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Context dependent fungal and bacterial soil community shifts in response to recent wildfires in the Southern Appalachian Mountains

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ABSTRACT

Decades of fire suppression coupled with changing climatic conditions have increased the frequency and intensity of wildfires. The Southern Appalachia region of the United States is predicted to be particularly susceptible to climatic changes, with predicted increases in fire severity and occurrence. Following the record breaking fire season in 2016 in Southern Appalachia, we examined wildfire impacts on soil chemistry and below ground communities (fungi and bacteria – Illumina MiSeq) within two substrates (duff and soil) at two adjacent locations with similar plant communities (Great Smoky Mountains National Park – 'Chimney Top 2' Fire (GRSM) and Nantahala National Forest – 'Cliffside' Fire (NNF)) from replicate plots representing a range of fire severities (unburned, low severity, moderate severity, severe). Differing fire severities changed community composition, and fire severity played a stronger role in structuring bacterial communities than in structuring fungal communities. Further, fire impacts on soil communities and functional guilds responses were location- and substrate-specific with NNF responding more strongly to fire than GRSM. Additionally, using a novel analysis tool (Axis Weighted Ordination Distance – AWOrD), domain and location specific responses to wildfire severity are demonstrated. Taken together, our results suggest context-dependency in microbial responses to fire that must be accounted for to generate ecosystem-wide recovery predictions.

1. Introduction

With the projected increase in drought frequencies and durations, North American forests are predicted to be dramatically affected by tree mortality and extreme events (IPCC, 5th Assessment Report, 2014). Extended droughts in forests can increase combustible fuel loads, markedly increasing the probability of fire and increase fire severity (Williamson et al., 2009). Specifically, in the Southeast USA, general circulation models predict a 1.5–3 °C temperature increase over the following five decades (Mitchell et al., 2014), resulting in a substantial risk of severe droughts and wildfires. In the southern Appalachian region of the United States, fire suppression and prevention since the early 1900 s has shifted plant communities from those dominated by *Quercus* spp. to those with a greater proportion of fire-sensitive *Acer rubrum* (Abrams, 1992; Nowaki and Abrams, 2008). For most of the 20th century, prescribed fire has not been commonly implemented in these southeastern forests, thus increasing fire susceptibility in Southeast USA.

Fire changes soil properties and chemistry, but these responses often depend on fire intensity, severity and burn duration (Certini, 2005). For example, fire may alter nutrient pools, including reduction on the quantity and quality of soil carbon (Neff et al., 2005), general nutrient availability (Wan et al., 2001; Certini, 2005), and soil pH (Anderson and Menges, 1997). Furthermore, areas affected by fire, when patchy, often result in variable organic layers and plant litter residues associated with ash (Zavala et al., 2014). High intensity fires consume most vegetation and seed sources in the topmost soil profiles necessitating long recovery periods. Severe fires may result not only in more variable organic matter composition, but also in long-term effects on soil carbon storage and release (González-Pérez et al., 2004) therefore altering

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major available nutrient pools. Such alterations to carbon stocks in forest soils are generally greater in forests with long-term fire suppression (Nave et al., 2011), such as those in the southeastern United States.

Extreme fires can have profound effects on the soil microbial communities that mediate ecosystem function and soil carbon- and nitrogen-cycling (DeLuca et al., 2006; Allison and Treseder, 2008). Soil microbial biomass can be substantially reduced depending on burn severity (Dooley and Treseder, 2012) and severe burns may effectively destroy local habitat with microbial biomass being reduced for years or decades (Holden et al., 2016). Microbial community responses to fire have been well documented in studies of previous wildfire events (*see* Goberna et al., 2012, Ferrenberg et al., 2013, Fultz et al., 2016). These studies indicate that not only do bacterial and fungal communities shift with fire, but also that specific functional groups may be selected for (e.g. nitrogen-fixing bacteria; Yeager et al., 2005) or against (mycorrhizae and litter decomposers; Hebel et al., 2009, Holden et al., 2013). These responses may correlate with soil physicochemistry, such as pH or nutrient pool sizes or availability, exemplifying microbe–-environment feedbacks in response to extreme disturbances. Furthermore, selection for disturbance tolerant microbial communities and declines in microbial biomass likely result in long-term effects on essential ecosystem functions, such as exoenzyme production and decomposition (Dooley and Treseder, 2012; Holden et al., 2013). Thus, addressing wildfire effects on nutrient pool and forest soil microbial communities in parallel is imperative to gain a fine-tuned understanding of ecosystem resilience to future fires and to better understand recovery dynamics and potential.

The largest wildfire in the state of Tennessee history (as well as the largest fire within Great Smoky Mountain National Park (GRSM)) started in November 2016 (Chimney Tops 2 fire – with flareups and smolder continuing until April 2017). Due to extreme drought conditions in the southeastern USA and coinciding high winds, fire ignition was widespread and merged with wildfires outside of GRSM resulting in a burn area greater than 7,000 ha (~70 km^2). Simultaneously with the GRSM fire, multiple wildfires in Greater Appalachia occurred including a smaller but equally severe fire in the Cliffside region of Nantahala National Forrest (NNF) in North Carolina, where approximately 50 ha (~0.5 km^2) burned (Cliffside Fire – USFS, unpublished data). In response to these extreme wildfires, we investigated the short-term impacts of wildland fire of differing severities on fungal and bacterial communities in soil and duff in two nearby locations (GRSM, NNF) with a recent history of fire suppression. Our goal was to discern how soil and duff microbial communities (bacteria and fungi) respond to a gradient of burn severities in southern Appalachia in conjunction with soil nutrient pool size changes due to pyrolysis. GRSM and NNF are found in the same ecoregion, are geographically close [~70 km apart], and have similarly aged forest stands. We hypothesized that microbial communities would respond more strongly to burn severity than to wildfire locations (GRSM vs. NNF). We also discuss the need for better integration of multi-domain and mechanistic investigations into wildfire impacts to better model and understand ecosystem recovery and resilience.

2. Materials and methods

2.1. Sampling locations

We sampled sites within the Great Smoky Mountains National Park (GRSM) and Nantahala National Forest (NNF) impacted by the Chimney Top 2 Fire near Gatlinburg, Tennessee, USA and by the Cliffside Fire near Highlands, North Carolina, USA, respectively. Plots to compare responses to recent wildland fires (November 2016-January 2017) affected by different burn severities were established in May 2017; some of the plot locations were still smoldering as late as April 2017. Based on the Composite Burn Index (Key and Benson, 2006), eight plots in four burn severities were established in both GRSM and NNF for a total of 64 plots (7 × 7 m plots). Fire severity categories were demarcated using the following parameters: (1) *low severity*: litter mostly consumed, duff < 50% consumed, shrub layer with partial canopy scorch, and overstory trees with char height at the base of trees of < 2 m; (2) *moderate severity*: litter consumed, duff > 50% consumed, shrub layer canopy partially to all consumed, heavy scorch, overstory trees having > 3 m char height at the base of trees, and needles/leaves in the canopy completely scorched to partially consumed; and (3) *high severity*: litter fully consumed, duff almost completely consumed, shrub layer completely consumed or charred main stems remaining, overstory trees charred to full tree height; and canopy needles/leaves mostly to completely consumed, and (4) unburned reference plots which were as near to the burned plots as possible.

