads for given taxa to all sequence reads of any taxa within treatment group. Three most dominant genera of fungi displayed for unburned controls, and genera with highest relative abundance displayed for each burned group. Sequence reads produced using Qiime 2 (Bolyen et al. 2019).

| Forest Type | Fire Severity | Year Post-Fire | Soil Depth | Dominant Taxon | Relative Abundance (%) | |
|---------------|---------------------|----------------|-----------------------|-----------------------|------------------------|-------|
| Lower Montane | Unburned | Control | Surface | <i>Russula</i> | 16.32 | |
| | | | | <i>Inocybe</i> | 12.20 | |
| | | | | <i>Mortierella</i> | 10.34 | |
| | | | Depth | <i>Russula</i> | 17.73 | |
| | | | | <i>Inocybe</i> | 17.56 | |
| | | | | <i>Mortierella</i> | 7.44 | |
| | Low | 1 | Surface | <i>Leucosporidium</i> | 20.56 | |
| | | | | <i>Geminibasidium</i> | 34.18 | |
| | | | Depth | <i>Geminibasidium</i> | 34.18 | |
| | | | | <i>Geminibasidium</i> | 34.18 | |
| | | | 2 | Surface | <i>Calyptrozoma</i> | 16.26 |
| | | | | | <i>Calyptrozoma</i> | 16.26 |
| High | 1 | Surface | <i>Geminibasidium</i> | 28.43 | | |
| | | | <i>Geminibasidium</i> | 28.43 | | |
| | | Depth | <i>Geminibasidium</i> | 34.18 | | |
| | | | <i>Geminibasidium</i> | 34.18 | | |
| | | 2 | Surface | <i>Calyptrozoma</i> | 28.02 | |
| | | | | <i>Calyptrozoma</i> | 28.02 | |
| Depth | <i>Calyptrozoma</i> | 22.32 | | | | |
| | <i>Calyptrozoma</i> | 22.32 | | | | |
| Subalpine | Unburned | Control | Surface | <i>Piloderma</i> | 16.45 | |
| | | | | <i>Mortierella</i> | 13.11 | |
| | | | | <i>Amphinema</i> | 11.29 | |
| | | | Depth | <i>Mortierella</i> | 21.05 | |
| | | | | <i>Piloderma</i> | 18.65 | |
| | | | | <i>Tylospora</i> | 15.34 | |
| | Low | 1 | Surface | <i>Leucosporidium</i> | 17.95 | |
| | | | | <i>Leucosporidium</i> | 16.69 | |
| | | | Depth | <i>Leucosporidium</i> | 16.69 | |
| | | | | <i>Leucosporidium</i> | 16.69 | |
| | | | 2 | Surface | <i>Mortierella</i> | 17.07 |
| | | | | | <i>Mortierella</i> | 17.07 |
| Depth | <i>Mortierella</i> | 17.24 | | | | |
| | <i>Mortierella</i> | 17.24 | | | | |
| High | 1 | Surface | <i>Leucosporidium</i> | 27.98 | | |
| | | | <i>Leucosporidium</i> | 27.98 | | |
| | | Depth | <i>Leucosporidium</i> | 18.39 | | |
| | | | <i>Leucosporidium</i> | 18.39 | | |
| | | 2 | Surface | <i>Calyptrozoma</i> | 46.99 | |
| | | | | <i>Calyptrozoma</i> | 46.99 | |
| Depth | <i>Calyptrozoma</i> | 41.95 | | | | |
| | <i>Calyptrozoma</i> | 41.95 | | | | |

Table 4: PERMANOVA analysis results of weighted UniFrac fungal community composition dissimilarity. Pairwise comparisons between treatment groups are stratified by forest type, fire severity, and soil depth to quantify proportion of inter-group variability to intra-group variability expressed as pseudo-F (psF). Greater psF represents greater dissimilarity in fungal composition between groups. Total proportion of inter-group variation is summed for each forest type and fire severity comparison, expressed as Σ psF (Σ psF). Analysis run at default of 999 permutations using Qiime2 (Bolyen et al. 2019). Type-severity-depth relationships not significantly different based on False Discovery Rate (FDR) adjusted p-value (q-value) shown in red.

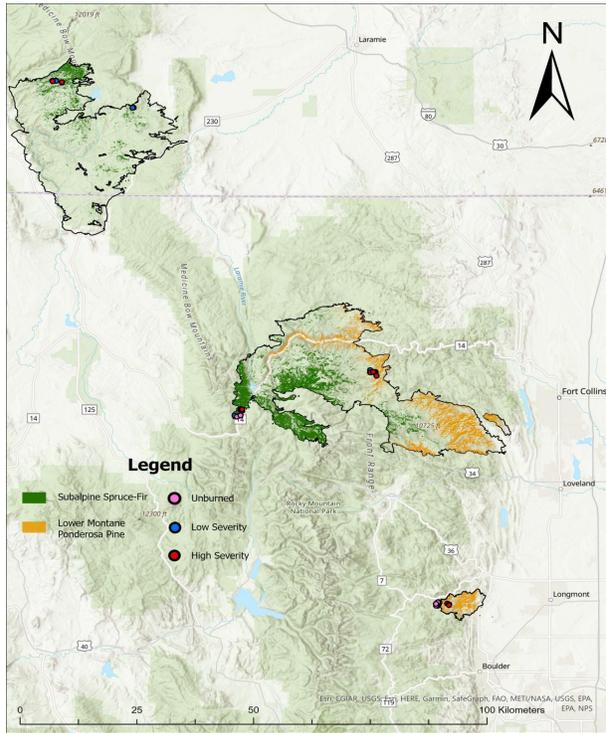
| Year Post-Fire | Forest Type | Fire Severity | Soil Depth | Proportion Inter-Group Variation (psF) | FDR adj. p-value (q-value) | Total Inter-Group Variation (Σ psF) | |
|--|--|---------------|------------|--|----------------------------|---|--------------------|
| 1 | Lower Montane | Low | High | Surface | 4.86 | q = 0.003 | 6.93 |
| | | | | Depth | 2.07 | q = 0.043 | |
| | Subalpine | Low | High | Surface | 7.91 | q = 0.005 | 14.04 |
| | | | | Depth | 6.13 | q = 0.008 | |
| | Lower Montane/ Subalpine | Low | | Surface | 5.13 | q = 0.003 | 8.53 |
| | | | | Depth | 3.40 | q = 0.004 | |
| | | High | | Surface | 10.02 | q = 0.003 | 16.45 |
| | | | | Depth | 6.43 | q = 0.003 | |
| | Prop. of Significant Inter-Group Variation (%) | | 59.65 | 35.41 | 4.94 | | |
| | 2 | Lower Montane | Low | High | Surface | 1.42 | q > 0.05 |
| Depth | | | | | 1.45 | q > 0.05 | |
| Subalpine | | Low | High | Surface | 8.87 | q = 0.004 | 16.96 |
| | | | | Depth | 8.09 | q = 0.004 | |
| Lower Montane/ Subalpine | | Low | | Surface | 6.35 | q = 0.005 | 14.81 |
| | | | | Depth | 8.46 | q = 0.004 | |
| | | High | | Surface | 14.35 | q = 0.005 | 24.13 |
| | | | | Depth | 9.78 | q = 0.004 | |
| Prop. of Significant Inter-Group Variation (%) | | 69.66 | 28.14 | 7.26 | | | |

Table 5: PERMANOVA analysis results of weighted UniFrac fungal community composition dissimilarity between Unburned (control) and fire-affected (low- and high-severity) plots sampled in Year 2. Pairwise comparisons between treatment groups are stratified by forest type, fire severity, and soil depth to quantify proportion of inter-group variability to intra-group variability expressed as pseudo-F (psF). Greater psF represents greater dissimilarity in fungal composition between groups. Total proportion of inter-group variation is summed for each forest type and fire severity comparison, expressed as Σ pseudo-F (Σ psF). Analysis run at default of 999 permutations using Qiime2 (Bolyen et al. 2019). Type-severity-depth relationships not significantly different from unburned controls based on False Discovery Rate (FDR) adjusted p-value (q-value) shown in red.

