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Fire-associated microbial shifts in soils of western conifer forests with Armillaria root disease

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ABSTRACT Fires in coniferous forests throughout the northern United States alter ecosystem processes and ecological communities, including the diversity and composition of microbial communities living in the soil. In addition to its influence on ecosystem processes and functions, the soil microbiome can interact with soilborne pathogens to facilitate or suppress plant disease development. Altering the microbiome composition to promote taxa that inhibit pathogenic activity has been suggested as a management strategy for forest diseases, including Armillaria root disease caused by *Armillaria solidipes*, which causes growth loss and mortality of conifers. These forest ecosystems are experiencing increased wildfire burn severity that could influence *A. solidipes* activity and interactions of the soil microbiome with Armillaria root disease. In this research, we examine changes to the soil microbiome following three levels of burn severity in a coniferous forest in northern Idaho, United States, where Armillaria root disease is prevalent. We further determine how these changes correspond to the soil microbiomes associated with the pathogen *A. solidipes,* and a putatively beneficial species, *A. altimontana*. At 15-months post-fire, we found significant differences in richness and diversity between bacterial communities associated with unburned and burned areas, yet no significant changes to these metrics were found in fungal communities following fire. However, both bacterial and fungal communities showed compositional changes associated with burn severity, including microbial taxa with altered relative abundance. Further, significant differences in the relative abundance of certain microbial taxa in communities associated with the three burn severity levels overlapped with taxa associated with various *Armillaria* spp. Following severe burn, we observed a decreased relative abundance of beneficial ectomycorrhizal fungi associated with the microbial communities of *A. altimontana,* which may contribute to the antagonistic activity of this soil microbial community. Additionally, *A. solidipes* and associated microbial taxa were found to dominate following high-severity burns, suggesting that severe fires provide suitable environmental conditions for these species. Overall, our results suggest that shifts in the soil microbiome and an associated increase in the activity of *A. solidipes* following high-severity burns in similar conifer forests may result in priority areas for monitoring and proactive management of Armillaria root disease.

IMPORTANCE With its influence on ecosystem processes and functions, the soil microbiome can interact with soilborne pathogens to facilitate or suppress plant disease development. These forest ecosystems are experiencing increased wildfire frequency and burn severity that could influence the fungal root pathogen, *Armillaria solidipes*, and interactions with the soil microbiome. We examined changes to the soil microbiome following three levels of burn severity, and examined how these changes correspond with *A. solidipes*, and a putatively beneficial species, *A. altimontana*. Following severe burn, there was a decreased relative abundance of ectomycorrhizal fungi associated *A. altimontana*. *A. solidipes* and associated microbial taxa dominated following high-severity burns, suggesting that severe fires provide suitable environmental conditions for

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these species. Our results suggest that shifts in the soil microbiome and an associated increase in the activity of *A. solidipes* following high-severity burns in conifer forests may result in priority areas for monitoring and proactive management of Armillaria root disease.

KEYWORDS root disease, fire, ectomycorrhizal fungi, *Armillaria solidipes*, *Armillaria altimontana*

Fires in forest ecosystems are important disturbance events that alter ecosystem processes and ecological communities, including the soil microbial community. Fire influences on soil microbial communities have broader implications for nutrient cycling, plant–microbe, and microbe–microbe interactions (1, 2). Fires can alter microbial communities through direct, heat-induced mortality or indirectly through changes to vegetation and soil properties, yet predicting fire impacts on a given community is difficult due to interactions among edaphic and fire characteristics that affect heat transfer to the soil (2). Commonly observed changes to microbial communities following fire include reduced biomass, diminished diversity, and changes in community composition due to selection for taxa adapted to withstand fire or effectively colonize post-disturbance conditions (3–8). Such changes may be attributable to common post-fire edaphic changes, including increased soil pH and decreased organic matter (1, 9). Further, extensive mortality of dominant overstory plants following severe fires can be especially detrimental to populations of microbial symbionts (2, 10).

Recent research has accentuated the importance of the soil microbiome in creating conditions that are either conducive or unfavorable to plant disease development, termed a pathobiome or a disease suppressive soil, respectively (11–13). Transitions from a healthy microbiome to a pathobiome with the onset of disease, including changes in the relative abundance of key microbial taxa, have been demonstrated in multiple agricultural settings (14, 15). Plants can also selectively secrete exudates in response to biotic or abiotic stressors to promote the abundance of microbial taxa in the rhizosphere that assist in resistance or recovery, thereby fostering the formation of a plant-associated disease suppressive soil (12, 16–18). Consequently, post-fire changes to microbial community composition can indirectly affect plant disease development by altering the diversity and abundance of taxa that play key roles in a pathobiome or disease suppressive soil.

Fungal pathogens that cause Armillaria root disease are responsible for mortality and growth reduction of woody plants in agronomic, horticultural, and natural settings worldwide (19–23). In the northwestern United States, the native species *Armillaria solidipes* (formerly North American *A. ostoyae*) acts as a virulent pathogen, causing a white rot of primarily conifer hosts that reduces structural integrity of the roots and lower bole, diminishes growth, and increases susceptibility to additional mortality agents (23, 24). The resulting tree mortality can alter forest structure and composition, with individual genets of *A. solidipes* capable of encompassing over 900 ha of land (25). Current control methods for Armillaria root disease, such as root collar excavation and soil fumigation, are economically and logistically impractical at this forest landscape scale (21, 26).

In contrast to *A. solidipes*, the native species *A. altimontana* [formerly North American biological species (NABS) X] is considered a secondary pathogen (27). Evidence from research conducted at the Priest River Experimental Forest in Idaho, United States, suggests that *A. altimontana* may be beneficial to the health of western white pine (*Pinus monticola*) on sites where it co-occurs with *A. solidipes* (28). A tree health assessment at 16-years post-planting showed that trees associated solely with *A. altimontana* had increased height, diameter at breast height (DBH), and survival rates compared with trees associated with *A. solidipes*. Trees associated with *A. altimontana* also had greater height and survival rates compared with trees associated with both *Armillaria* spp. and with neither *Armillaria* spp. (28). Further, the distribution of these two *Armillaria* spp.

across the field site indicated that *A. altimontana* was gradually outcompeting *A. solidipes* and thus reducing disease prevalence.

The benefits to tree health and competitive advantage of *A. altimontana* suggest a putative disease suppressive soil that could effectively control *A. solidipes*. When compared to the *A. solidipes* microbial communities, the soil microbial community of *A. altimontana* showed a higher relative abundance of three families of ectomycorrhizal (ECM) fungi (29). ECM fungi form symbiotic relationships with tree hosts and assist with the uptake and transport of key nutrients and water from the soil (30), possibly contributing to the increased survival rates and tree growth associated with *A. altimontana* in Warwell et al. (28). In contrast, a higher relative abundance of the bacterial family Enterobacteriaceae was found in the microbial community of *A. solidipes* when compared with an *A. altimontana*-associated community, suggesting a potential role for Enterobacteriaceae in the *A. solidipes* pathobiome (29). In coniferous forests where *A. solidipes* is prevalent, practices to shift the composition of the soil microbial community toward taxa from a putative disease suppressive community could be part of an effective strategy for landscape scale management of Armillaria root disease.

While previous research has demonstrated how soil microbial communities change in response to fire (e.g., 7, 10) and examined the potential importance of microbial communities influencing the activity of *A. solidipes* (29), previous studies have not yet examined how burn severity interacts with the soil microbiome to affect Armillaria root disease. Impacts from these interacting influences are increasingly important, as climate change and anthropogenic activity continue to raise fire severity and frequency in the regions of North America where *A. solidipes* is prevalent (31). The objective of this study was to determine how different levels of burn severity influence soil microbial communities in coniferous forest ecosystems where *A. solidipes* and *A. altimontana* co-occur, particularly how burn severity affects taxa that compose putative pathobiomes and disease suppressive soils for Armillaria root disease. To investigate these interactions, we examined differences in composition and diversity of bacterial and fungal communities in soils associated with three different burn-severity levels (high, low, and unburned) at 15-months post-fire in a coniferous forest in northern Idaho, United States, where Armillaria root disease caused by *A. solidipes* naturally occurs. The results presented here can foster the development of novel management strategies for Armillaria root disease by determining how burn severity influences disease development through its impact on the soil microbiome.