Samples within GRSM were located on mostly westerly slopes (due to local topography, a few plots were eastern and northern slopes) near the Baskins Creek Trail (783–823 m asl, and slope aspects of 10–54%), Cherokee Orchard (582–665 m asl, 23–45% slopes), Bullhead Trail (*ca.* 805 m asl, 6–17% slopes), and Cove Mountain Trail (545–823 m asl, 23–67% slopes). The vegetation predominantly consisted of *Quercus montana* (chestnut oak) with some of the higher elevation sites dominated by *Pinus rigida* (pitch pine) with a *Rhododendron maximum* (rosebay rhododendron) understory. While soil types vary dramatically with topological features in Appalachia, soils in GRSM were predominantly loamy soils (mixtures of channery soil and fine sandy loam). Samples within NNF were all collected near and around Cliffside Lake area (1049–1186 m asl, 22–60% slopes, southeastern aspect). Vegetation there was dominated by *Pinus strobus* (eastern white pine) mixed with various *Quercus* spp. throughout, with an *R. maximum* and *Kalmia latifolia* (mountain laurel) understory. Soils at NNF consisted of a mixture of gravelly fine sandy loam and loam (*see* Table A1 for complete sampling locations and descriptions). Samples within GRSM were collected across an area of *ca.* 1400 ha. (separated by 5.4 km between the most distant samples) while samples within NNF were collected across *ca.* 60 ha. (1 km distant). The GRSM and NNF locations are *ca.* 75 km from each other and separated by the crest of the Blue Ridge Mountains.

2.2. Sampling

Subsoil and duff were sampled from each plot within GRSM and NNF. Because of the potential danger and safety concerns outlined in the federal permits, the sampling was conducted immediately following stoppage of residual smoldering and after being cleared by authorities. Soils were collected by sampling from 10 random points within each plot using hand spades to collect the top ~15 cm of duff and soil. Each soil sample was divided to duff (O horizon; ~5–12 cm thick) layers and mineral subsoil (A-B horizons), providing ~100 g of duff (in unburned, low, and moderate burn severity plots, severely burned plots had duff fully consumed) and ~200 g of mineral soil. While O, A, and B horizons are not homogenous in depth, and thus may be differentially impacted by fire, we did our best to minimize inter-sample soil depth variability to allow for the most representative sample possible. Samples were placed into new plastic zip-top bags and transported to the laboratory on ice, where they were stored at −20 °C. Within 48 h after collection, all subsamples (within plot) were homogenized using a Warring Blender for 1 min with the machine cleaned and allowed to cool between samples to prevent excessive heat transfer to soil. This protocol produced a composite sample for each plot (duff and soil mixed separately). After mixing, a subsample was stored at −80 °C for subsequent soil chemical analysis. For each plot, the composite sample was further mixed, and 1 g from each composite sample was used for DNA extraction following Veach et al. (2018). Duff was completely consumed in the severe plots resulting in a total of 112 samples (64 soil, 48 duff).

2.3. Soil chemical analyses

We recorded pH, dry-weight percentages of organic matter (OM), total nitrogen (TN), and total carbon (TC) following soil preparation (50 °C drying overnight and grinding to pass through a 2 mm sieve) using the following methods: pH was measured in a 1:1 soil [10 g] to deionized water slurry and read on a Skalar Inc. SP50 Robotic Analyzer System [Burfurd, GA, USA]); organic matter content was measured via loss on ignition in a muffle furnace; TN and TC were measured using a LECO CN 2000 combustion analyzer (Saint Joseph, MI, USA). Additionally, we calculated C:N from TC and TN values.

2.4. DNA extractions and MiSeq library preparation

Genomic DNA was extracted from soil and duff samples using PowerSoil DNA Isolation Kits (MoBio Laboratories, Carlsbad, CA, USA) following manufacturer's protocol with the modification of a homogenization step using a Biospec Mini-beadbeater (Biospecc, Bartlesville, OK, USA) for 2 min with the addition of the lysis buffer prior to remaining extraction steps. DNA was quantified on a NanoDrop 1000 Spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA) and normalized to a working concentration of 10 ng μL^{-1} prior to amplicon generation to maximize comparability between samples.

Fungal and bacterial amplicon libraries were generated by amplifying the Internal Transcribed Spacer Region 2 (ITS2) and bacterial 16S (V4) regions in triplicate using a two-step amplification process (*following* Brown et al., 2018). Briefly, the ITS2 (fungal) and the V4 (bacterial) regions of the ribosomal RNA (rRNA) gene repeat were amplified using the primer pairs nexF-N[3-6]-fITS7 and nexR-N[3-6]-ITS4 for fungi and nexF-N[3-6]-515f and nexR-N[3-6]-806r for bacteria where fITS7 (Ihrmark et al., 2012) and ITS4 (White et al., 1990) are fungal gene primers and 515f and 806r (Caporaso et al., 2011) are bacteria gene primers. N[3-6] represents four identical primers with the exception of containing a range of ambiguous nucleotides (3-6) mixed to equal molarity to increase nucleotide diversity during sequencing, and nexF and nexR are Nextera forward and reverse sequencing primers.

Triplicate primary PCRs were conducted in 25 μL reactions using 5 μL DNA template (50 ng), 5 μL 5X Phusion High-fidelity Buffer, 200 μM each dNTP, 0.5 μM of each forward and reverse primer, 0.25 μL Phusion HotStart II DNA Polymerase (0.02 U/μL final concentration; ThermoFisher Scientific), and 9.25 μL molecular grade H_2O with the PCR parameters of 98 °C for 30 s, 25 cycles of 98 °C for 20 s, annealing temperature for 30 s (51 °C for fungi and 52.5 °C for bacteria), and 72 °C for 40 s, followed by a final extension at 72° for 10 min, all ramp rates were 1 °C/second. This resulted in a final 1° PCR construct of nexF-N[3-6]-primer-{ITS2 or V4}-primer-N[3-6]-nexR. After primary PCR and positive visualization using gel electrophoresis (1.5% agarose *w:v* in TBE), the triplicate samples were pooled. Secondary PCR reactions were conducted in 20 μL reactions using the forward primers that include the P5-i5-overlap and the reverse primers P7-i7-overlap where P5 and P7 are the Illumina Adaptor sequences, i5 and i7 are 8 bp unique Molecular Identifiers (MIDs – barcode), and the overlap is the partial nexF and nexR sequence that acts as the annealing site for the 2° PCR. The forward and reverse barcoded 2° primers were mixed in a combinatorial fashion to generate unique dual barcoded primers in a working concentration of 10 μM (5 μM for each primer). The 2° PCR reactions contained 2 μL of 1° PCR product, 4 μL 5X Phusion High-fidelity Buffer, 200 μM each dNTP, 0.5 μM of each forward and reverse primer, 0.2 μL Phusion HotStart II DNA Polymerase (0.02 U/μL final concentration; ThermoFisher Scientific), and 11.4 μL molecular grade H_2O with the PCR parameters of 98 °C for 30 s, 8 cycles of 98 °C for 20 s, 50 °C 30 s and 72 °C for 40 s, followed by a final extension at 72° for 10 min. This produced the final amplicon constructs of P5-i5-nexF-N[3-6]-primer-{ITS2 or V4}-primer-N[3-6]-nexR-i7-P7 using a total of 32 cycles. Secondary PCR products were cleaned using Axygen AxyPrep Mag PCR clean up breads (Axygen Biosciences, Union City, CA, USA) following kit protocol with the modification using a 1:1 bead solution to reaction volume ratio (Brown and Jumpponen, 2014). Negative controls (molecular grade water instead of gDNA) were included and remained free of observable amplification via gel electrophoresis. Cleaned PCR products were quantified using Qubit 3.0 fluorometric assays (dsDNA HS Assay Kit; ThermoFisher Scientific). Fungal and Bacterial PCR products were separately pooled into libraries at equal concentrations. The amplicon libraries were sequenced on one Illumina MiSeq (v.3, 300PE) sequencing run with a 65% Fungi: 35% Bacteria loading ratio at the Kansas State University Integrated Genomics Facility (Manhattan, KS, USA). Demultiplexing of the raw sequence data using the unique i5 and i7 sequence combinations provided individual paired fastq files for each of the 112 samples (for both fungal and bacterial libraries (see Table A2 for primer and MID sequences).