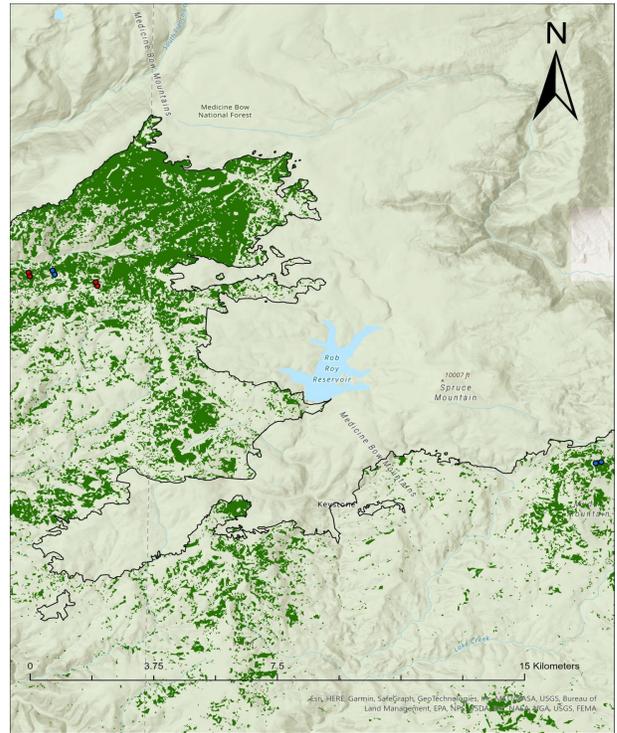
| Forest Type | Compared to Unburned Control | Soil Depth | Proportion Inter-Group Variation (psF) | FDR adj. p-value (q-value) | Total Inter-Group Variation (Σ psF) |
|-----------------------------|------------------------------|------------|--|----------------------------|---|
| Lower Montane | Low | Surface | 5.38 | q = 0.007 | 8.91 |
| | | Depth | 3.53 | q = 0.031 | |
| | High | Surface | 8.20 | q = 0.014 | 13.53 |
| | | Depth | 5.33 | q = 0.021 | |
| Subalpine | Low | Surface | 1.56 | q > 0.05 | 2.98 |
| | | Depth | 1.42 | q > 0.05 | |
| | High | Surface | 15.19 | q = 0.019 | 25.27 |
| | | Depth | 10.08 | q = 0.038 | |
| Lower Montane/ Subalpine | Unburned | Surface | 2.25 | q > 0.05 | 4.70 |
| | | Depth | 2.45 | q > 0.05 | |

Figures

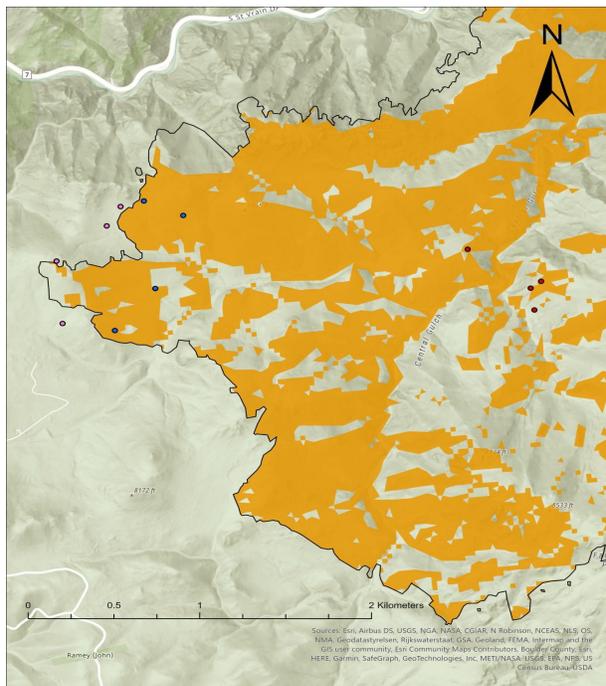
(a)



(b)



(c)



(d)

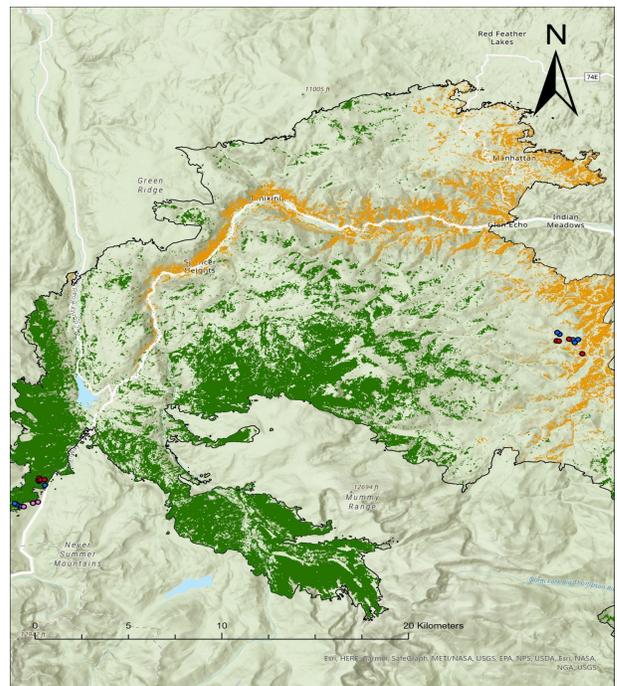


Figure 1: Fire perimeters and plot locations sampled during the summers of 2021 and 2022 (a). 41 plots were established to study post-fire effects of the 2020 wildfire season for the Mullen (b), Calwood (c), and Cameron Peak (d) Fires in two distinct forest types.