MATERIALS AND METHODS

Sample collection

In July 2021, portions of a mature conifer forest within the Nez Perce-Clearwater National Forest in northern Idaho, United States, experienced a wildfire (the Sand Mountain Fire), which was caused by lightning and burned approximately 650 ha (1,600 acres), creating areas of tree mortality. During the fire, forest managers attempted to manage the spread of the fire with controlled burns, producing areas of low-severity burn in which the trees had experienced fire but remained alive. Unburned areas were also interspersed within the burn areas, resulting in a study site with three levels of burn severity: high, low, and unburned (Fig. 1; Fig. S1). Further, Armillaria root disease caused by *A. solidipes* is known to occur on the site (G.I. McDonald and J.W. Hanna, unpublished observations). Before the fire, the study area consisted of predominantly mature, high density, and relatively even-aged forest, with canopy cover generally 70%–100% and most diameters at breast height ranging between 38 and 51 cm. Dominant species were grand fir (*Abies grandis*) and Douglas-fir (DF; *Pseudotsuga menziesii*), with lesser amounts of western hemlock (*Tsuga heterophylla*) and western redcedar (*Thuja plicata*) on the lower slopes and riparian draws. Western larch (*Larix occidentalis*), lodgepole pine (*Pinus contorta*), western white pine (WWP), and ponderosa pine (*Pinus ponderosa*) were infrequently scattered throughout. Stands originated approximately 120–180 years ago,

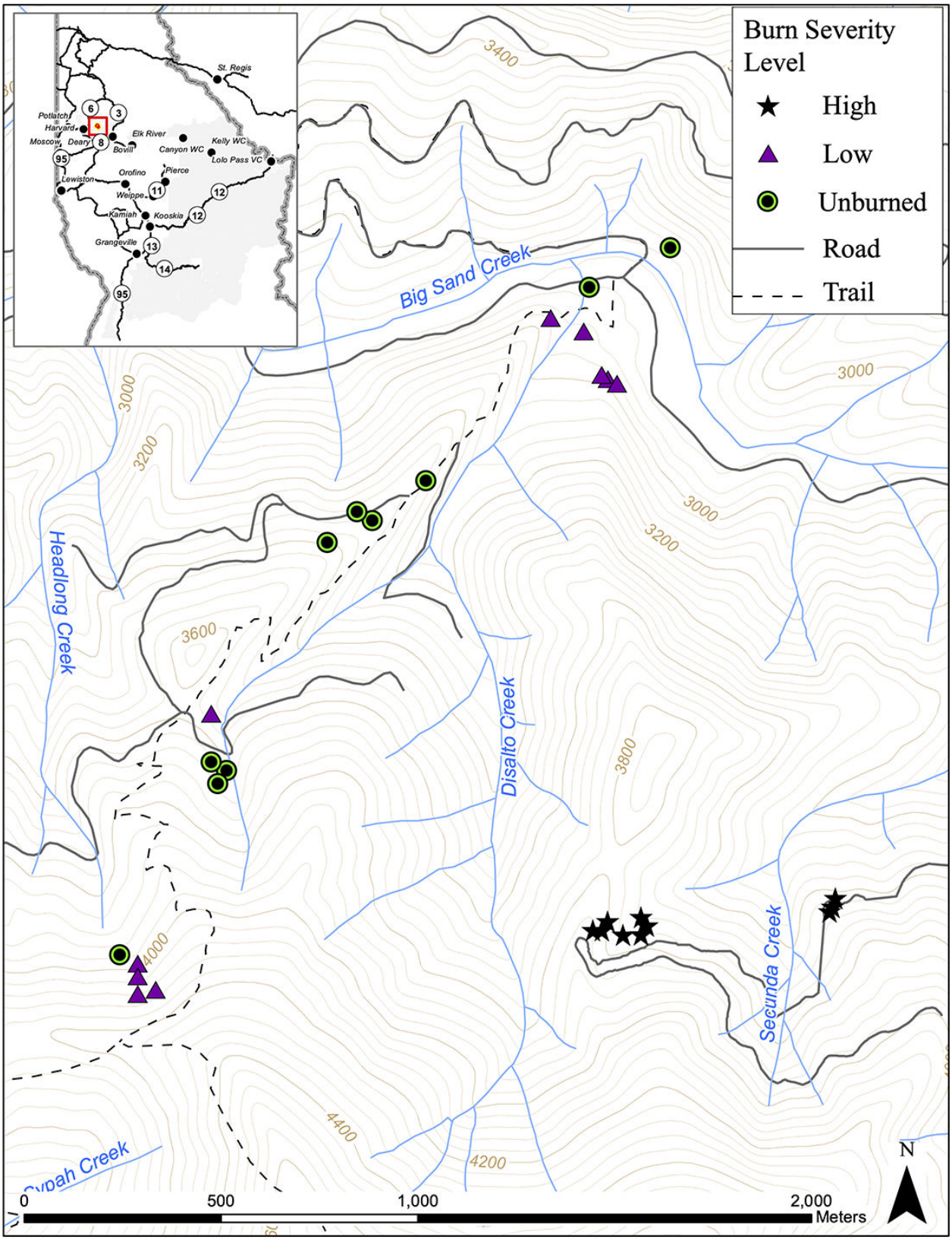

FIG 1 Site map of sampled trees in mature conifer forest within the Nez Perce-Clearwater National Forest (near Laird Park Campground) in northern Idaho, United States that experienced a fire (Sand Mountain Fire). Burn severity level associated with each tree is indicated by color and shape. The map was created with ArcMap, using USFS corporate GIS layers for the roads, trail, creeks, towns, and topography.

though grand fir and DF trees 80–100 years old were most widespread at the time of the fire. There are no records of vegetation management or fire disturbance within our study area for at least 100 years. The landscape has historically experienced low frequency, high-severity fires causing stand replacement.

Within each of the three burn severity levels, five mature WWP (DBH 40 to 98 cm) and five mature DF (DBH 41 to 68 cm) trees were identified for sampling, resulting in 30 sampled trees (15 WWP and 15 DF). Sampled trees were spaced at least 20 m apart. In October 2022, two soil cores were collected near the base of each tree, for a total of 60 soil cores, 2 m from the bole and in opposite cardinal directions. The top layers (1–5 cm), composed of forest floor litter, were removed, resulting in cores with a depth of 5–20 cm. The two soil cores from each tree were homogenized and 2 g from the homogenized soil were placed in 4 mL of LifeGuard Preservation Solution (MO BIO Laboratories, Inc., Carlsbad, CA, USA). The remaining soil samples and tubes containing soil in LifeGuard solution were stored at −20°C until later use.

Surveys for *Armillaria* spp. were performed by excavating main lateral roots (ca. 0.5 m deep and 1 m away from the bole) of each sampled tree to collect rhizomorph samples, and the outer bark of the roots and lower bole were removed to collect mycelial fan samples. Rhizomorph samples were placed in 15 mL Falcon tubes, while mycelial fans were stored in paper bags. All *Armillaria* samples were stored in a refrigerator at 4°C for 2–4 days until isolation. Surveyed trees were re-sampled in June 2023 for *Armillaria* rhizomorphs and mycelial fans.

Armillaria isolation and identification

In a laminar flow hood, *Armillaria* mycelial fan samples were separated from the bark using a sterile blade, and a small section of fungal tissue was excised from each fan. These samples were plated in a Petri dish on modified malt extract agar media for *Armillaria* cultures (Armillaria MEA: 3% malt extract, 3% dextrose, 1.5% peptone, 1.5% agar).

For surface disinfestation, rhizomorph samples were first soaked in a 20% commercial bleach solution (1.5% NaClO) for 8 min, then rinsed with sterile, distilled water, and the samples were transferred to 3% H_2O_2 for 7–10 min. Samples were moved to the laminar flow hood and soaked in sterile distilled water for 2–10 min. Treated rhizomorphs were then cut into sections approximately 1 cm long and embedded vertically into Armillaria MEA in Petri dishes. All *Armillaria* sample plates were incubated in the dark at 20°C.

To obtain mycelia for DNA extractions, *Armillaria* isolates were subcultured onto sterile, 0.22-µm pore MF-Millipore Membrane nylon flters (MilliporeSigma, Burlington, MA, USA) overlayed on modified Armillaria MEA (1.5% malt extract, 1.5% dextrose, 0.5% peptone, 1.2% agar) in Petri dishes. SYNERGY 2.0 Plant DNA Extraction Kits (OPS Diagnostics, Lebanon, NJ) and manufacture protocols were used to extract DNA from the *Armillaria* mycelia. Extracted DNA was quantified with a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and diluted with sterile deionized (DI) H_2O to a concentration of 10 ng/µL. Polymerase chain reaction (PCR) with primers EF983F (5′- GCYCCYGGHCAYCGTGAYTTYAT - 3′) (32) and ARMEF-R (5′-TACCCGTTCGGC GATCAATCT-3′) (33) or 2218R (5′- ATCATGACACCRACRGCRACRGTYTG-3′) (32) and an Eppendorf Mastercycler Pro Thermal Cycler (Eppendorf, Hamburg, Germany) were used to amplify the translation elongation factor 1-alpha (*tef1*) region of the DNA. This region was used due to its utility in informing the identification of closely related *Armillaria* spp. (34). The PCR cycle was 1 min at 94°C, 40 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 45 s, before a final 10 min at 72°C. Amplicons were visualized using gel electrophoresis, cleaned with ExoSAP-IT PCR Product Cleanup Reagent (Thermo Fisher Scientific, Santa Clara, CA, USA), and sent to Eurofins Genomics (Louisville, KY, USA) for sequencing using either the previously mentioned *tef1* primers or four *Armillaria*-specific sequencing primers (ARMEF-F3A, ARMEF-R2, ARMEF-FI2, and ARMEF-RI2) (34). The resulting sequences were used with the BLASTn search of the NCBI (National Center for

Biotechnology Information) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) database to identify the *Armillaria* spp.