2.5. Bioinformatics

Sequence data were processed using the program mothur (v.1.39.5; Schloss et al., 2009). The forward and reverse sequences were contiged and screened to cull sequences with ambiguous bases, or greater than 10 homopolymers and merged into single fasta files for fungi and bacteria and trimmed to eliminate primer sequences. Bacterial sequences were aligned against the SILVA (release 132; www.arb-silva.de) reference alignment and filtered to exclude non 16S V4 regions (fungal ITS sequences cannot be reliably aligned). Sequences were preclustered to remove basepair variation due to sequence chemistry errors (following Huse et al., 2010 as implemented in mothur), screened for chimeras (mothur implemented VSEARCH; Rognes et al., 2016), and putative chimeras were culled. Sequences were screened for off-target amplification (non-fungal or non-bacterial in origin) by classifying all sequences using a mothur implemented Naïve Bayesian Classifier (Wang et al., 2007) against the RDP training set (bacteria; v.10) or a UNITE non-redundant database (fungi; v6, Kõljalg et al., 2013) that was locally modified to increase representation of Plantae, Protista, and Oomycota. Non-target lineages were culled and distance matrices for bacteria (not punishing terminal gaps) and fungi (pairwise sequence distances using Needleman-Wunsch alignments and not punishing terminal gaps) were generated. Sequences were clustered into operational taxonomic units (OTUs) at a 3% dissimilarity threshold using OptiClust (Westcott and Schloss, 2017) which optimizes clustering success and OTU assignments to maximize the Matthews Correlation Coefficient through iteration. OTUs with fewer than 10 sequences globally were considered potentially spurious and culled (Brown et al., 2015, Oliver et al., 2015). Bacterial OTUs were assigned taxon affinities based on the most representative sequence of the OTU (centroid) whereas fungal OTUs were classified to the level of Species Hypothesis (UNITE). Further, OTUs that did not have 100% bootstrap support for phylum level identities were manually confirmed using MOLE-BLAST (NCBI) against GenBank (nr/nt) using BLASTn with exclusion of environmental sequences; non-target OTUs were culled. Final fungal taxonomic identities were determined using massBLASTer (BLAST + 2.4.0) in the PlutoF toolkit (Abarenkov et al., 2010) against the UNITE database with the inclusion of the global INSD sequences and exclusion of environmental sequences. After all sequence quality control, our uneven library sequencing loads resulted in $\sim 5.1 \times 10^6$ fungal and $\sim 1.2 \times 10^6$ bacterial high quality and verified reads. We calculated relative OTU richness (S_{obs}), Diversity (Compliment of Simpson's diversity index; 1-*D*), and Evenness (Simpson's Evenness, E_D) for duff and soil samples by implementing an iterative subsampling approach (1000 iterations) to maximize inter-sample comparability. Fungal and bacterial samples were queried at a subsampling depth of 10,000 and 5,000 sequences per sample, respectively, and average diversity values used for downstream analyses.

2.6. Statistical analysis

Soil chemistry and nutrient pools (pH, %OM, TN, TC, C:N) were compared using Analysis of Variance (ANOVA) models across burn severities, locations (GRSM, NNF), and their interactions separately by substrates (soil, duff). Where significant, Tukey's HSD was preformed to examine which treatments differ. Microbial diversity estimators were analyzed using similar ANOVA models as above. Diversity and evenness were transformed using logit functions prior to data analyses to meet ANOVA assumptions of normality. Furthermore, sequences belonging to each taxonomic group at each taxonomic rank (for fungi and bacteria) were tabulated and the two most abundant lineages per rank were tested using two-way ANOVAs (on relative abundances) to determine whether abundant taxa differ in relative abundance with location, burn severity, or their interactions.

A permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) of average Bray-Curtis dissimilarity values derived from iterative subsampling of OTU × Sample matrices (1000 iterations with a subsampling depth of 10,000 for fungi and 5,000 for bacteria using mothur) was used to test if communities differ with burn severity, location, and burn × location interactions for soil and duff communities. PERMANOVA and post-hoc multiple comparisons were conducted using the packages *vegan* (function *Adonis,* Oksanen et al., 2017) and *RVAIDeMemoire* (function *pairwise.perm.manova* with FDR correction, Hervé, 2017) in the program R (R Core Team; v.3.3.3). Additional PERMANOVAs were run with the exclusion of severely burned soils (without duff) to examine differential fire impacts on soil and duff for these microbial communities.

We used path analysis to examine how microbial communities are influenced by soil nutrient pools and chemistry across burn severity treatments and locations. Path analyses between soil chemical and nutrient data with microbial community composition as the response variable (on NMDS axis loading scores for the axis with the highest R^2 value – mothur implemented NMDS with 1000 iterations on average Bray-Curtis values subsampled as above) were generated for location (NNF and GRSM), burn severity (within locations), and bacteria and fungi separately. Briefly, multivariate correlations between microbial ordination axes loading scores and soil chemistry were calculated (using JMP Pro v 13.2.0). Using nonparametric comparisons (Kendall's Tau), and where significant correlations were observed, partial correlations (scaled negative inverse correlations) were calculated between variables (this adjusts for effects of all other variables on each other). Only significant correlations (where a direct or indirect path between soil chemistry variables and communities) were retained and path diagrams were generated.

To discern the magnitude and directionality of fire impacts on microbial communities, we developed the following new metric: the Axis Weighted Ordination Distance (AWOrD; Eq. (1)). The AWOrD metric quantifies a modified Manhattan distance between any two points in ordination space whilst accounting for ordination axes explanatory power (axes coefficients of determination). This allows determining *how* dissimilar communities (or samples) are and allows for comparisons within or between groups but does not require a balanced experimental design.

$$\sum_{i=1}^{n} [|(a_i - b_i)|(R^2{}_i)] \quad (1)$$

where i is the ordination axis number (axis number 1,2,3,…,i), a is the axis loading score coordinate for a given sample on axis i and b is the axis loading score coordinate for a different sample on axis i and R^2 is the proportion of the community variance that axis i explains. This allows axes that explain more community variance to be more heavily weighted in the calculation. It is important to note that this AWOrD measure is not a true distance, but the higher the value, the more dissimilar two samples are. Simply, this estimates *how different* communities are across the treatments whilst only accounting to major explanatory axes, not *only* if they differ categorically. The AWOrD measure may facilitate increased sensitivity to discern treatment differences (compared to Bray-Curtis or other measures). This is due to dimensionality reduction associated with ordinations which may mitigate zero-inflated concerns associated with analysis of Bray-Curtis data directly (Clarke et al., 2006) and omission of dimensions that consist of negligible community variation which may occlude observed patterns. This may be especially true when inherent community dispersion patterns are unknown or heterogeneous (Anderson and Walsh, 2013).

We calculated pairwise AWOrD values between all fire samples and all unburned samples for GRSM and NNF and for soil and duff separately. We used as many axes as needed to explain > 90% of community variation (first 13 axes for fungi and 8 for bacteria). NMDS axes were resolved from average Bray-Curtis dissimilarity matrices determined from iterative subsampled OTU × Sample input matrices (as implemented in mothur) using 1000 iterations to optimally resolve the ordinations. Using pairwise AWOrD measures by treatment, we tested for a positive relationship between the burn severity and AWOrD values, which would indicate that communities from more severely burned plots are more dissimilar to unburned controls than burns of lower severity. These were tested using a combination of one-way ANOVAs (soils; UB vs. LB compared to UB vs. MB and UB vs. SB) or Student's t-tests (duff; UB vs. LB compared to UB vs. MB).