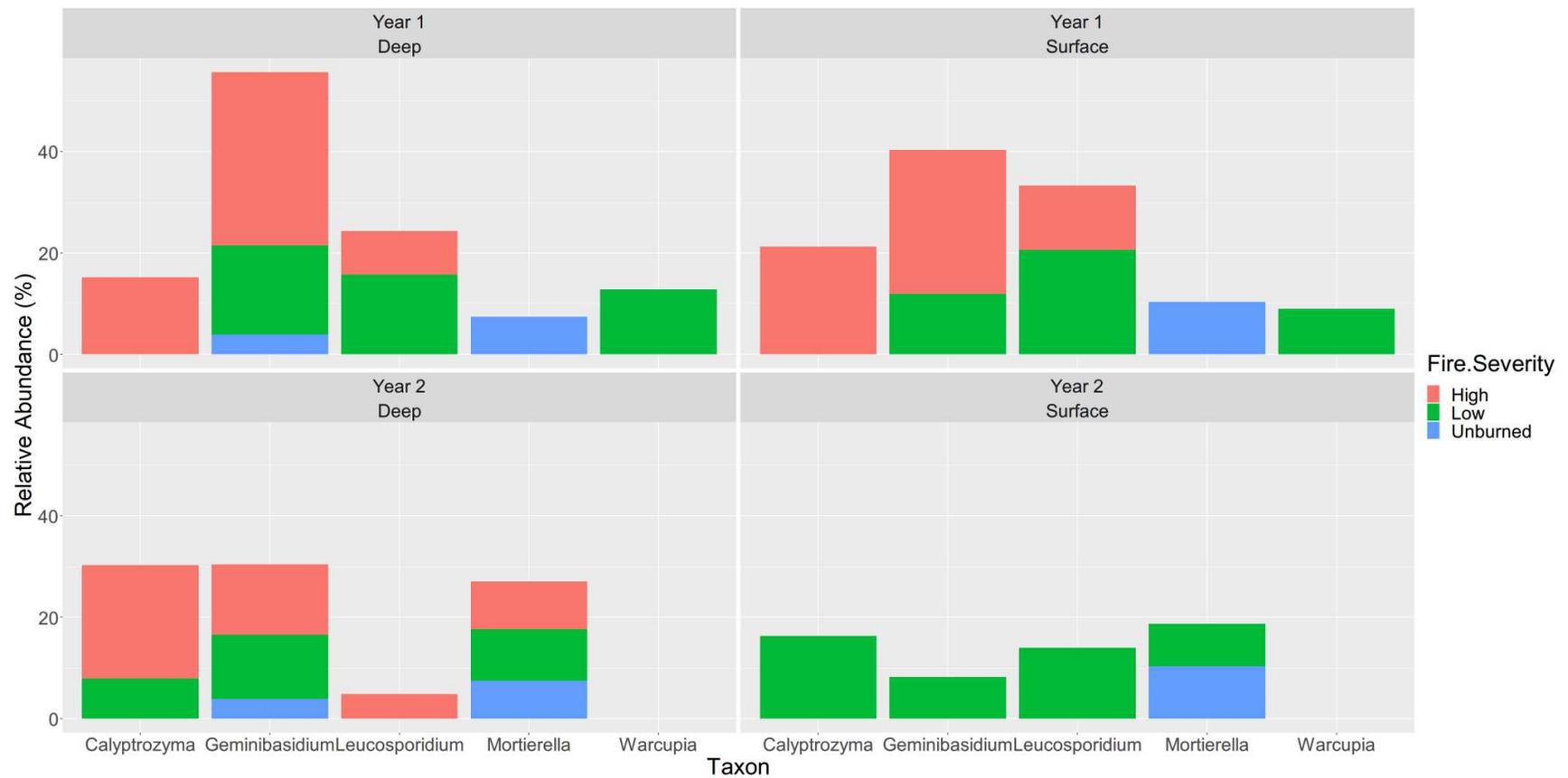


Figure 2: Five overall dominant taxa of fungi sequenced from post-fire soil collection in lower-montane forests. “Dominance” defined as highest levels of relative abundance within all burned lower-montane treatment groups. Relative abundance quantified as proportion (percent) of sequence reads for given taxon to sequence reads of all taxa within treatment group. Results stratified by soil depth (columns) and year post-fire. Relative abundance of taxa in unburned controls (blue) graphed to compare presence/absence of fungal genera in burned and unburned plots. Taxon not graphed in Year 2 (*Warcupia*) results from lower relative abundance and loss of top-five relative abundance dominance in that treatment group, though it may be dominant in lower-montane forests overall. Visualized using ggplot2 in R (Wickham, 2016).

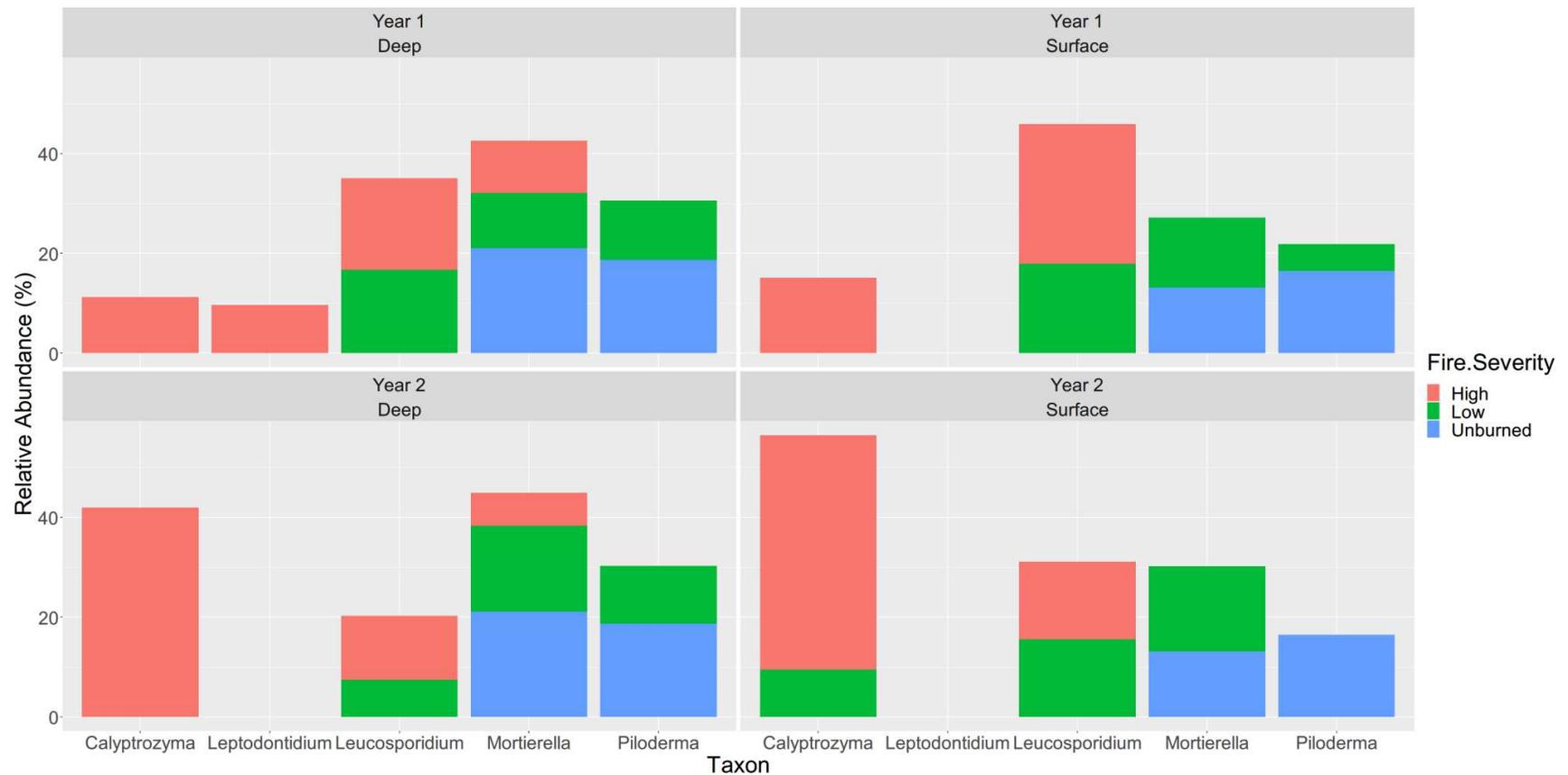


Figure 3: Five overall dominant taxa of fungi sequenced from post-fire soil collection in subalpine forests. “Dominance” defined as highest levels of relative abundance within all burned subalpine treatment groups. Relative abundance quantified as proportion (percent) of sequence reads for given taxon to sequence reads of all taxa within fire severity treatment group. Results stratified by soil depth (columns) and year post-fire. Relative abundance of taxa in unburned controls (blue) graphed to compare presence/absence of fungal genera in burned and unburned plots. Taxon not graphed in all four grid panels (*Leptodontidium*) results from overall dominant status in subalpine plots, but less dominant relative abundance within fire-depth-year treatment groups. Visualized using ggplot2 in R (Wickham, 2016).