Soil DNA extraction and sequencing

For soil DNA extraction, one tube containing soil and LifeGuard Preservation Solution was thawed at room temperature and centrifuged for 5 min at 12,300×*g* to consolidate soil materials. The supernatant was discarded, and approximately 250 mg of saturated soil was placed in a sterile tube. DNA extractions were performed using Qiagen DNeasy Power Soil Pro kits (Qiagen, Carlsbad, CA, USA) following manufacture protocols. The quantity and quality of the extracted DNA were assessed with a Nanodrop 1000 spectrophotometer, and approximately 50 µL of each sample was sent to Novogene Corp. Inc (Sacramento, CA) for metabarcoding. Amplicon metabarcoding was performed using 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) (35) and 907R (5′-CCGTCAATTCCTTTGAGTTT-3′) (36) primers to amplify the 16S V4–V5 region of bacteria, and ITS3 (5′-GCATCGATGAAGAACGCAGC-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (37) primers to amplify the ITS2 rDNA region of fungi. These data are available from the NCBI database (BioProject ID PRJNA1098642), which includes both the bacterial and fungal sequencing results.

Characterization of the soil microbiome

The program FastQC (38) was used to remove adapters and low-quality reads from the soil metabarcoding sequences. All remaining analyses were performed through RStudio (version 1.1.456; RStudio Team, 2016). The dada2 package (39) was used to construct an amplicon sequence variant (ASV) table of bacterial and fungal ASVs. Identification of the 16S and ITS2 ASVs was performed by matching to the ITGDB (40) and UNITE (41) databases, respectively. Sequencing of the ITS2 region of sample rDNA included ASVs from other eukaryotic organisms within the sample; these non-fungal ASVs were removed from further analyses.

The phyloseq package (42) was used to calculate microbial community richness and alpha diversity, as measured by the Shannon's diversity index (*H′*) and the inverse Simpson's index (1*/D*). Both indices account for the richness, number of unique ASVs, and evenness, how evenly abundant these ASVs are within the sample. However, Shannon's diversity index gives more weight to increased sample richness, while inverse Simpson's index prioritizes evenness (43). Using the car package (44), type III analysis of variance (ANOVA) analyses were used to determine the influence of burn severity (high, low, and unburned), host tree species (DF and WWP), and association with *Armillaria* spp. (*A. solidipes*, *A. altimontana, A. solidipes* and *A. altimontana*, *A. sinapina,* and none) on microbial community richness and alpha diversity. ANOVA assumptions were first assessed through diagnostic plots and Shapiro–Wilk (45) and Levene (46) tests, and appropriate data transformations were applied as needed. Additional pairwise comparisons were performed with Tukey adjusted *P*-values in the emmeans package (version 1.8.4–1; 47) if there was a significant effect ($P < 0.05$) of burn severity, host species, or *Armillaria* spp. association.

Compositional differences between communities grouped by burn severity, host species, burn severity and host species (BurnSpecies), and *Armillaria* spp. (*A. solidipes*, *A. altimontana, A. solidipes* and *A. altimontana*, *A. sinapina,* and none) association were visualized through three-dimensional non-metric multidimensional scaling (NMDS) plots created using the vegan package (version 2.6–4; 48) and two-dimensional NMDS and principal coordinate analysis (PCoA) plots created with the phyloseq package. A permutational multivariate ANOVA (PERMANOVA) was used to assess statistical differences among communities using the vegan package. Permutests were also used to check for homogeneity of variance among communities. Pairwise comparisons using PAST software (version 4.14; 49) were performed on communities with statistically significant differences in composition and Bonferroni-adjusted *P*-values were calculated.

The ggplot2 package (50) was used to create pie charts representing bacterial genera present at ≥2.5% relative abundance and fungal genera present at ≥5% relative

abundance in BurnSpecies communities. Bacterial and fungal ASVs were consolidated by genus; those present in significantly different relative abundance (adjusted $P < 0.05$) among burn severity and *Armillaria* spp. (*A. solidipes*, *A. altimontana,* and none) association communities were identified using the metagenomeSeq package (51). Taxa present in only one sample were removed to obtain a more accurate representation of genera responsible for differences. Relative abundance data were used to generate Venn diagrams depicting genera that proliferated in communities associated with each burn-severity level or *Armillaria* spp. when compared with the others.

Bacterial and fungal isolation from soils

Within 8 weeks of collection, the homogenized soil samples were thawed, and serial dilutions were performed. From each sample, 2 g of soil was added to 5 mL of sterilized, deionized water (DI H_2O), inverted five times to mix, and left to settle for 30 s. From the resulting supernatant, 200 µL was added to 1.8 mL of DI H_2O, which was again inverted and left to settle. This process was repeated three more times, for dilutions of 1:10, 1:100, 1:1,000, and 1:10,000. For each dilution, 150 µL was placed on culture medium in a Petri dish and spread with a sterilized glass probe. Four plates of each dilution were made: two with nutrient agar (2.3% nutrient agar) and two with quarter-strength potato dextrose agar (PDA) with antibiotics (0.975% potato dextrose agar, 1.125% agar, 0.01% streptomycin, 0.005% chloramphenicol), for a total of 16 plates per soil sample. The plates were placed in a dark incubator at 23°C and monitored. As microbial growth appeared, unique bacterial and fungal colonies were visually identified and transferred to new plates. These microbial isolates were incubated to allow growth in pure culture before subsequent DNA extraction.

A modified colony PCR procedure was used to extract DNA from bacterial cultures. A small sample of the bacterial colony was mixed with 40 µL of molecular grade H_2O followed by heating at 98°C for 15 min to lyse the cells. Tubes were vortexed and then briefly centrifuged. Amplification of the 16S V4–V5 region of bacterial DNA was performed with the Eppendorf Mastercycler Pro Thermal Cycler with primers fD1 (5′-AGAGTTTGATCCTGGCTCAG-3′) and rP2 (5′-ACGGCTACCTTGTTACGACTT-3′) (52). The PCR cycle was 95°C for 10 min, 35 cycles of 95°C for 2 min, 54°C for 1 min, and 72°C for 2 min, before a final 10 min at 72°C.

To extract DNA from fungal cultures, a pipette tip was used to scrape a small amount of mycelia from the edge of the colony, which was added to a tube containing 100 µL of 5% Chelex 100 Resin solution (Bio-Rad Laboratories, Hercules, CA, USA). Tubes were heated at 98°C for 20 min using an Eppendorf Mastercycler Pro Thermal Cycler, then briefly centrifuged. The resulting supernatant was used as the DNA template for PCR. The ITS region of fungal DNA was amplified with primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (37). The PCR cycle was 95°C for 2 min, 35 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min, before a final 2 min at 72°C.

Fungal cultures for which initial DNA extraction failed were subjected to a second round of extractions using a modified NaOH procedure (53). Again, a pipette tip was used to add a small amount of mycelia from each culture to a tube containing 300 µL 0.5 M NaOH and five glass beads, then vortexed for 1 min and centrifuged for 1 min at 15,000×*g*. From the resulting supernatant, 5 µL of DNA was added to a new tube with 495 µL 100 uM Tris-HCl pH 8.0. Primers and PCR settings from the Chelex procedure were used to amplify the ITS region.

Cultures that failed extractions three times were removed from further analyses. Successful amplicons were visualized using gel electrophoresis, cleaned with ExoSAP-IT PCR Product Cleanup Reagent (Thermo Fisher Scientific), and sent to Eurofins Genomics (Louisville, KY) for Sanger sequencing in the forward direction. These sequences were identified using the BLASTn search function of the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). For comparison with metabarcoding data, isolates that could not be confidently identified to the genus level were removed from analysis.

Influence of soil characteristics

Approximately 250 g of each soil sample was sent to Ward Laboratories, Inc. (Brighton, MI, USA) for analysis of soil chemical characteristics. Pearson's correlation analysis was used to determine which soil characteristics were highly correlated. One of two characteristics with a correlation coefficient above 0.80 or below −0.80 were removed to improve the strength of the model (Table S1a through j). Significant predictor variables from the filtered list were selected through stepwise analysis using Akaike Information Criterion (AIC). Type III ANOVA analyses were performed using the car package to evaluate the effect of soil characteristics on microbial richness and alpha diversity. Diagnostic plots and Shapiro–Wilk tests were used with each model to confirm ANOVA assumptions had been met, and data transformations were applied as necessary.

Type III ANOVA analyses were also used to determine the effect of burn severity on each soil characteristic. If linear models failed to meet the ANOVA assumptions, as evaluated by diagnostic plots and Shapiro–Wilk and Levene tests, the necessary data transformations were applied, or Kruskal–Wallis tests (54) were performed as a non-parametric alternative. If a significant effect ($P < 0.05$) of burn severity was found, additional pairwise comparisons were performed using the emmeans package, and Tukey-adjusted *P*-values were calculated.