To identify biomarker OTUs that are differentially abundant across treatments, we used a Linear Discriminant Analysis Effect Size (LEfSe) approach (Segata et al., 2011). All OTUs were tested for differential abundance among burn severity categories (class) using Kruskal-Wallis tests and pairwise Wilcoxon signed rank tests were then calculated across classes for OTUs that were identified as differentially abundant. Signed Linear Discriminant Analysis (LDA) log scores (along with associated p-values) were calculated. These LEfSe analyses were conducted for soil and duff separately. We chose to separate these analyses by location and substrate as PERMANOVA results indicated OTU composition between locations and substrates were divergent and our primary goal is to understand how burn severity alters community membership.

To elucidate functional microbial shifts with burn severity, we classified OTUs to putative functional groups using FUNGuild for fungi (v.1; Nguyen et al., 2016) and FunGene for bacteria (Fish et al., 2013). All species and/or genus identities were screened in FUNGuild using a reduced OTU list (OTUs that had BLAST bit scores > 300, percent identity > 97% where confidence ranking for functional IDs were either 'Highly Probable' or 'Probable' with FUNGuild) were queried. We only retained the functional groups Ectomycorrhizal, Plant Pathogens, and Saprobes as the other groups were highly depauperate. Bacterial putative functional groups were identified on a reduced OTU subset by screening genus level OTU affinities (reduced OTU list only where genus identity was supported by > 90% bootstrap support) against the FunGene database for putative presence of nitrogenase genes (*nifD* and *nifH*) and lignin degrading capabilities (*LigE*). While many OTUs aligned with these bacterial functional groups, only putative taxa that matched with a bit score > 300 were included (see Table A3). Functional assignments were purposely highly conservative to reduce the probabilities of false assignments, this led to a highly reduced subset of all OTUs. While this conservative approach maximized confidence of functional assignments, inferred community wide functional shifts based on these analyses must be perceived to potentially be under-representative. Total functional group relative abundances (by sample) were arcsine transformed and ANOVAs were used to test if functional groups changed with burn severity.

2.7. Accession numbers

Sequence data are archived at the Sequence Read Archive (SRA) at NCBI under the accessions: BioProject (PRJNA546006) and BioSamples

(SAMN11947873-SAMN11947920; SAMN11948738-SAMN11948753).

3. Results

Soil chemistry and nutrient pools shift with fire severity and were shown to be location dependent (Table A4). In general, OM, TN, and TC (% dry weight) decreased with increased burn severity, whereas soil pH increased with burn severity. Further, pH in duff samples, and C:N for both duff and soil did not change with burn severity. Soil chemical responses show significant location × burn interactions (with exceptions of TN and C:N in soil) indicating location specific responses.

After sequence quality control and elimination of non-target OTUs, 5,123,521 fungal and 1,176,637 bacterial sequences remained representing 1620 OTUs and 6873 OTUs, respectively. Fungal communities were dominated by the Phyla Ascomycota (54.8% sequences, 87.5% OTUs) and Basidiomycota (31.0% sequences, 8.6% OTUs), with Archaeorhizomycetaceae representing the most abundant Family. Bacterial communities were dominated by the Phyla Proteobacteria (32.3% of sequences, 22.9% OTUs) and Acidobacteria (17.2% of sequences, 17.8% OTUs) with Planctomycetaceae representing the most abundant bacterial Family (*see* Tables A5–A7) for taxa abundances. Analysis of the dominant taxa relative abundances with location, burn severity, and their interactions (see Table A8 for complete statistics) indicate that the fungal phylum Ascomycota decreases in relative abundance with burn severity coupled with a concomitant increase of Basidiomycota ($F_{3,105} = 4.87$, $P = 0.038$ and $F_{3,105} = 6.63$, $P < 0.001$ respectively). Lower fungal taxonomic tests indicate that the Ceratobasidiaceae decrease with burn severity ($F_{3,105} = 3.97$, $P = 0.10$) and *Diplogelasinospora* sp. increases in abundance with burn severity ($F_{3,105} = 33.06$, $P < 0.001$) among others. Bacterial analyses indicate a general decrease in the abundance of Bacteroidetes ($F_{3,105} = 37.03$, $P < 0.001$), the Sphingobacteriales ($F_{3,105} = 38.47$, $P < 0.001$), and members of unresolved Gp2 lineages ($F_{3,105} = 11.04$, $P < 0.001$) with burn severity. The groups within the Actinobacteria ($F_{3,105} = 7.17$, $P < 0.001$), the Burkholderiales ($F_{3,105} = 17.67$, $P < 0.001$), and *Mucilaginibacter* sp. ($F_{3,105} = 34.86$, $P < 0.001$) all increased in abundance with burn severity (among others).

Results of two-way ANOVA analyses indicate that fungal richness and diversity are lower in soil and duff samples from more severe fires, regardless of location (80% reduction in richness in GRSM soils with severe burns, 51% reduction in NNF soils with severe burns, 58% reduction in GRSM duff with moderate burns compared to unburned samples) whereas fungal evenness estimates remained stable across burn severities (Table 1, Fig. 1, Table A9). Similarly, bacterial richness and diversity decreased with burn severity (52% reduction of richness in GRSM soils with severe burns, 25% reduction in NNF soils with severe burns, 20% reduction in duff in moderate burns compared to unburned samples). However, in contrast to fungi, bacterial evenness decreased with more severe burns. Soil fungi had higher richness in NNF compared to GRSM overall. There were significant interactions between burn severity and location for both soil fungal and bacterial diversity, evenness and for duff richness, indicating location-based differences in microbial diversity responses. Both fungal and bacterial richness in soil decreased with increasing fire severity, whereas richness in duff was greatest with light burns (Tables 1, A9). Similarly, there were differences between how soil and duff responded to fire depending on location (Table A9; GRSM or NNF). Richness, diversity, and evenness often declined at GRSM with fire severity, whereas NNF soil and duff had greatest fungal and bacterial richness and diversity in low burn severity but were reduced in more severely burned sites, generally following the intermediate disturbance hypothesis (Table A9).

Fire severity was the strongest driver of microbial communities for both fungi and bacteria (Table 2). Despite significant community responses to fire severity ($R^2 = 0.21$ for fungi in soils, $R^2 = 0.15$ for fungi in duff, $R^2 = 0.29$ for bacteria in soils, $R^2 = 0.18$ for bacteria in duff), location also accounted for 3.4% of variation for soil fungi, 3.7% for duff fungi and 4.3% for soil bacteria, whereas location had no significant effect on duff bacteria. There were significant interactions between burn severity and location for all samples (Table 2). Fungal communities within low severity GRSM soils were indistinguishable from unburned reference plots. Further, fungal communities within GRSM duff were indistinguishable between burn severities (but all differ from unburned samples) which is in contrast with NNF duff samples that consistently changed among fire severities. This suggests that there are fundamental site-specific factors that regulate fungal responses to fire. In contrast, bacterial responses to fire were similar across the two sites (Table 2). Soil bacteria were more fire responsive than soil fungi – burn severity explained ~10% more community variation for bacteria than for fungi, suggesting that fungi on the whole may be more resilient to wildfire than bacteria. This is in contradiction to previous literature (Bárcenas-Moreno and Bååth, 2009) which suggests that bacteria should be more resilient to fire responses than fungi due to life history strategies and faster growth rates. To examine if observed community shifts were driven by severe plots (soil only), an additional series of PERMANOVA tests were conducted with severe samples omitted. In these, we see that the initial strong difference between soil and duff fungal communities were attenuated ($R^2 = 0.12$ for soil fungi and $R^2 = 0.15$ for duff fungi) but soil bacterial communities remained more impacted by burn severity than duff, albeit greatly reduced in magnitude ($R^2 = 0.21$ for soil and $R^2 = 0.17$ for duff).