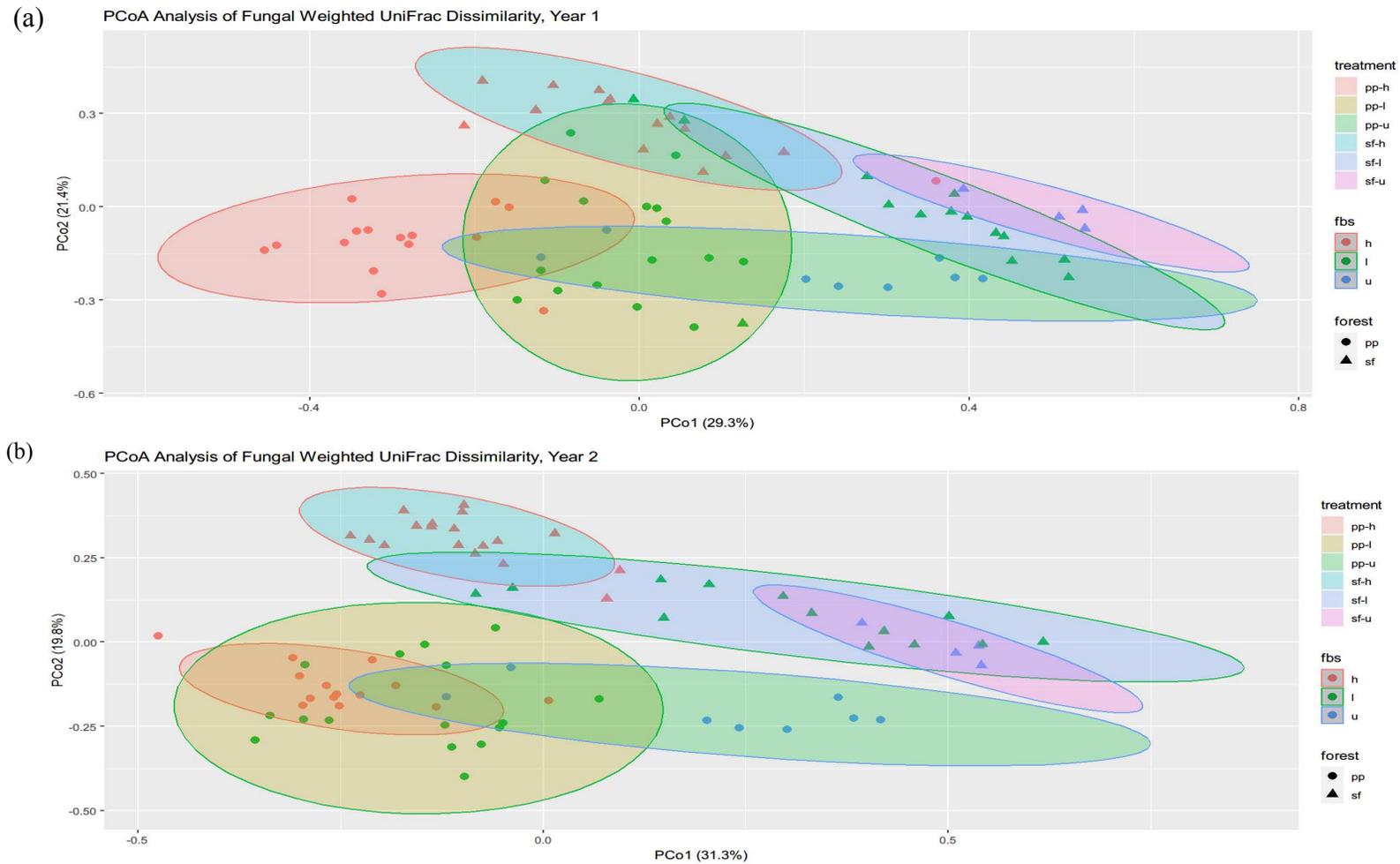


Figure 4. Principle Coordinate Analysis (PCoA) of Weighted Unique Fraction (UniFrac) fungal diversity dissimilarity stratified by forest type and fire severity one (a) and two (b) years post-fire. Axes represent principle coordinates explaining greatest (PCo1) and penultimate (PCo2) percent of variability in fungal beta-diversity for each year. Points placed closer together on each graph represent greater phylogenetic similarity of fungal species composition between samples. Ellipses represent 95% confidence interval of point distribution for each forest type-fire severity treatment group. Fungal OTUs sequenced via ITS primer PCR replication. Analysis performed using Qiime 2 (Bolyen et al. 2019) and visualized using ggplot2 in R (Wickham, 2016).

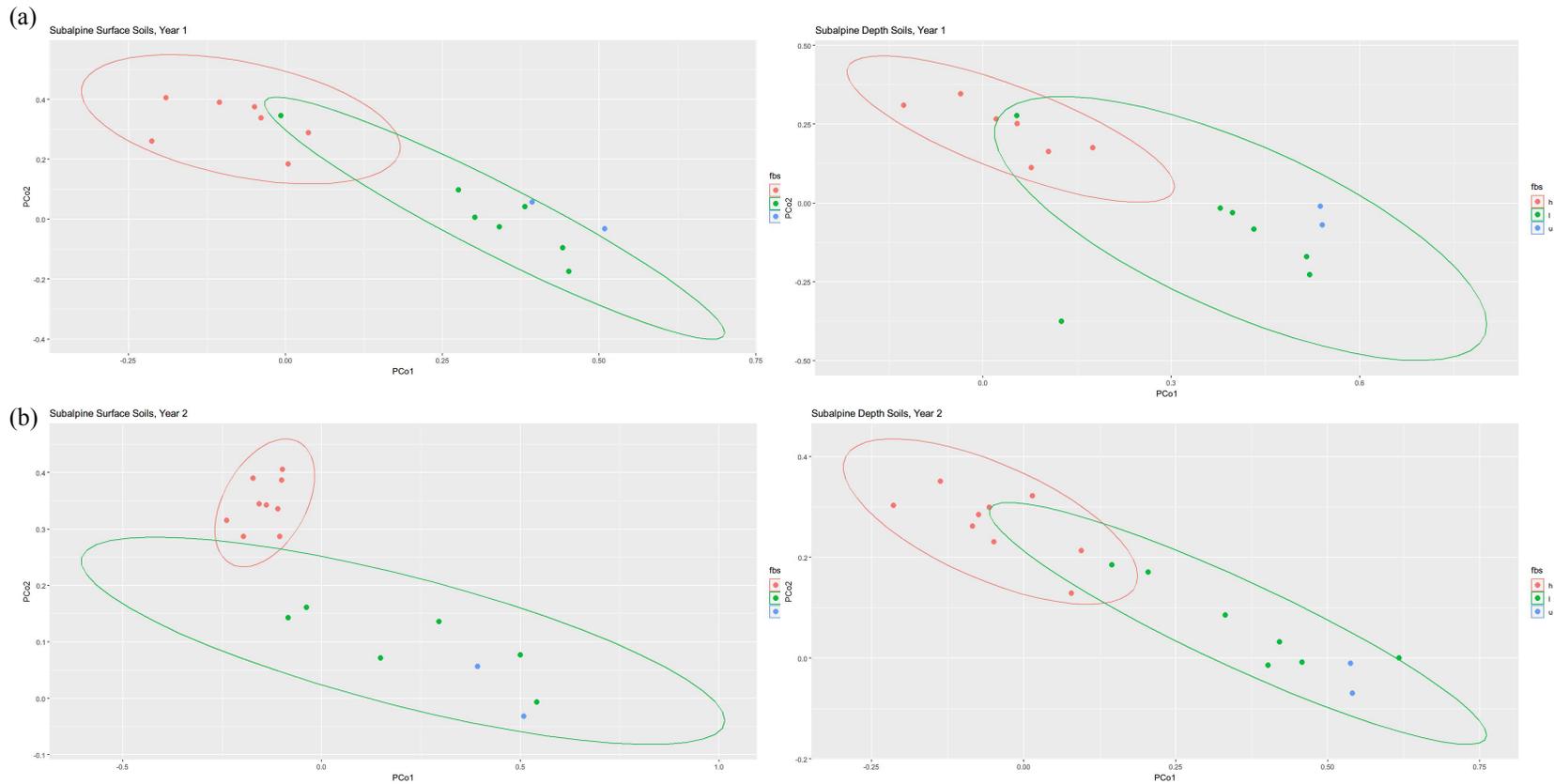


Figure 5: Principle Coordinate Analysis (PCoA) of Weighted Unique Fraction (UniFrac) fungal diversity dissimilarity in subalpine forests stratified by fire severity and soil depth one (a) and two (b) years post-fire. Axes represent principle coordinates explaining greatest (PCo1) and penultimate (PCo2) percent of variability in fungal beta-diversity for each year. Points placed closer together on each graph represent greater phylogenetic similarity of fungal species composition between samples. Ellipses represent 95% confidence interval of point distribution for each forest type-fire severity treatment group. Fungal OTUs sequenced via ITS primer PCR replication. Analysis performed using Qiime 2 (Bolyen et al. 2019) and visualized using ggplot2 in R (Wickham, 2016).