RESULTS

Armillaria collection and identification

A total of 18 rhizomorph and 11 mycelial fan samples were collected in October 2022 and June 2023 (Table 1). Pure cultures were obtained from most samples; however, four rhizomorph and three mycelial fan samples did not yield pure cultures due to contamination and/or absence of viable fungal tissue. Sequencing of the *tef1* region identified seven of the eight cultures obtained from mycelial fan samples as *A. solidipes* and one as *A. altimontana*. Of the 14 cultures obtained from rhizomorph samples, 10 were identified as *A. altimontana,* two *A. sinapina,* and two *A. solidipes*. Three rhizomorph isolates from June 2023 were duplicates of rhizomorph isolates collected in October 2022 from the same tree. In addition, both mycelial fan and rhizomorph samples were collected from four trees, including DF and WWP hosts from high-severity burn sites. On two of these trees, samples were identified as the same *Armillaria* spp.; however, on the other two trees, the rhizomorph samples were identified as *A. altimontana,* and the mycelial fan

TABLE 1 Identification of *Armillaria* isolates associated with sampled trees[c]

Tree number	Tree species[a]: burn severity	Sample type: rhizomorph (R), mycelial fan (MF)	*Armillaria* sp.[b] identification (*tef1*)
3	DF:High	MF	SOL
4	DF:High	R, MF	ALT (R), SOL (MF)
5	WWP:High	R, MF	SOL
6	WWP:High	MF	SOL
7	WWP:High	R, MF	ALT (R), SOL (MF)
8	DF:High	R, MF	ALT
10	DF:High	MF	SOL
14	DF:Unburned	R	ALT
15	DF:Unburned	R	SOL
16	WWP:Unburned	R	SIN
20	WWP:Unburned	R	SIN
24	DF:Low	R	ALT
26	WWP:Unburned	R	ALT
27	DF:Unburned	MF	SOL
29	DF:Low	R	ALT

[a]DF = Douglas-fir (*Pseudotsuga menziesii*), WWP = western white pine (*Pinus monticola*).
[b]SOL = *A. solidipes*, ALT = *A. altimontana*, SIN = *A. sinapina*.
[c]Isolate information, including GenBank accession numbers, can be found in Table S8.

samples were identified as *A. solidipes*. Six of the seven trees with *Armillaria* samples from high-severity burn sites were colonized by *A. solidipes* mycelial fans, which may indicate a preexisting Armillaria root disease pocket in the area and/or post-fire saprophytic colonization. In contrast, only two of the eight trees with *Armillaria* samples from low-severity burn or unburned sites were colonized by *A. solidipes*. GenBank accession numbers for the *Armillaria* isolate sequences can be found in Table S8.

Soil metabarcoding

All 30 soil DNA samples were used for sequencing of the ITS2 region; however, only 29 were used for the 16S region because of low DNA quality. A total of 3,870,864 reads were generated from sequencing of the 16S region, with an average of 133,478 reads per sample and a range of 64,209–148,974 reads. In total, 3,694,647 reads were produced from ITS2 sequencing, with an average of 123,155 reads per sample and a range of 17,961–148,725 reads. The sequencing depth of each sample was confirmed with rarefaction curves (Fig. S2). After filtering out low-quality reads, merging paired ends, and removing identical reads and chimeras, a total of 2,927,776 16S reads remained, averaging 100,958 reads per sample and ranging from 41,478 to 121,438 reads. Processing resulted in a total of 2,678,572 reads of the ITS2 region, with an average of 89,286 reads per sample and a range of 13,550–110,994 reads. Matching to the ITGDB and UNITE databases identified 20,692 unique ASVs from the sequencing of the 16S region and 4,679 unique ASVs from sequencing of the ITS2 region. After ASVs from non-fungal organisms were removed from the ITS2 results, 4,041 unique ASVs remained.

Characterization of the soil microbiome

Alpha diversity

Significant differences in alpha diversity of bacterial communities, as measured by richness and Shannon's diversity index, were identified based on burn severity ($F_{2,23}$ = 31.84, $P < 0.0001$ and $F_{2,23}$ = 9.33, $P = 0.001079$, respectively). Pairwise comparisons showed that differences in richness occurred between high-severity burn and unburned DF communities (t (23) = −3.970, $P = 0.0017$), high-severity burn and low-severity burn WWP communities (t (23) = −3.259, $P = 0.0093$), high-severity burn and unburned WWP communities (t (23) = −7.310, $P < 0.0001$), and low-severity burn and unburned WWP communities (t (23) = −3.633, $P = 0.0038$) (Fig. 2A). Pairwise comparisons of Shannon's diversity showed significant differences between high-severity burn and unburned WWP communities (t (23) = −3.159, $P = 0.0117$) and low-severity burn and unburned WWP communities (t (23) = −2.740, $P = 0.0302$) (Fig. 2C). Both bacterial community richness and Shannon's diversity increased progressively as burn severity decreased. No statistically significant differences in bacterial richness or alpha diversity, as measured by Shannon's diversity index or inverse Simpson's index, were identified based on host tree species, and no significant differences were identified in the bacterial community inverse Simpson's index alpha diversity based on burn severity.

No significant differences were found in the richness or alpha diversity of fungal communities based on burn severity or host tree species (Fig. 2B and D), and no significant differences in richness or alpha diversity of fungal or bacterial communities occurred based on the association with different *Armillaria* spp.

Beta diversity

PERMANOVA tests revealed significant differences among bacterial and fungal community composition based on burn severity and BurnSpecies (Table 2). However, fungal BurnSpecies communities failed to pass the permutest, indicating that significant PERMANOVA results may be from unequal dispersion within the groups, rather than true compositional differences. Bonferroni-adjusted *P*-values generated from pairwise comparisons indicated significant differences between high-severity burn and unburned groups and between low-severity burn and unburned groups for bacterial and fungal

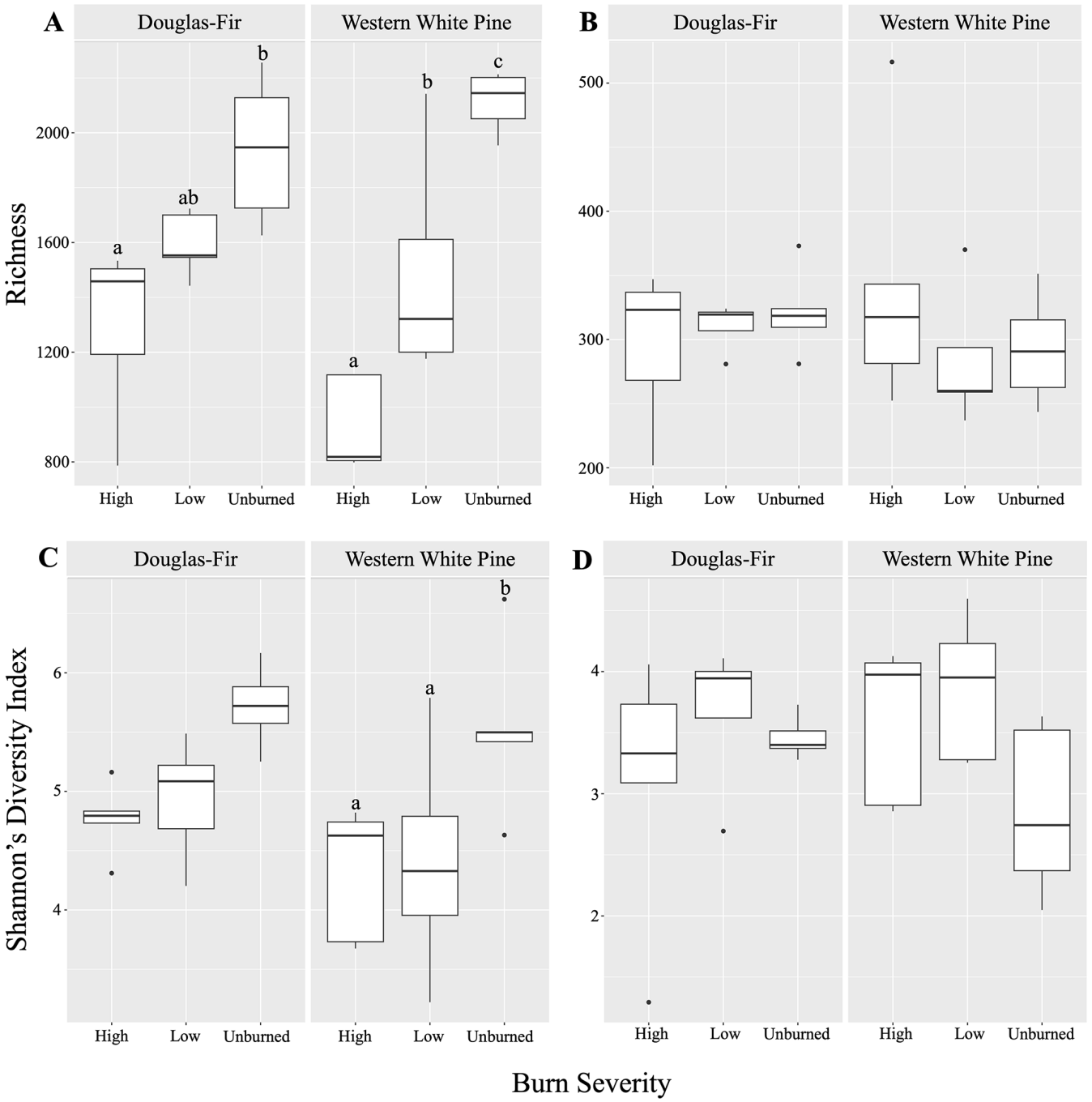

FIG 2 Alpha diversity, as measured by richness and Shannon's diversity index, of bacterial (A, C) and fungal (B, D) communities separated by burn-severity level (high, low, and unburned) and host tree species [Douglas fir (*Pseudotsuga menziesii*) and western white pine (*Pinus monticola*)].

communities (Table S1b). Further, visual inspection of the NMDS plots suggests an increased variance in community composition associated with high-severity burns (Fig. 3 and 4), which was also supported by the PCoA plots (Fig. S3). Pairwise comparisons with Bonferroni adjustment found no significant differences between bacterial BurnSpecies communities. Due to permutest failure, pairwise comparisons were not run on fungal BurnSpecies communities. However, the NMDS plots show overlap among communities, especially low-severity burn and unburned communities (Fig. 4C and D). No significant effects were found for host tree species or *Armillaria* spp. association on microbial community composition (Fig. S4).