The AWOrD analyses indicate that fire impacts on belowground communities are location-specific (Fig. 2). For NNF soil and duff fungal samples, pairwise AWOrD distance decreased with increasing burn severity ($P < 0.001$ and $P = 0.007$ respectively). So, fungal communities in low and moderate severity fires were more different from unburned controls than those in most severe fires. In contrast, fungi within GRSM

Table 1
ANOVA test statistics for fungal and bacterial Richness (S_{obs}), Diversity (1-D), and Evenness (E_D) for soil and duff samples across location (GRSM and NFF), burn severity (unburned, low, moderate, and severe [soil only]), and location by burn severity interactions. Richness data were untransformed, and Diversity and Evenness data were logit transformed. Significant factors are bolded and italicized.

Response	Location (F_{df}; P)	Burn severity (F_{df}; P)	Location × Burn (F_{df}; P)
Fungal Richness - Soil	***$F_{1,60} = 6.77$; 0.012***	***$F_{3,57} = 25.61$; < 0.001***	$F_{3,57} = 2.51$; 0.069
Fungal Diversity - Soil	$F_{1,60} = 0.02$; 0.866	***$F_{3,57} = 7.34$; 0.003***	***$F_{3,57} = 5.96$; 0.001***
Fungal Evenness - Soil	$F_{1,60} = 3.26$; 0.076	$F_{3,57} = 2.67$; 0.056	***$F_{3,57} = 3.59$; 0.019***
Fungal Richness - Duff	$F_{1,46} = 3.06$; 0.087	***$F_{2,45} = 11.53$; < 0.001***	***$F_{2,45} = 5.57$; 0.007***
Fungal Diversity - Duff	$F_{1,46} = 1.39$; 0.244	***$F_{2,45} = 4.95$; 0.012***	$F_{2,45} = 2.31$; 0.111
Fungal Evenness - Duff	$F_{1,46} = 0.12$; 0.727	$F_{2,45} = 1.43$, 0.249	$F_{2,45} = 0.49$; 0.611
Bacterial Richness - Soil	$F_{1,55} = 0.08$; 0.768	***$F_{3,53} = 9.44$; < 0.001***	$F_{3,53} = 0.87$; 0.461
Bacterial Diversity - Soil	$F_{1,55} = 1.36$; 0.248	***$F_{3,53} = 10.71$; < 0.001***	***$F_{3,53} = 5.96$; 0.001***
Bacterial Evenness - Soil	$F_{1,55} = 1.75$; 0.191	***$F_{3,53} = 7.24$; < 0.001***	***$F_{3,53} = 4.51$; 0.007***
Bacterial Richness - Duff	$F_{1,46} = 1.49$; 0.228	***$F_{2,45} = 6.26$; 0.004***	***$F_{2,45} = 4.83$; 0.013***
Bacterial Diversity - Duff	$F_{1,46} = 1.51$; 0.224	***$F_{2,45} = 4.27$; 0.020***	***$F_{2,45} = 9.30$; < 0.001***
Bacterial Evenness - Duff	$F_{1,46} = 0.39$; 0.535	***$F_{2,45} = 4.38$, 0.017***	***$F_{2,45} = 9.21$; < 0.001***

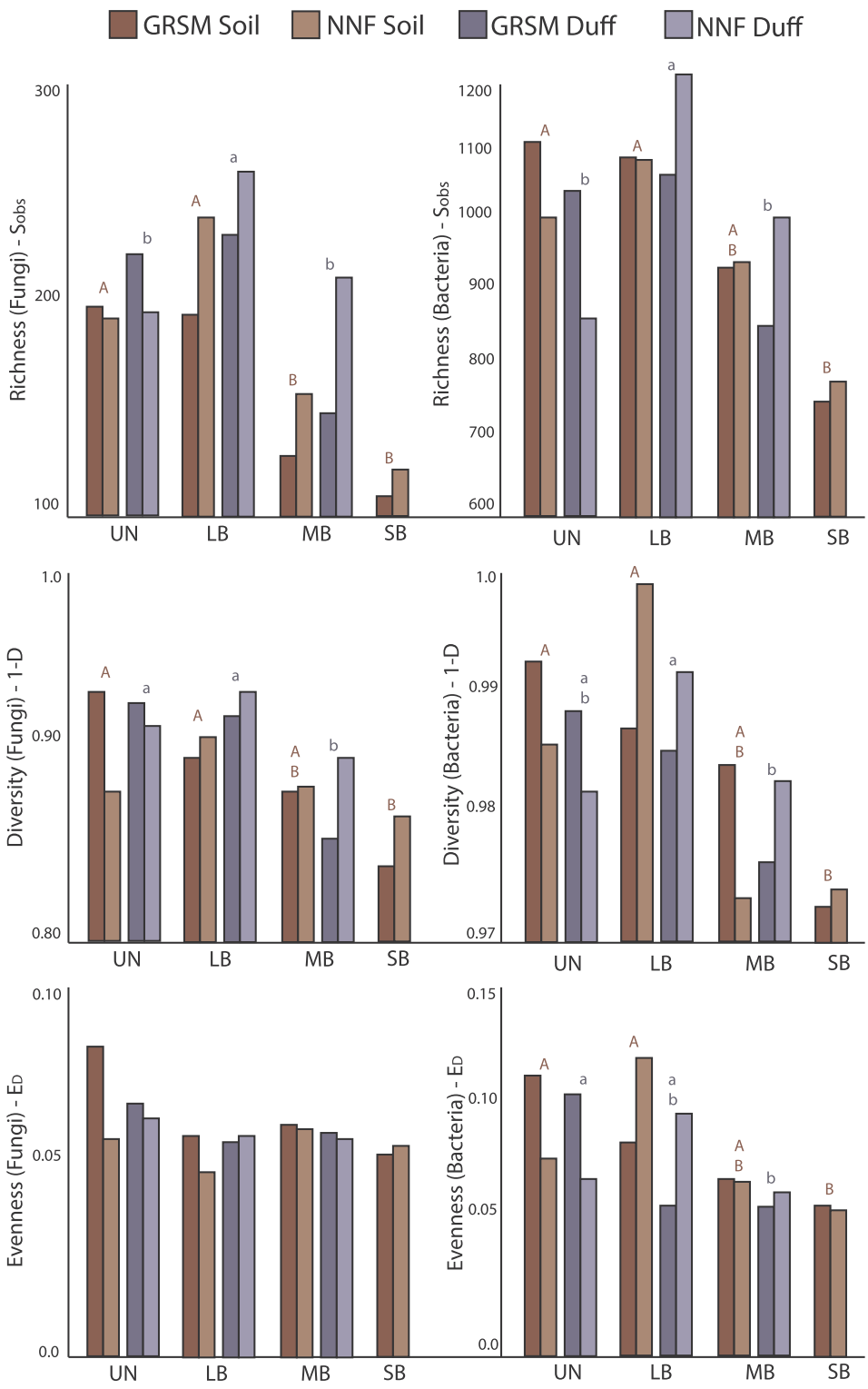

Fig. 1. Fungal (left) and Bacterial (right) richness (S_{obs}; top), diversity (1-D; middle), and evenness (E_D; bottom) estimates across burn severities (UN – unburned, LB - low severity, MB – moderate severity, and SB – severe within soils (red) and duff (blue) for both Great Smoky Mountain National Park (GRSM) and Nantahala National Forest (NNF) show strong impacts of fire intensity. Post-Hoc examination (presented here for all soil or duff samples combined, see Table A9 for additional analyses). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

were consistently different (independent of burn severity) from unburned plots (Fig. 2). For bacterial communities, NNF soil and GRSM duff AWOrD values consistently increased with more intense fires ($P < 0.001$ and $P = 0.007$ respectively; Fig. 2) indicating that bacterial communities in plots with greater fire severity became increasingly more distinct from the unburned references.