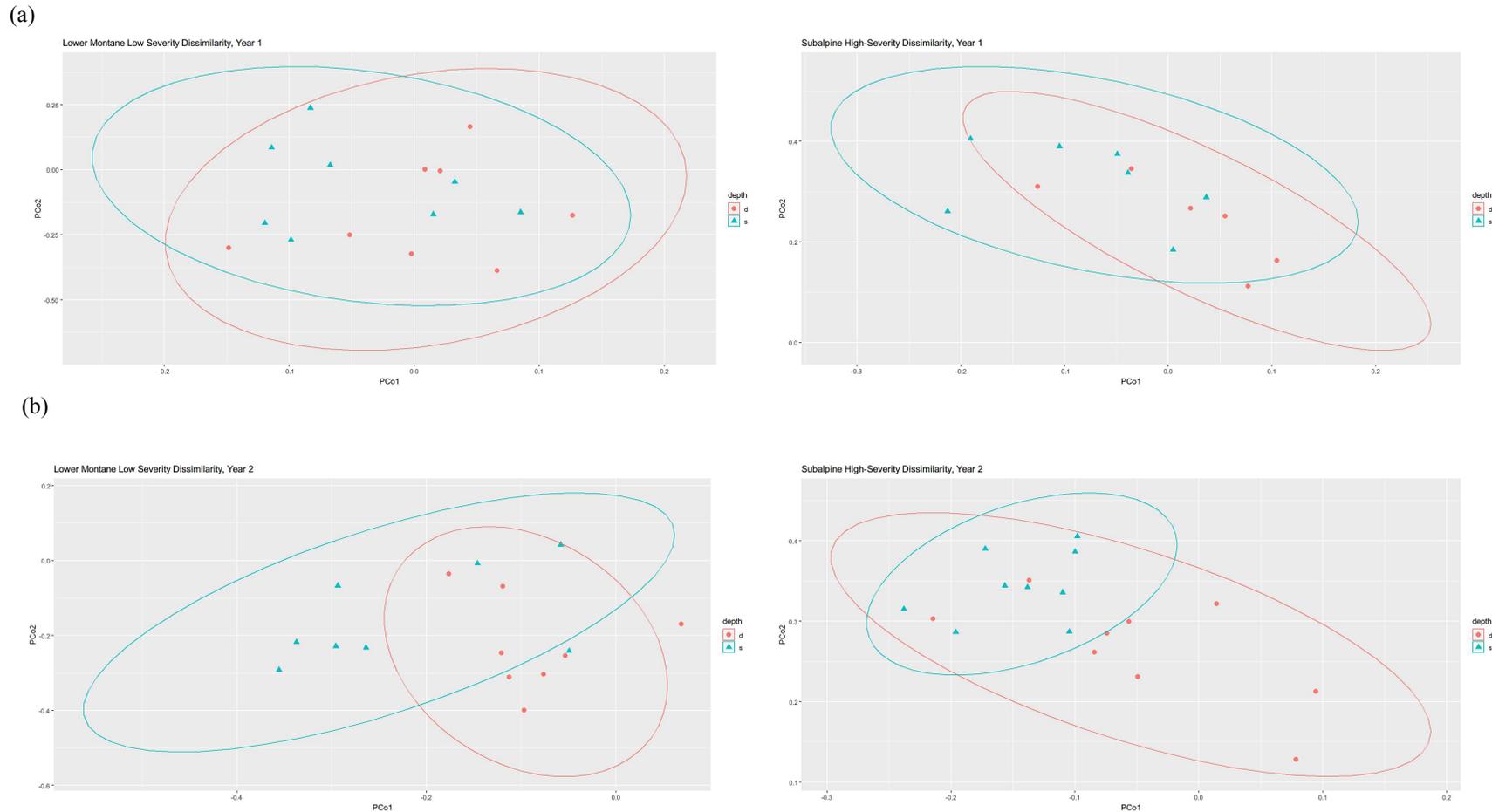


Figure 6: Principle Coordinate Analysis (PCoA) of Weighted Unique Fraction (UniFrac) fungal diversity dissimilarity between soil depths in low-severity lower montane and high-severity subalpine forests Year 1 (a) and Year 2 (b). Significant differences in fungal community composition were found between soil depths in both forest types. Graphed here, lower montane low-severity dissimilarity in Year 1 is the only relationship not statistically significant ($p > 0.05$) and is shown as a means of comparison for the same treatment group in Year 2 ($q = 0.043$). Axes represent principle coordinates explaining greatest (PCo1) and penultimate (PCo2) percent of variability in fungal beta-diversity for each year. Points placed closer together on each graph represent greater phylogenetic similarity of fungal species composition between samples. Ellipses represent 95% confidence interval of point distribution for each forest type-fire severity treatment group. Fungal OTUs sequenced via ITS primer PCR replication. Analysis performed using Qiime 2 (Bolyen et al. 2019) and visualized using ggplot2 in R (Wickham, 2016).