TABLE 2 Results from PERMANOVA tests indicating significant differences between bacterial and fungal community composition when grouped by burn severity[a] and BurnSpecies[b]

Community	Group	Df	F-value	*P*-value
Bacteria	Burn severity	2	2.3074	<0.001
	BurnSpecies	5	1.4332	0.003
Fungi	Burn severity	2	2.1756	<0.001
	BurnSpecies	5	1.5603	0.002

[a]Burn severity = high-severity burn, low-severity burn, and unburned.
[b]BurnSpecies = burn severity and host species [Douglas-fir (*Pseudotsuga menziesii*) and western white pine (*Pinus monticola*).

Taxonomic trends

In total, 11 genera were found to be present in at least 1% relative abundance in bacterial BurnSpecies communities (Fig. S5). *Pseudomonas* followed by *Pseudoarthrobacter* and *Yersinia* were found in high proportions across all communities. The genus *Ewingella* was also present at a high relative abundance in unburned WWP and DF and low-severity burn WWP communities.

A total of 14 genera were present in at least 5% relative abundance in fungal BurnSpecies communities (Fig. S6). Apart from low-severity WWP communities, each had a high relative abundance of *Ilyonectria.* Both high- and low-severity burn communities had a higher relative abundance of *Penicillium* than unburned communities.

Because host tree species did not have a significant effect on microbial community alpha or beta diversity, communities from DF and WWP hosts were combined by either burn severity or *Armillaria* spp. association for the remaining analyses. For these analyses, we use the terms "proliferated" or "abundant" to indicate taxa that had significantly greater relative abundance in the specified microbial community, when compared to others (for example, high-severity burn communities when compared to low-severity burn communities or in *A. solidipes* communities when compared to *A. altimontana* communities). Further, we add the term "consistently" to indicate taxa that were more abundant in the specified microbial community when compared to both opposing communities (for example, high-severity burn communities when compared to both low-severity burn and unburned communities), which are represented by "satellite"" circles in Fig. 5 and 6.

When comparing unburned, low-severity burn, and high-severity burn communities, unburned communities had the most relatively abundant and consistently abundant bacterial and fungal genera (Fig. 5). In bacterial and fungal communities, both unburned and high-severity burn communities shared the fewest relatively abundant genera, while unburned and low-severity burn communities shared the most.

When comparing bacterial communities associated with each *Armillaria* spp., *A. solidipes*-associated communities had the most consistently abundant genera (Fig. 6A). In contrast, communities associated with only *A. solidipes* or *A. altimontana* had the same number of consistently abundant fungal genera (Fig. 6B). For both bacterial and fungal communities, *A. solidipes*- and *A. altimontana*-associated communities shared the fewest relatively abundant genera.

Bacterial and fungal isolation from soils

From approximately 800 initial cultures, microbial species isolated from soil samples were identified as belonging to 34 bacterial and 19 fungal genera (Table S1g). A large proportion of bacterial isolates were identified as either *Bacillus* spp. or *Pseudomonas* spp., while *Penicillium* spp. and *Mortierella* spp. dominated the fungal isolates. Each of these genera was isolated from all three burn-severity levels. The number of isolates of bacterial and fungal genera was relatively evenly distributed among burn severity groups (Fig. 7). GenBank accession numbers and taxonomic identification for bacterial and fungal isolates can be found in Tables S1i and S1j, respectively.

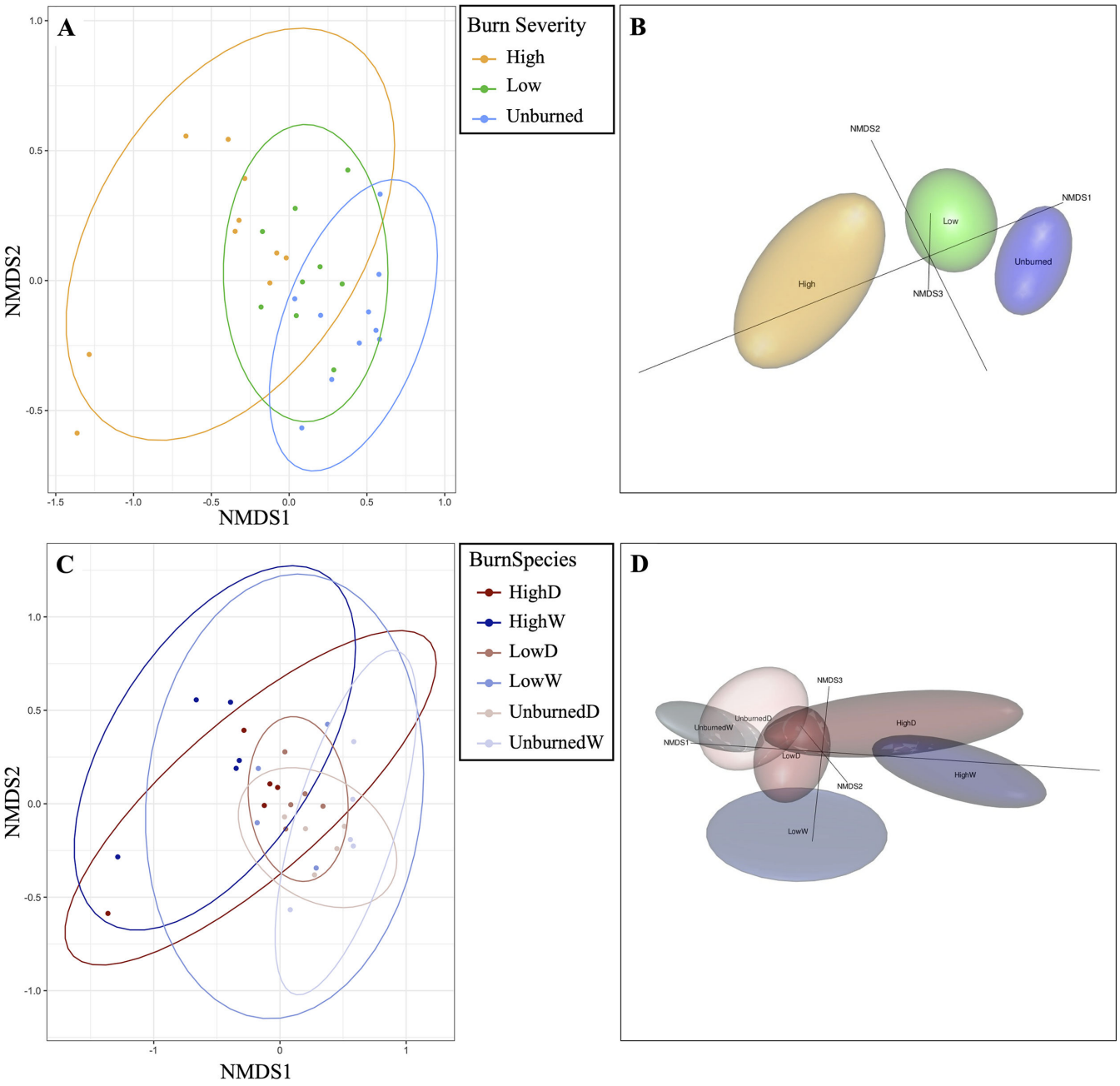

FIG 3 Two- and three-dimensional non-metric multi-dimensional scaling (NMDS) plots representing bacterial communities grouped by burn-severity level (A, B) and BurnSpecies (C, D) (stress value = 0.0888). HighD, LowD, and UnburnedD = high-severity burn, low-severity burn, and unburned Douglas-fir (*Pseudotsuga menziesii*), respectively, HighW, LowW, and UnburnedW = high-severity burn, low-severity burn, and unburned western white pine (*Pinus monticola*), respectively.