Microbial functional groups responded differently to burn severities and across the substrates (Table 3). Plant pathogens (within duff), saprobic fungi (within soil), lignin degrading bacteria (within soil), and diazotrophs (within soil) increased in relative abundance with increased burn severity, whereas ECM (within soil) and saprobic fungi (within duff) decreased with burn severity.

Table 2
Results from community-wide PERMANOVA tests for fungal and bacterial communities testing soil and duff communities are impacted by burn severity (Burn), location (GRSM or NNF) and burn × location interactions. (UB – unburned reference plots, LB – low-severity fires, MB – moderate-severity fires, and SB – severe fires). Presented are model terms, Pseudo-F-statistics, P-values, R^2 values and applicable post-hoc multiple comparisons (FDR adjusted) for burn responses by location (below).

Test	Pseudo-F_{df}	P-value	R^2	
Fungi soil				
Burn	$F_{3,54} = 5.331$	0.001	0.206	
Location	$F_{1,54} = 2.699$	0.007	0.035	
Burn × Location	$F_{3,54} = 1.636$	0.021	0.063	
Residuals			0.696	
Location	**UB**	**LB**	**MB**	**SB**
GSRM	A	AB	BC	C
NNF	A	B	B	C
Fungi duff				
Burn	$F_{2,42} = 4.221$	0.001	0.150	
Location	$F_{1,42} = 2.088$	0.010	0.037	
Burn × Location	$F_{2,42} = 1.794$	0.005	0.064	
Residuals			0.748	
Location	**UB**	**LB**	**MB**	
GSRM	A	B	B	
NNF	A	B	C	
Bacteria soil				
Burn	$F_{3,49} = 48.583$	0.001	0.296	
Location	$F_{1,49} = 3.743$	0.005	0.043	
Burn × Location	$F_{3,49} = 2.845$	0.002	0.098	
Residuals			0.563	
Location	**UB**	**LB**	**MB**	**SB**
GSRM	A	B	C	C
NNF	A	B	C	D
Bacteria duff				
Burn	$F_{2,42} = 5.266$	0.001	0.176	
Location	$F_{1,42} = 1.827$	0.055	0.031	
Burn × Location	$F_{2,42} = 2.678$	0.003	0.089	
Residuals			0.703	
Location	**UB**	**LB**	**MB**	
GSRM	A	B	C	
NNF	A	B	C	

Path analyses revealed complex edaphic-community interactions with burn severity (Fig. 3). While several treatment conditions showed no edaphic chemistry-community relationships (thus omitted from Fig. 3), several others indicate a complex connection between direct and indirect effects on community composition that differs with location, fire severity, and substrata. It is clear that soil chemistry nutrient pools affects communities, but often this effect is modulated via partial correlations with other soil parameters (for instance, TN often has an indirect control on communities via impacting OM or TC for fungi [e.g. moderate NNF duff, low GRSM soil, unburned GRSM duff] while sometime acting more directly [severe GRSM Soil]; see Fig. 3).

Biomarker OTUs also revealed location-specific responses to fire (see Tables 4, A10, A11). Fire-responsive fungal biomarker abundances in both soil and duff differed between GRSM and NNF; fungi in NNF compared to GRSM were more responsive whereas bacterial biomarkers were generally consistent between locations (Table A11). Interestingly, fire responsive fungal OTUs that are responsive to fires at one location but not the other are generally taxa whose identities cannot be discerned with these ITS2 sequences (OTU1, OTU5, OTU7, OTU10, and OTU13 among others). These were verified as fungi using tree based MOLE-BLAST, but cannot be identified below the kingdom level (Table A11, see Table A10 for taxonomic identification of these biomarkers). Interestingly, while there were observed many fire responsive biomarkers OTUs, the taxonomic identities of many of these taxa, particularly fungi, were unresolved and where taxonomic resolution was possible, were not among the commonly observed taxa that flourish post fire (phoenicoid fungi) (Carpenter and Trappe, 1985; McMullan-Fisher et al., 2011).

4. Discussion

We examined wildfire impacts on soil and duff microbial communities in the Southern Appalachian Mountains of the United States. Our study is unique because we were able to sample so soon after a near stand-replacing wildfire in mixed coniferous/deciduous forest thus representing one of the earliest analyses following such disturbances (*but see* Prendergast-Miller et al., 2017) – we sampled within a few weeks after flare ups and smoldering hotspots. Several recent studies addressing wildfire impacts on soil microbial communities using targeted gene sequencing have focused on one taxonomic group (Buscardo et al., 2015; Glassman et al., 2016; Prendergast-Miller et al., 2017; Rodríguez et al., 2018); less often do they simultaneously examine both fungal and bacterial communities (*but see* Pérez-Valera et al., 2018). Our study indicates that both fungal and bacterial community structure responded strongly to fire severity but that directionality of responses differ in certain responses by location (Egidi et al., 2016; Fig. 1, Fig. 2, Table 2). These location specific responses are likely due to differences in (1) initial soil nutrient pools (Table A4), (2) plant communities which may mediate soil responses to disturbance, also resulting in (3) fuel load and fire temperature variation among sites. In addition, the magnitude of these community shifts varied between bacteria and fungi with greater fire-sensitivity found for bacteria relative to fungi. Fungal groups which did respond included EcMs and saprobes, yet common phoenicoid fungi were not observed indicative that perhaps these adaptive groups are excluded from southeastern temperate habitats due to long-term fire suppression, or the short time between wildfire occurrence and sampling precluded the development or establishment of these groups.

Fungal community shifts to fire were stronger in NNF (Table 2) than in GRSM. At NNF, less severe fires resulted in community shifts that produced more dissimilar communities (compared to unburned), but with more severe fires, these communities were more similar (yet still distinct) to unburned plots (Fig. 2). Although these sites have similar fire histories, NNF had much greater nutrient pools in unburned soils relative to GRSM unburned soils (Table A4) which likely leads to greater N availability shortly after fires as predicted in late season fires (Hamman et al., 2008). Additionally, NNF has in general a more humid and warmer climate than GRSM which may combine to lead to higher soil moisture, which may induce larger effects (Holden et al., 2015); unfortunately, we are unable to test this explicitly with current data. Further, NNF substrata were more acidic (~10x) than those in GRSM. These initial edaphic physicochemical differences between NNF and GRSM were partially attenuated with burn severity (Table A4; expect duff C:N) with burned soils being more similar between GRSM and NNF. Likewise, pH may impact the viability of fungal community spore banks (Carpenter et al., 1987). A greater flush of nutrients and significant shifts in pH may in fact stimulate microbial growth immediately after fire (Rutigliano et al., 2007) resulting in stronger effects for locations with greater N and C pools. Furthermore, the composite burn index used to delineate burn severity may be occluding underlying differences in burn intensity at sites. NNF fires may have been more intense via greater energy output due to longer heating durations or overall temperature (Keeley, 2009) thereby causing greater microbial community shifts. The ultimate underlying cause of these different responses remains unknown and are likely due to several interacting factors.