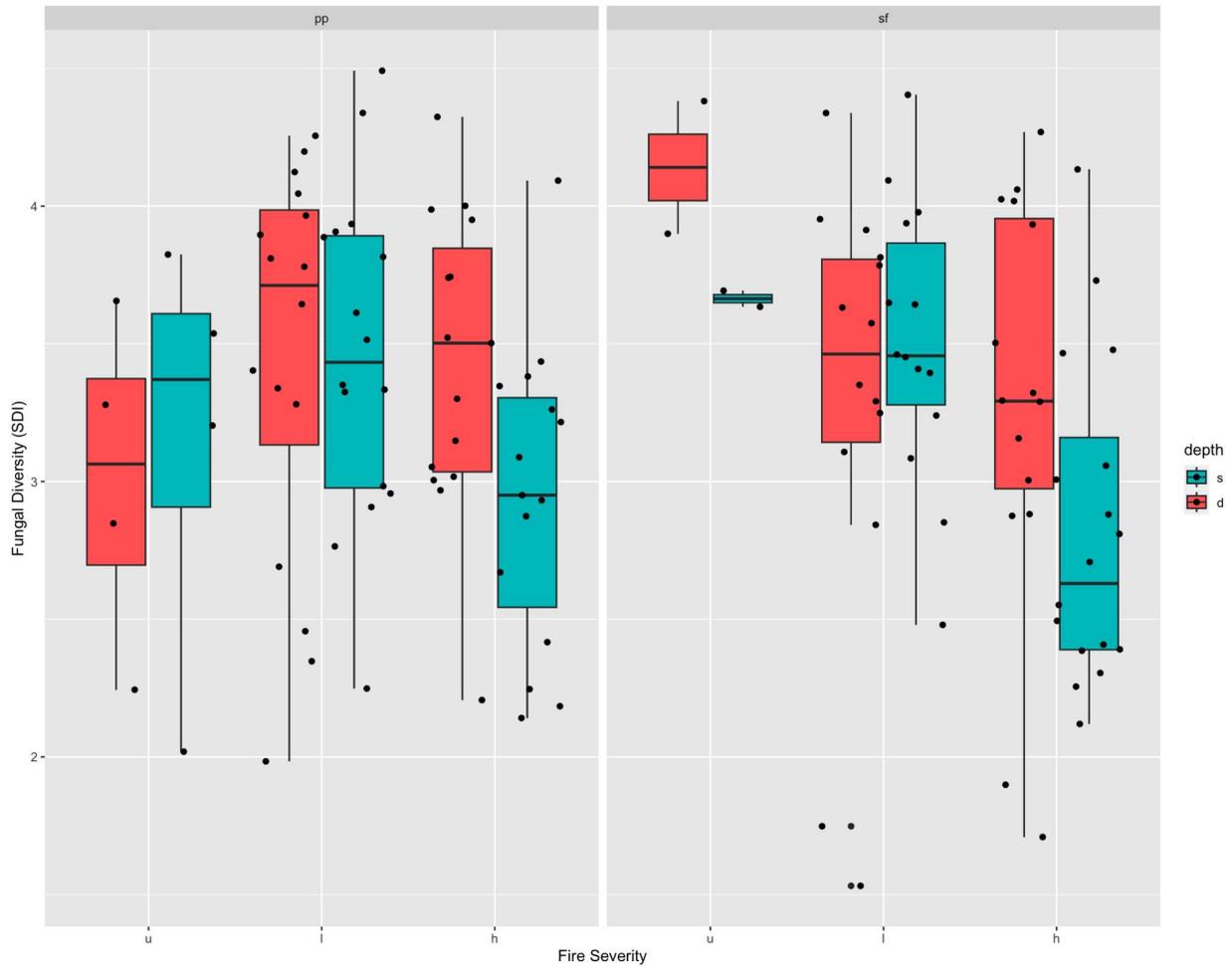


Figure 7: Shannon's Diversity Index (SDI) quantified for fungal alpha-diversity and represented as means stratified by fire severity and soil depth. Fungal diversity is greatest in low-severity burn plots ($p = 0.009$), particularly in depth (d) soils versus surface (s) soils (fire severity x soil depth $p = 0.013$). Unburned (u) control plots added to graph for fungal diversity comparison against low- (l) and high- (h) severity plots. SDI quantified and visualization created using vegan and ggplot2 in R, respectively.

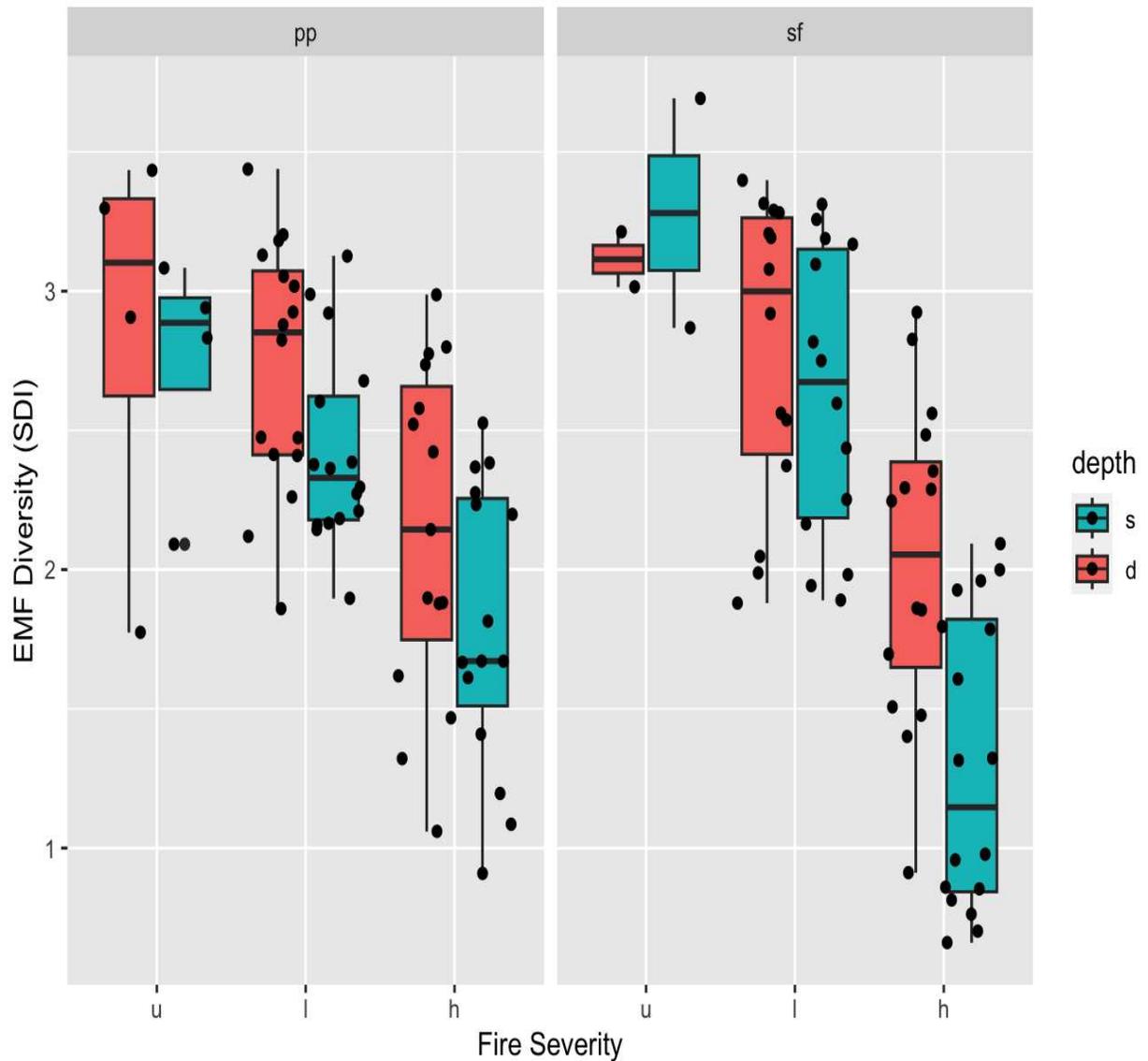


Figure 8: Alpha-diversity of ectomycorrhizal fungi (EMF) stratified by forest type, fire severity, and soil depth, quantified using Shannon's Diversity Index (SDI). Fire severity and soil depth are the most significant effects on EMF diversity (both $p < 0.001$), though the magnitude of diversity dissimilarity driven by fire severity varies between ponderosa pine and spruce-fir forests (forest type \times fire severity $p = 0.23$). SDI quantified and visualization created using `vegan` and `ggplot2` in R, respectively.