Analysis of soil characteristics

Multiple linear regression models were used to determine soil characteristics that significantly predicted bacterial and fungal community richness and alpha diversity, as measured by Shannon's diversity index and inverse Simpson's index (Table 3). For bacterial and fungal communities, every soil variable selected for the model using AIC was a significant predictor of inverse Simpson's index. Further, more soil characteristics were associated with bacterial community diversity compared with fungal community diversity. Opposite trends (positive versus negative) in changes to the alpha diversity of bacterial and fungal communities in response to changing edaphic characteristics

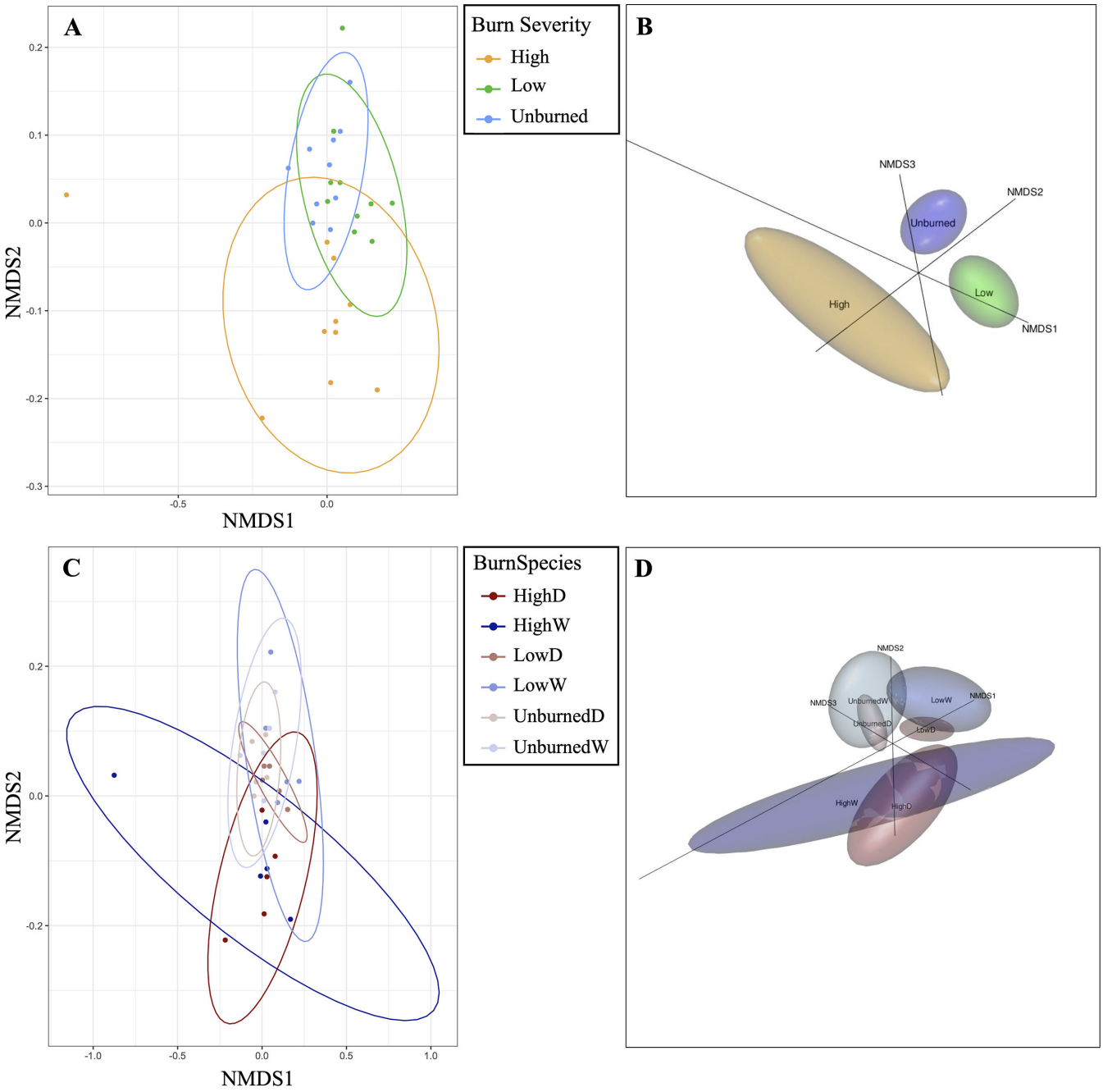

FIG 4 Two- and three-dimensional non-metric multi-dimensional scaling (NMDS) plots representing fungal communities grouped by burn-severity level (A, B) and BurnSpecies (C, D) (stress value = 0.1374). HighD, LowD, and UnburnedD = high-severity burn, low-severity burn, and unburned Douglas-fir (*Pseudotsuga menziesii*), respectively, HighW, LowW, and UnburnedW = high-severity burn, low-severity burn, and unburned western white pine (*Pinus monticola*), respectively.

were observed for soil N content, organic matter loss on ignition (LOI), cation exchange capacity (CEC), and K saturation.

Burn severity had a significant effect on soil N content (kg N per hectare) ($F_{2,27}$ = 4.33, P = 0.0235) and Fe content (Fe parts per million [ppm]) ($F_{2,27}$ = 6.62, P = 0.0046). Further pairwise comparisons found significantly higher N content in high-severity burn soils when compared with unburned soils (t (27) = 2.928, P = 0.0182). Pairwise comparisons indicated significant differences in soil Fe content between high-severity burn and unburned soils (t (27) = −3.43, P = 0.0054) and low-severity burn and unburned soils (t

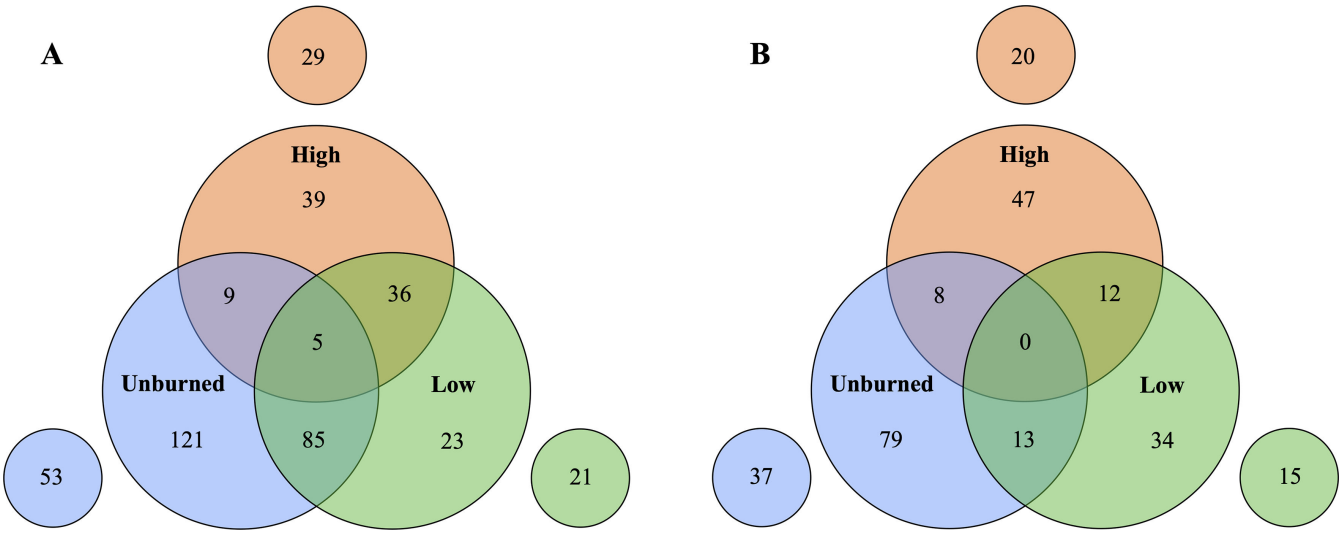

FIG 5 Numbers of bacterial (A) and fungal (B) genera that were significantly more abundant in microbial communities under each level of burn severity (high, low, and unburned) when compared with the other communities. Satellites indicate the number of genera that consistently proliferated in the associated burn severity when compared with the other two groups. List of genera can be found in Tables S1c (bacteria) and S1d (fungi).

(27) = −2.761, $P = 0.0268$), with unburned soils having higher soil Fe content than burned soils.

DISCUSSION

Our study provides a new understanding of the interactions among fire, soil microbial communities, and Armillaria root disease by examining changes to bacterial and fungal communities following low- and high-severity burns in coniferous forests where *Armillaria* spp. commonly occur. We observed compositional changes in microbial communities following fire that correspond with the microbial communities of various *Armillaria* spp. Importantly, following high-severity burns, we observed a decreased relative abundance of many ECM fungi, which likely contribute to the benefits to plant growth associated with *A. altimontana* (28, 29). Additionally, we found that *A. solidipes*