There is a relative lack of overall common phoenicoid fungi observed in our sequence data (and in concurrent sporocarp collections, unpublished data). Phoenicoid fungi are often observed in high abundance after burns and are often “early colonizers” after fire events (Carpenter and Trappe, 1985, Glassman et al., 2016, Reazin et al., 2016). While there is no compendium of phoenicoid fungi that always occur after fire, there are several taxa that are often found (McMullan-Fisher et al., 2011, Baynes et al., 2012) and include the genera *Anthracobia, Morchella*, and *Pyronema* among others. However, in this study we observed none of these common phoenicoid taxa despite the

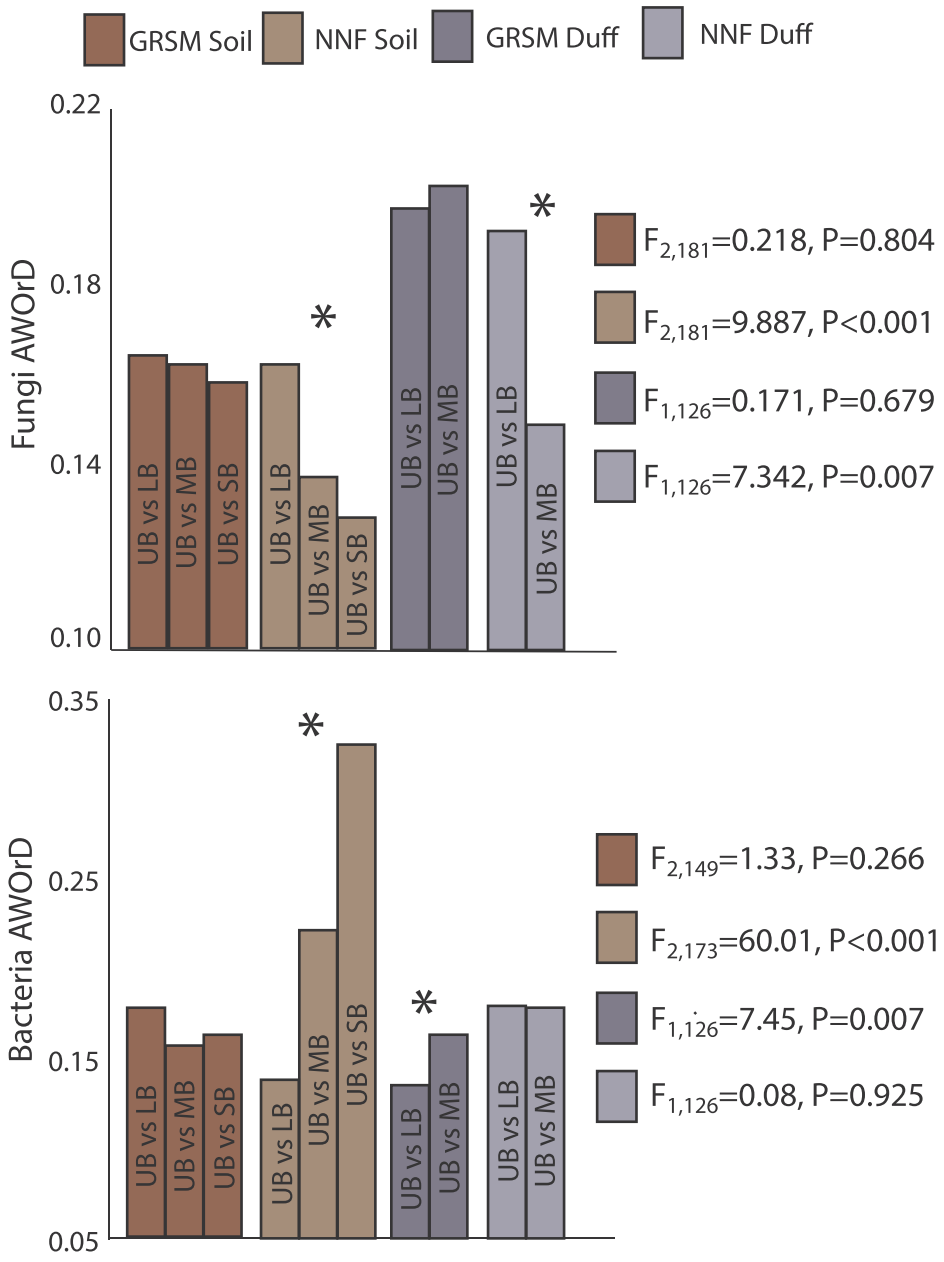

Fig. 2. Results of pairwise Axis Weighted Ordination Distance (AWOrD) comparison between burn severities [low severity (LB), moderately severity (MB) and severe burned (SB)] and unburned reference plots (UN), plots within soils (red) and duff (blue) for both locations within Great Smoky Mountain National Park (GRSM) and Nantahala National Forest (NNF). Test statistics are presented and where significant, asterisks are presented. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

numerous fire responsive OTUs. Approximately one-third of demarcated fungal OTUs could only be classified at the kingdom level indicating highly dissimilar sequenced from any ITS2 representatives within the combined global genetic repositories. These OTU representatives may represent a reservoir of novel or poorly defined fungal taxa that are fire-responsive. Whilst it is not uncommon that a large proportion of microbial OTUs are classified into taxonomically uncertain categories, this study has perhaps a larger portion of unclassified taxa than most. Further, most studies on wildfire impacts on microbial communities or fungal sporocarp surveys have their initial sampling longer after a fire event, due to the proximity of sampling so soon after the wildfires, we perhaps captured a more dynamic, transitory, and unknown microbial community. It remains to be seen if samples collected after a longer period of time will capture more of these expected phoenicoid taxa.

Soil chemistry plays a large role in microbial community assembly, particularly pH for bacterial taxa (Fierer and Jackson, 2006; Rousk et al., 2010; Tripathi et al., 2018). Additionally, nutrient quality and quantity often explain community structure for both fungi and bacteria (Delgado-Baquerizo et al., 2017; Glassman et al., 2017). Our path analyses indicate that pH is only a significant predictor for bacterial communities in low-severity GRSM soils and that C and N directly and indirectly impact fungal communities in low and severe GRSM soils (Fig. 3). These data indicate that the effects of fire on soil nutrient pools and impacts on communities were site-dependent and through correlative modelling and more hypothesis-driven experimentation, such as controlled pyro-mesocosms with and without nutrient additions, we may be better able to uncover mechanistic drivers of microbial responses to fire under differing chemical conditions. Recent work suggests that nutrient (particularly nitrogen) loads may be more influential

Table 3
One-way ANOVA of the effect of burn severity on the relative abundance of putative functional groups analyzed separately for duff and soil samples. Presented are F-statistics, p-values and directionality of community responses (where significant). Relative abundance data were Arcsine transformed prior to analyses (ECM – Ectomycorrhizal fungi, LigE – lignin degradation capability).