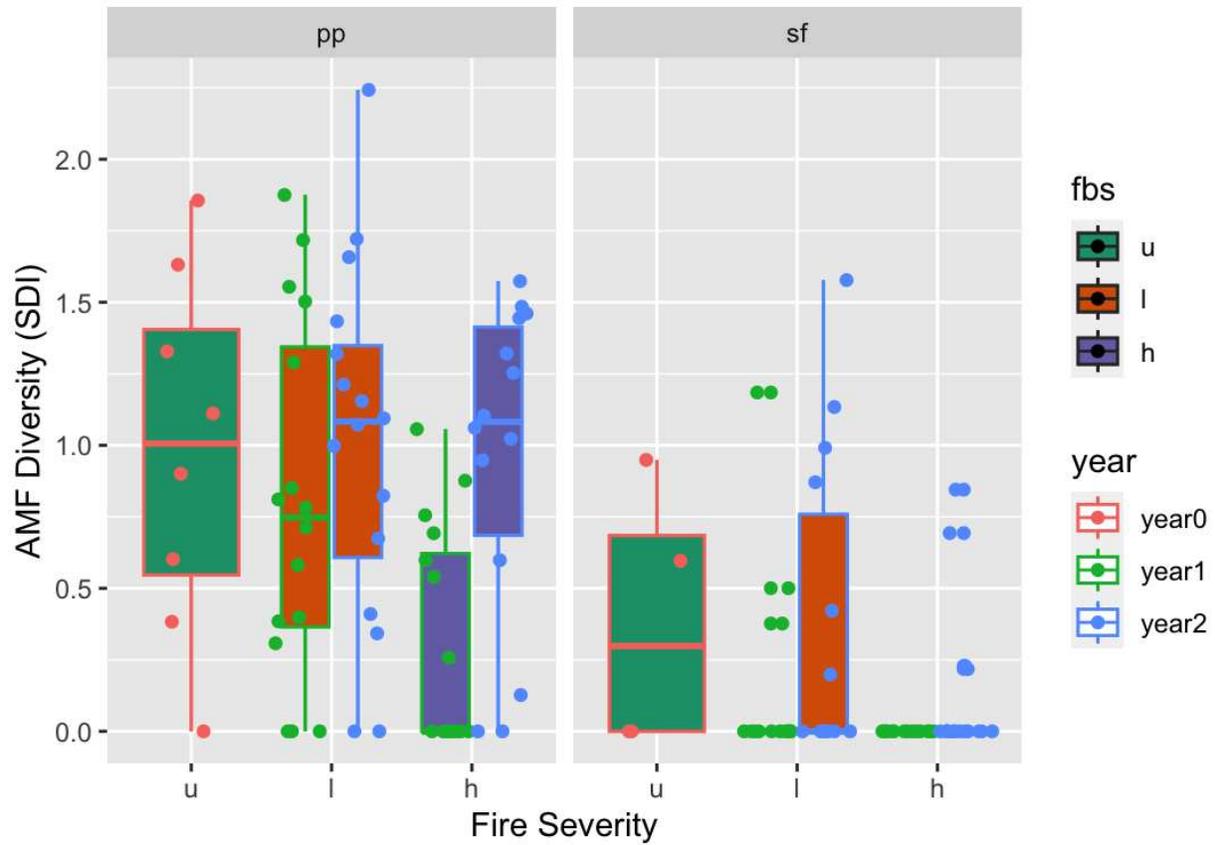


Figure 9: Shannon’s Diversity Index (SDI) quantified for arbuscular mycorrhizal fungi (AMF) as a function of fire severity, forest type, and year post-fire. Forest type and year post-fire are the most significant drivers of AMF alpha-diversity (both $p < 0.001$). Very few AMF OTUs were detected in spruce-fir surface soils collected in Year 1, and thus a boxplot distribution of the data is not possible. Unburned (u) control plot AMF diversity quantified as “Year 0” for comparison against low- (l) and high- (h) severity plot diversity. SDI quantified and visualization created using `vegan` and `ggplot2` in R, respectively.

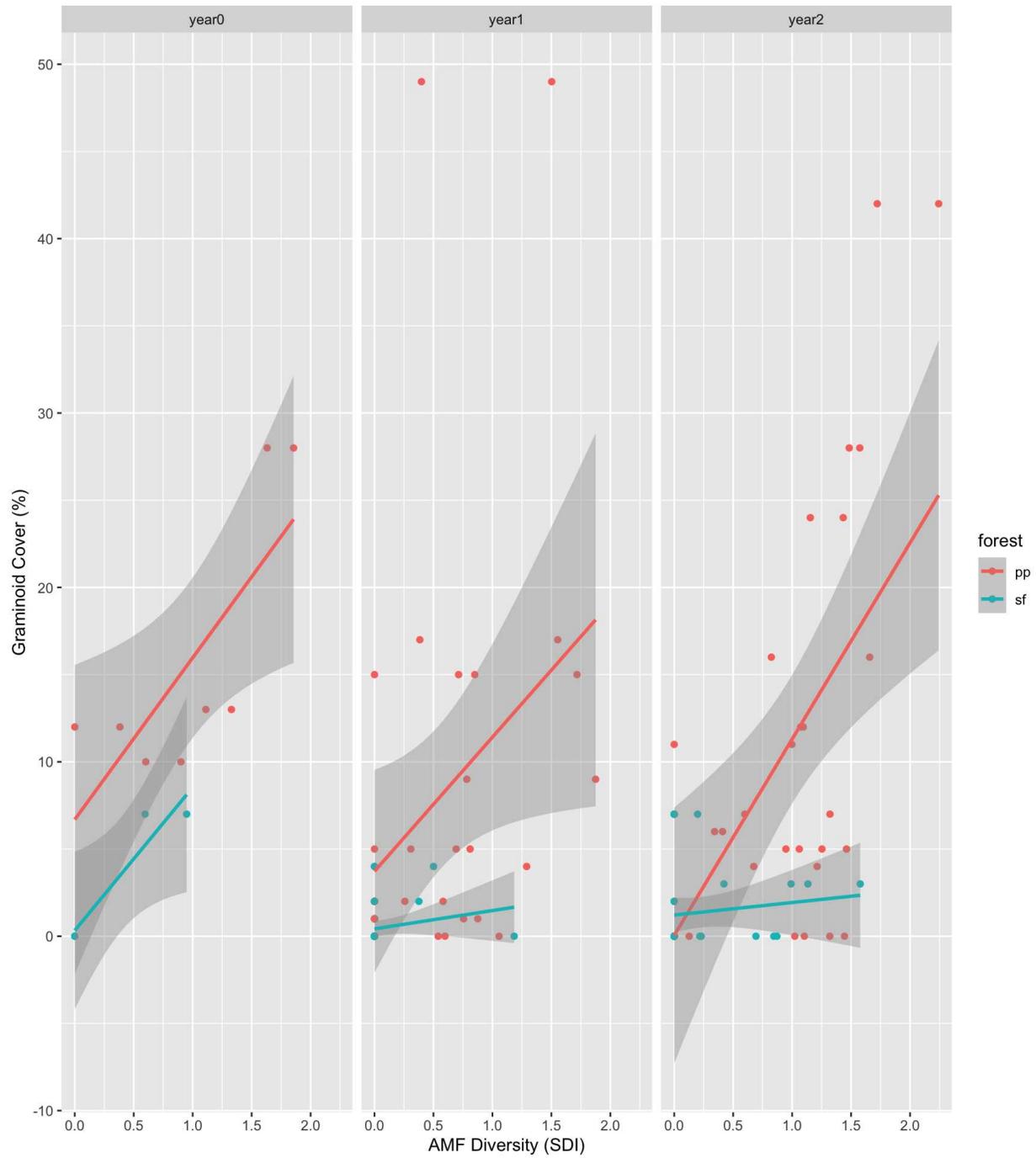


Figure 10: Mixed-effects linear regression model between alpha-diversity of soil arbuscular mycorrhizal fungi (AMF) and understory percent graminoid cover stratified by forest type and year post-fire. Alpha-diversity measured using Shannon’s Diversity Index (SDI). Graminoid cover in lower montane forests is generally greater than subalpine forests at similar levels of AMF SDI ($p = 0.005$). Graminoid cover increases more strongly with AMF diversity in Year 2 than Year 1 ($p = 0.033$). Unburned control diversity and cover expressed as “Year 0” to compare against burned treatment groups. SDI quantified and visualization created using `vegan` and `ggplot2` in R, respectively.