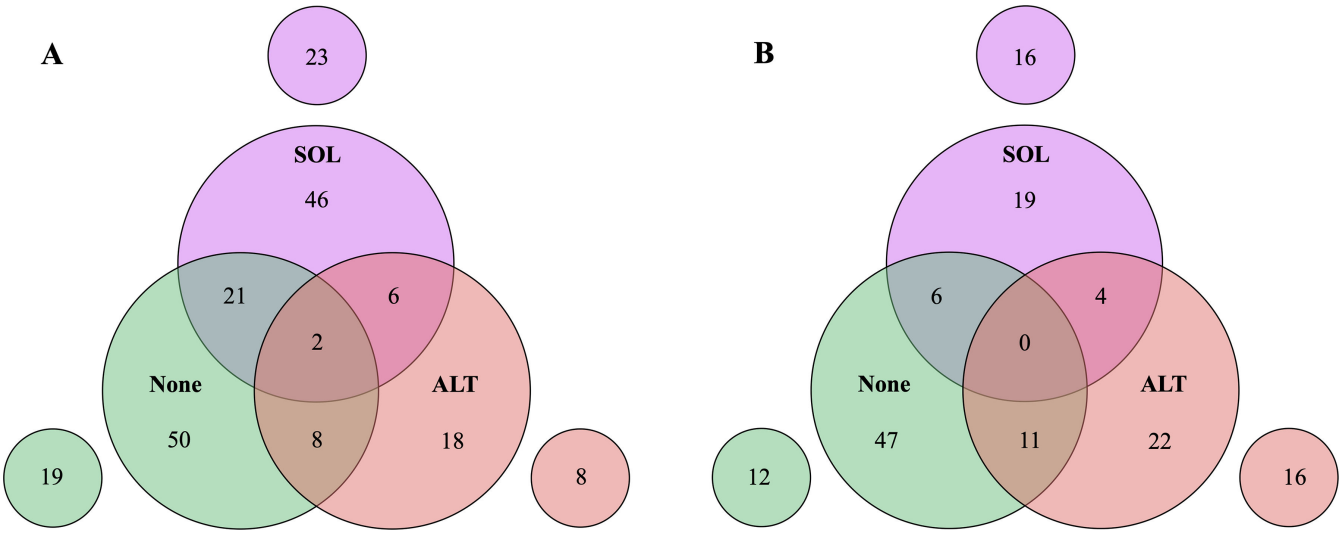

FIG 6 Numbers of bacterial (A) and fungal (B) genera that were significantly more abundant in microbial communities associated with *Armillaria solidipes* (SOL), *A. altimontana* (ALT), or neither (None) when compared with the other communities. Satellites indicate the number of genera that consistently proliferated with the indicated *Armillaria* spp. when compared with the other two groups. List of genera can be found in Tables S1e (bacterial) and S1f (fungi).

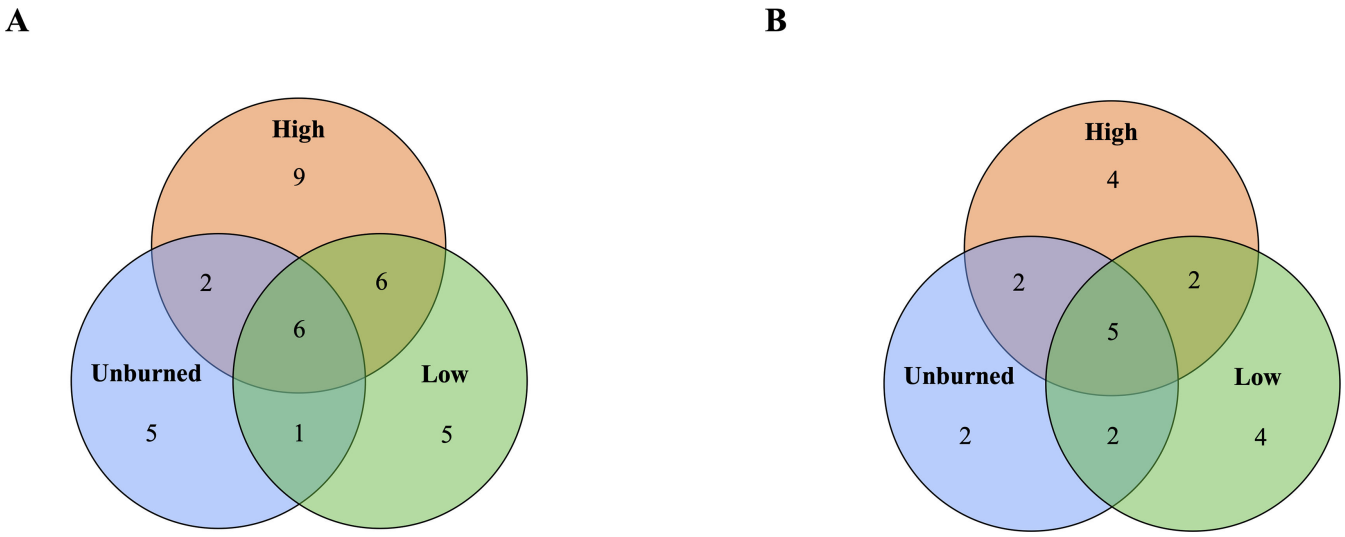

FIG 7 Numbers of bacterial (A) and fungal (B) genera isolated in culture from soils collected in high-severity burn, low-severity burn, and unburned areas. List of genera can be found in Table S1g.

and associated taxa were prevalent in high-severity burn areas, suggesting an increased need for post-fire monitoring and management for Armillaria root disease. These results further elucidate microbial taxa that may be critical components of the microbiomes associated with *A. solidipes* and *A. altimontana* in western United States coniferous forests and provide initial indications of how management strategies could be altered to accommodate changes in the soil microbiome following fire.

At 15 months post-fire, we observed that burn severity continued to have a significant impact on bacterial community richness and Shannon's diversity index, while no significant impact to fungal communities was found. The observed changes to bacterial alpha diversity are consistent with previous research showing that fire, especially high-severity burns, is associated with decreased bacterial community richness and Shannon's diversity index across ecosystems (4, 5, 7). Because decreases in fungal richness and diversity following fire have been previously reported (4, 6, 8, 55), we expected that fungal communities in our study would also follow this trend. In contrast, we found no change in fungal community richness or diversity following fire. Hopkins et al. (56) similarly found no change in the richness or alpha diversity of fungal communities following burns in two pyrophilic ecosystems managed with frequent prescribed fire. Further, studies separating fungal communities by soil depth show insignificant changes

TABLE 3 Soil characteristics with significant effects on bacterial and fungal richness (R) and alpha diversity, as measured by Shannon's diversity index (H') and inverse Simpson's index ($1/D$)[b]

Soil characteristic	Bacteria			Fungi		
	R	H'	$1/D$	R	H'	$1/D$
Soil pH		+*				
Organic matter LOI[a]			+**	-*	-*	-*
Kg N per hectare	-**	-*	-**		+**	+**
K parts per million (ppm)			-***			
Zn ppm			-***			
Mn ppm			+*			
Na ppm		-**	-*			
Cation exchange capacity		+**	+***		-*	-**
K saturation			+***		-**	-**

[a]LOI, loss on ignition.
[b]Direction of the coefficient (+ or -) produced by the linear model is indicated. *, significance at 5% level; **, significance at 1% level; ***, significance at 0.1% level.

in overall fungal richness and diversity in lower soil layers following fire (4, 10). Additionally, Pérez-Izquierdo et al. (10) found that the richness of saprophytic soil ascomycetes in boreal forests increased following fire, despite decreased or unchanged total fungal community richness. Separating our fungal communities by ecological lifestyle or soil depth may have revealed similar trends in how specific fungal groups respond to burn severity.

Meta-analyses of typical changes to soil characteristics following fire report decreased organic matter, increased pH, and increased available nitrogen (1, 9). In contrast, we observed that only soil N content and Fe content varied significantly in response to burn severity. The discrepancies between the results of this study and previous studies are perhaps attributable to our sampling time, which was over 1-year post-fire, as another study reported a return to near pre-fire conditions after only 1 year (7). Further, it seems reasonable that our results were again affected by pooling soils from different depths, given that Pérez-Izquierdo et al. (3) found differences among soil properties in the organic layer following fire but insignificant changes in the underlying mineral soil. Our results suggest that post-fire changes to edaphic characteristics have a greater influence on microbial evenness than richness, while vegetative shifts can have the opposite effect. For example, we observed that inverse Simpson, which prioritizes community evenness, was influenced by more soil characteristic changes than were richness or Shannon's diversity index, especially for bacterial communities (Table 3). In contrast, our burn-severity levels (unburned, low, and high) were determined by post-fire tree survival and roughly corresponded with the tree-related burn severity metric used in Pérez-Izquierdo et al. (3), which included factors, such as tree mortality and stem soot height. In our study, these tree-related changes, represented by our burn severity levels, corresponded with changes in bacterial community richness and Shannon's diversity index, but not changes to inverse Simpson's index.

We observed that fire resulted in changes to the composition of our soil microbial communities, concurring with previous research (3–5, 7, 8, 55, 56). Previous studies have reported increased clustering of soil microbial communities in the upper soil layers following high-severity burns (4, 10) as fire selects for taxa that can withstand high temperatures and post-disturbance conditions; however, no change or decreased clustering post-fire has also been observed (55, 56). We found that high-severity burn communities consistently showed higher variance in composition compared with our low-severity burn communities; this trend was also observed when communities were separated by host species, apart from WWP bacterial communities. We speculate that, in our study, exudates from trees that remained alive following low-severity burns may have promoted a stress-resistant soil microbiome, resulting in more similar microbial communities composed of beneficial taxa that could expedite tree recovery.