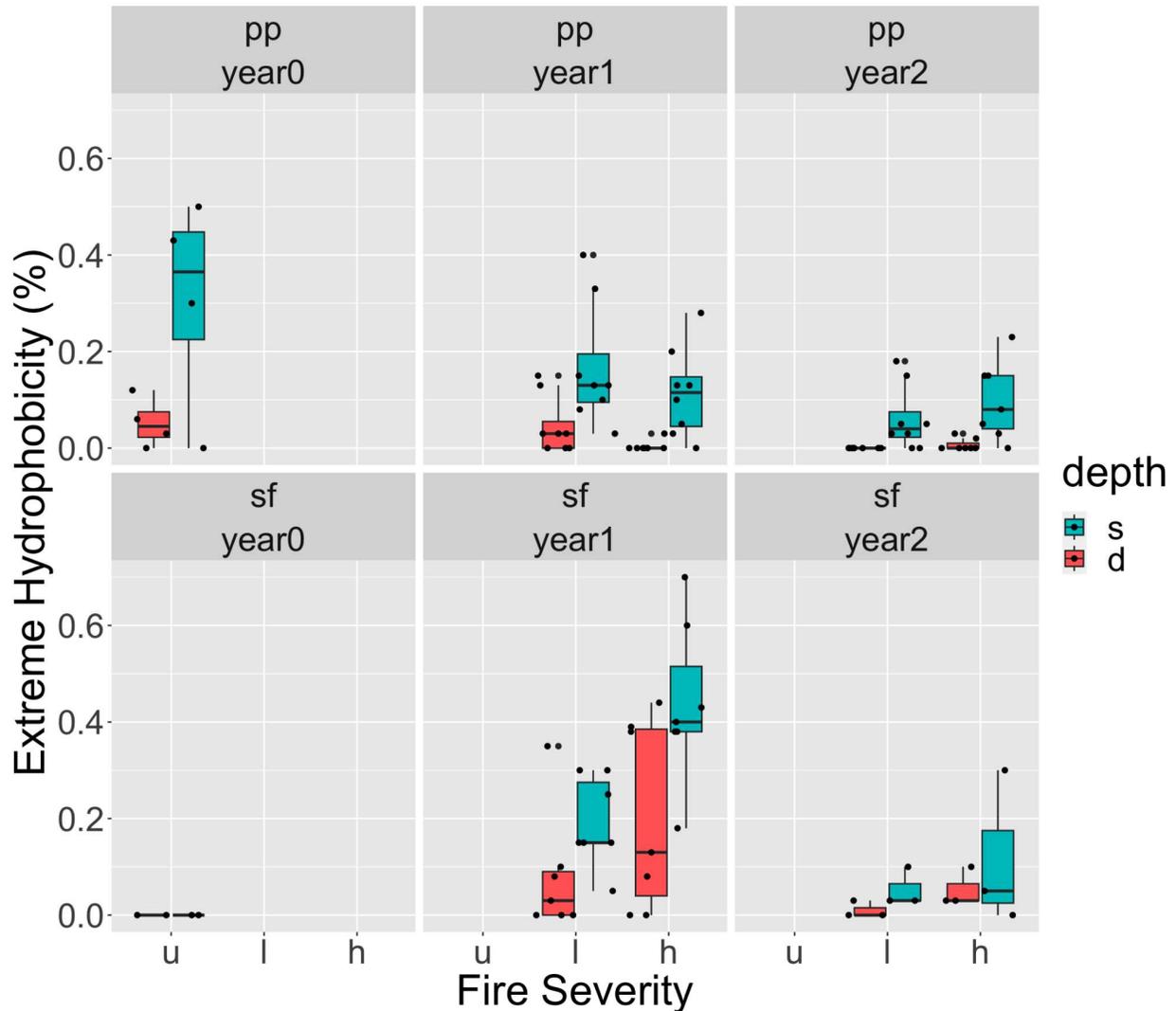


Figure 11: Observed presence of extreme soil hydrophobicity expressed in relation to fire-severity. Extreme hydrophobicity calculated as percentage of soil depths requiring 15+ seconds for complete water infiltration based on Water Droplet Penetration Time (WDPT) test. Analysis stratified by soil depth (surface soil at 0-5 cm and deep soil at 5-10 cm), forest type (top row lower-montane and bottom row subalpine), and year post-fire (columns). Unburned control observed hydrophobicity expressed as “Year 0” to compare against burned treatment groups, though unburned control plots were far fewer in quantity than burned plots. Visualized using ggplot2 in R (Wickham, 2016).

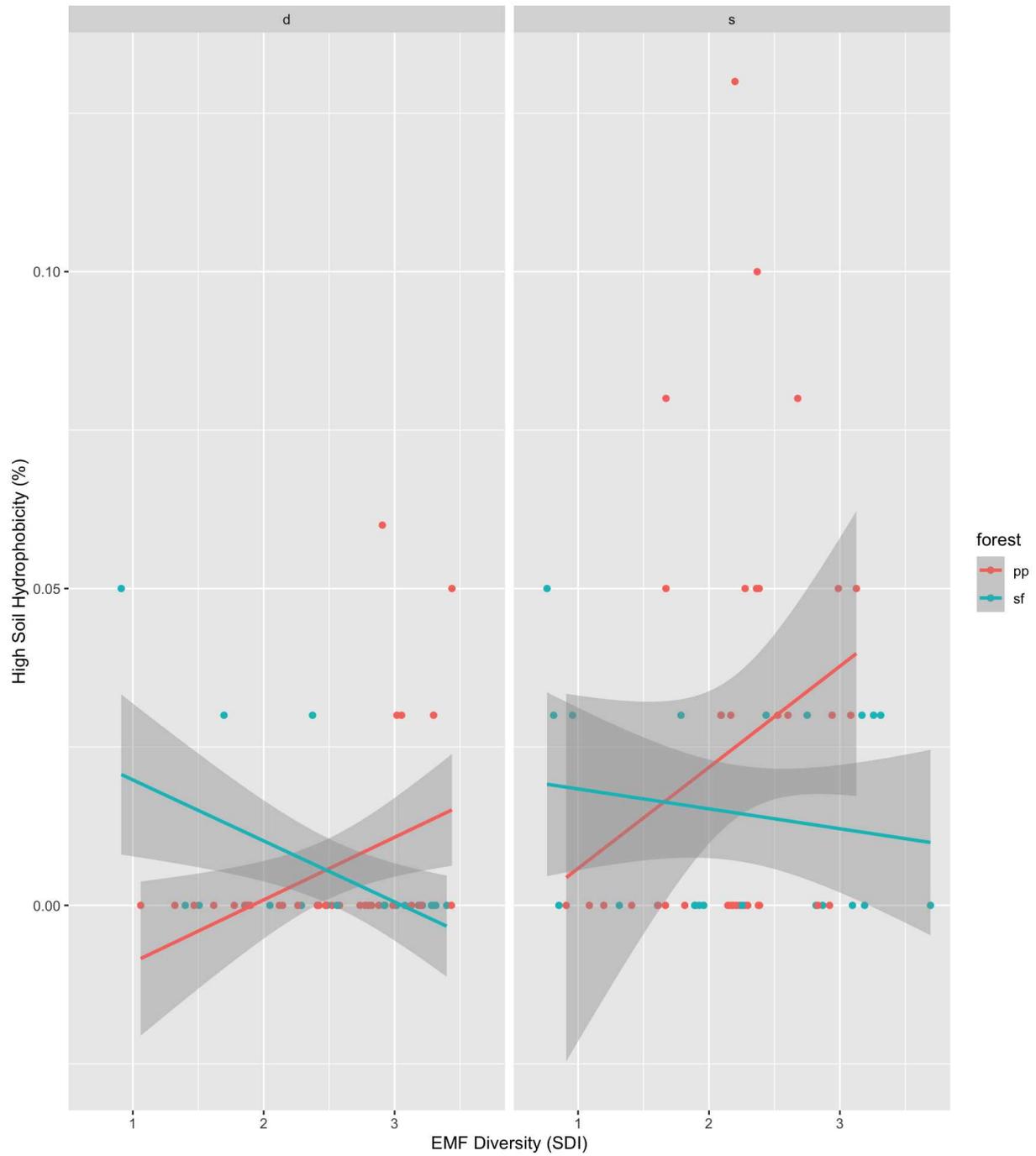


Figure 12: Mixed-effects linear regression model between ectomycorrhizal fungal (EMF) alpha-diversity and percent high soil hydrophobicity observed, stratified by forest type and soil depth. High hydrophobicity is positively influenced by surface (s) soils versus deep (d) soil, and correlations are inverted between lower montane (pp) and subalpine (spruce-fir) forests. Alpha-diversity measured using Shannon's Diversity Index (SDI). SDI quantified and visualization created using `vegan` and `ggplot2` in R, respectively.