Fire is known to change the relative abundance of specific microbial taxa within the soil community as the disturbance selects for bacteria and fungi that are adapted to withstand heat or efficiently colonize under post-fire environmental conditions (4, 6, 7, 55–57). For example, across a variety of ecosystems, the bacterial genera *Arthrobacter* and *Massilia* and the Phylum Firmicutes tend to increase in relative abundance following fire (4, 5, 7, 55, 57), as do the fungal genera *Pyronema*, *Penicillium*, and *Geopyxis* (6, 8, 10, 57). In contrast, fungi from the family Umbelopsidaceae, including *Mortierella* species, and ECM fungi tend to decrease following fire (8, 10, 55, 57). In this study, we found some contrasting results in changes to specific taxa based on both our metabarcoding and culture-based analyses. For example, many of these fungal genera, including *Geopyxis*, *Pyronema*, and *Mortierella*, were present on our study sites yet did not drive significant differences among the composition of microbial communities in our metabarcoding analyses. *Arthrobacter* was more abundant in high-severity burn communities compared with low-severity burn communities, but also consistently proliferated in unburned communities compared with other burn-severity levels. Further, in our culture-based analyses, species belonging to *Penicillium* and *Mortierella* were isolated from all three burn-severity levels, reflecting the widespread distribution of these taxa in the soil.

However, fire-associated changes in the presence or abundance of certain taxa reflected results from previous studies. For example, in this study, ASVs belonging to *Penicillium*, which was associated with fire in Day et al. (6) and Whitman et al. (57), proliferated in high-severity burn communities when compared with unburned communities. We also isolated *Massilia,* a genus that was previously found to be abundant in the burned communities of managed *Eucalyptus* forests (4), from soils of high- and low-severity burns, but not from unburned soils. Similarly, the fungal genus *Paraphoma* (recently separated from *Phoma*), which was associated with higher severity fire in Canadian boreal forests (6), was isolated from a sample of high-severity burn soil in this study.

Changes to communities of ECM fungi following fire are especially relevant to Armillaria root disease management due to their potential role in a putative disease suppressive soil and/or promoting tree growth and survival. Decreases in relative abundance, richness, and diversity of ECM fungi are typically observed following fire (6, 8, 55, 58), especially severe fires that lead to the mortality of their tree hosts (2, 3). In our study, changes to the relative abundance of specific ECM genera following fire varied in comparison with results from other studies. For example, the relative abundances of the ECM fungi *Russula* and *Cortinarius*, reported to be indicators of unburned conditions in boreal forests in Pérez-Izquierdo et al. (10), were not significantly different between burned and unburned communities in our study. Further, this same study found that the ECM genus *Laccaria* was more relatively abundant in burned conditions; however, we found that *Laccaria* consistently proliferated in unburned communities of our study. We also found that the ECM genera *Inocybe* and *Tomentella* consistently proliferated in unburned communities when compared with both low-severity and high-severity burn communities, which concurs with the findings of Reazin et al. (8) that showed decreased relative abundance of these genera following fire in ponderosa pine (*P. ponderosa*) forests. We also found fire-associated changes in the relative abundance of *Suillus* and *Truncocolumella*, two ECM genera in the family Suillaceae, which were previously reported to proliferate in soil microbial communities associated with *A. altimontana* (29). In our study, *Suillus* showed higher relative abundance in low-severity burn communities when compared with unburned communities, while *Truncocolumella* showed higher relative abundance in unburned communities when compared with low-severity burn communities. Previous studies have indicated that *Suillus* spp., which have wind-dispersed spores, are effective post-fire colonizers (10, 59, 60), potentially supporting their role as important ECM symbionts for tree regeneration following disturbance.

In agreement with Caballero et al. (29), we found no significant differences in the alpha or beta diversity of our microbial communities based on association with different *Armillaria* spp. Further, we also found similarities between taxa enriched in the communities of *A. altimontana* or *A. solidipes.* This included a proliferation of *Suillus* and *Truncocolumella* in *A. altimontana*-associated communities when compared with *A. solidipes*-associated communities, providing support for the hypothesis that these beneficial symbionts are playing a role in the positive plant growth effects connected to *A. altimontana* (28). Similarly, we found an increased abundance of the bacterial genus *Solirubrobacter* in *A. solidipes*-associated communities compared with *A. altimontana*-associated communities, which supports a result that Ibarra Caballero et al. (29) found at the family level (Solirubrobacteraceae). In addition, three genera belonging to Enterobacteriaceae, a large bacterial family previously associated with *A. solidipes* communities (29), were significantly different among our microbial communities. However, instead of being affiliated with *A. solidipes*, our study found that two genera (*Trabulsiella* and *Buttiauxella*) consistently proliferated in *A. altimontana*-associated communities, and one genus (*Cedecea*) consistently proliferated in communities associated with no *Armillaria* spp. Interestingly, two bacterial genera that have been found enriched in the microbiome of plants under pathogen attack, *Chitinophaga* and *Chryseobacterium* (13, 61), were consistently abundant in *A. solidipes*-associated communities in our study. While the majority of microbial communities where both *A. solidipes* and *Chitinophaga* were present were in high-severity burn soil samples, one community was from an unburned

soil sample. This could possibly suggest that this particular tree (tree 27) was secreting exudates to promote a stress-resistant microbiome against the pathogen.

Interpretations of comparisons among the microbial communities from different burn-severity levels and *Armillaria* spp. are complicated by the uneven distribution of *Armillaria* spp. across our burn-severity groups. For example, the majority of trees with only *A. solidipes* isolates were found in high severity burn areas, which may explain why fire-associated bacteria, including *Massilia* and *Arthrobacter*, were found to proliferate in *A. solidipes*-associated communities. Similarly, many trees associated only with *A. altimontana* were from low-severity burn or unburned areas. Further, in our high-severity burn sites, *A. solidipes* was likely existing as a saprophyte on trees killed by the fire, and this microbial community may differ from the community present when *A. solidipes* is acting as a pathogen. However, because an exact overlap among communities from various burn-severity levels and *Armillaria* spp. was not observed, we examined shared taxa that consistently proliferated in microbial communities grouped by burn severity and *Armillaria* spp. There were no genera that consistently proliferated in both low-severity burn communities and *A. solidipes*-associated communities, while high-severity burn communities and *A. solidipes*-associated communities showed the most overlap in consistently abundant bacterial and fungal genera. Similarly, we found no shared bacterial or fungal genera that consistently proliferated in *A. altimontana*-associated communities and those that consistently proliferated after high-severity burns, while consistently abundant genera were shared among *A. altimontana*-associated communities and low-severity burn or unburned communities.

In conclusion, we found that incremental increases in burn severity altered the diversity and composition of soil microbial communities in a northern Idaho forest where Armillaria root disease from *A. solidipes* is present. Further, the high occurrence of *A. solidipes* in high-severity burn areas and the overlap between consistently abundant taxa in high-severity burn and *A. solidipes* communities indicates that these high-severity burn sites may provide ideal conditions for *A. solidipes* and its microbial community to inhabit. Although *A. solidipes* has a saprophytic lifestyle on dead trees in areas of high-severity burns, this could produce an inoculum source for both adjacent regions with remaining live trees and regenerating trees on the severely burned site, increasing the presence of Armillaria root disease over time. For this reason, severely burned conifer forests where *A. solidipes* occurs should be identified as potential priority areas for monitoring and preventative management for Armillaria root disease. In contrast, the association between the ECM genus *Suillus* and *A. altimontana* and its increased relative abundance following low-severity burns may suggest that controlled, low-severity burns have the potential to be part of a management strategy for *A. solidipes* by shifting the soil microbiome towards taxa that potentially comprise a disease suppressive soil. However, a deeper understanding of the soil microbiome in coniferous forests, including the importance of ECM fungi in the putative disease suppressive soils associated with *A. altimontana* and other specific microbial taxa associated with the virulence of *A. solidipes*, and the influences of burn severity on its ecological functions are needed to help integrate fire into an effective landscape-scale management strategy. Overall, our research provides insight on the importance of understanding the complex interactions among burn severity and soil microbial communities and the consequent management of prevalent forest diseases.

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ADDITIONAL FILES

The following material is available online.

Supplemental Material

Figure S1 (AEM01312-24-s0001.tif). Pictures highlighting differences in understory vegetation and tree mortality between high-severity burn, low-severity burn, and unburned field sites.
Figure S2 (AEM01312-24-s0002.pdf). Rarefaction curves for bacterial (A) and fungal (B) metagenome data.
Figure S3 (AEM01312-24-s0003.pdf). Principal coordinate analysis (PCoA) plots for bacterial (A, C) and fungal (B, D) communities separated by burn severity (A, B) and BurnSpecies (C, D). HighD, LowD, and UnburnedD = high-severity burn, low-severity burn, and unburned Douglas-fir (*Pseudotsuga menziesii*), respectively. HighW, LowW, and UnburnedW = high-severity burn, low-severity burn, and unburned western white pine (*Pinus monticola*), respectively.
Figure S4 (AEM01312-24-s0004.pdf). Non-metric multi-dimensional scaling (NMDS) plots for bacterial (A) and fungal (B) communities grouped by *Armillaria* species association.
Figure S5 (AEM01312-24-s0005.pdf). Pie charts depicting bacterial genera present in at least 1% relative abundance in communities separated by BurnSpecies. DF = Douglas-fir (*Pseudotsuga menziesii*) host, WWP = western white pine (*Pinus monticola*) host.
Figure S6 (AEM01312-24-s0006.pdf). Pie charts depicting fungal genera present in at least 5% relative abundance in communities separated by BurnSpecies. DF = Douglas-fir (*Pseudotsuga menziesii*) host, WWP = western white pine (*Pinus monticola*) host.
Table S1a-j (AEM01312-24-s0007.xlsx). Extra information.

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