Test	F_{df}; P-value	Directionality
Fungi		
ECM - Duff	$F_{2,45}$ = 1.330; 0.276	
Plant Pathogens - Duff	***$F_{2,45}$ = 3.240; 0.048***	***Increase with burn severity***
Saprobes - Duff	***$F_{2,45}$ = 3.786; 0.030***	***Decrease with burn severity***
ECM - Soil	***$F_{3,60}$ = 6.390; 0.001***	***Decrease with burn severity***
Plant Pathogens - Soil	$F_{3,60}$ = 2.129; 0.106	
Saprobes - Soil	***$F_{3,60}$ = 2.941; 0.040***	***LB and MB higher than UB and SB***
Bacteria		
Diazotroph - Duff	$F_{2,45}$ = 1.509; 0.232	
LigE - Duff	***$F_{2,45}$ = 9.071; < 0.001***	***Increase with burn severity***
Diazotroph - Soil	***$F_{3,60}$ = 3.122; 0.032***	***Increase with burn severity***
LigE - Soil	***$F_{3,60}$ = 18.883; < 0.001***	***Increase with burn severity***

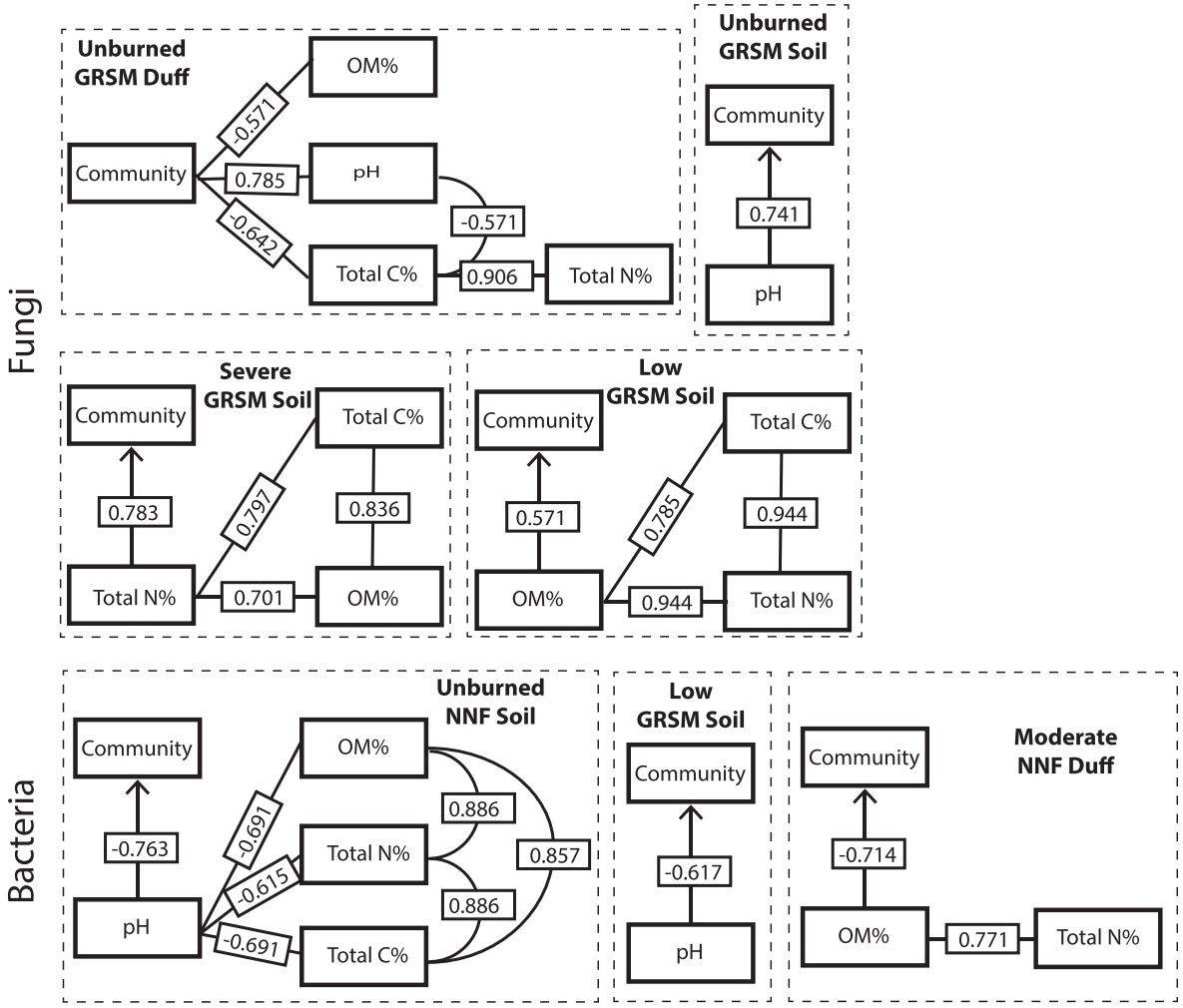

Fig. 3. Path diagram of soil nutrient pools and chemistry that influences fungal (top) and bacterial (bottom) communities (most explanatory NMDS axes: 43.29% community variation for fungi and 54.9% for bacteria) separated by sampling location (GRSM and NNF) soil and duff samples by burn severity. Only factors that significantly (Kendall's Tau correlation; $p < 0.05$) influence microbial communities directly or indirectly are presented (severities where chemical factors do not significantly impact microbial communities are omitted for simplicity).

in structuring microbial communities than prescribed burning (Carson et al., 2019) and this interaction between nutrients and burning may partially explain our divergent responses between NNF and GRSM.

We demonstrate that increasing burn severity results in a decrease in mycorrhizal fungi with a concurrent increase of saprobic fungi, lignin degrading bacteria, and nitrogen-fixing bacteria in soils (Table 3). Further, we see a decrease of the fungal family Ceratobasidiaceae with burn severity. The Ceratobasidiaceae are an ecologically diverse group that includes orchid mycorrhizae (Yagame et al., 2008) but are generally considered saprobic, although some obligate plant pathogens are within this family. Most interesting here is the strong association of the Ceratobasidiaceae with pectinase generation (pectin degradation; Sweetingham et al., 1986, Paduano et al., 2011). Pectin makes up a not insignificant component of many plant cell walls. With tree mortality,

Table 4
Percentage of significant biomarker taxa (based on LEfSe analyses and the top 100 OTUs) for fungal and bacterial communities across substrate (duff and soil) and locations (GRSM and NNF) that were biomarker for fire severity category. Additionally, the results of Fisher's Exact Tests are presented testing if GRSM and NNF samples differ in the proportion of biomarkers (bold where significant).

	Unburned	Low intensity	Moderate intensity	Severe intensity	P-Value
Fungi duff					
GRSM	12%	9%	6%		**0.050**
NNF	6%	13%	12%		
Fungi soil					
GRSM	18%	10%	2%	5%	**0.022**
NNF	13%	18%	9%	10%	
Bacteria duff					
GRSM	19%	7%	15%		0.101
NNF	21%	20%	31%		
Bacteria soil					
GRSM	28%	26%	12%	12%	0.523
NNF	35%	26%	11%	23%	

one might expect an increase in pectin degradation potential, not less as we see here (based on this abundant family). However, pectin depolymerizes and thermally degrades at temperatures as low as 210 °C (Aburto et al., 2015), well below the general temperature of wildfire, thus it is likely that fires vastly reduce pectin concentrations, and this may drive the decrease in abundances of this family. Additionally, we see an increase of the genus *Diplogelasinispora* which may reduce ketones (Carballeira et al., 2004) suggesting a potential increase in complex hydrocarbon utilization with fire. Of the interesting bacterial shifts (Table A8), we see an increase in the relative abundances of Burkholderiales and Sphingobacteriaceae, which contain many nitrogen-fixing and autotrophic members (Santi et al., 2013; Lambiase, 2014). This is congruent with the observed increases of putative diazotrophs with burn severity (Table 3). Additionally, we see a marked increase of the abundant genus *Mucilaginibacter* with burn severity, which are often found in acidic soils and may have xylan-, laminarin-, and general polysaccharide-degradation capabilities (Pankratov et al., 2007). While full ecological roles of the *Mucilaginibacter* are unknown (de Alencar et al., 2016), this, and other evidence presented here, hints toward polysaccharides and other plant cell wall components playing a large role in microbial responses to fire and warrants additional examinations.

Mycorrhizal fungi, particularly ectomycorrhizae, have previously been reported as very sensitive to fire (Holden et al., 2013) as confirmed here. This functional 'changing of the guards' is likely a direct response to increased plant mortality with fire and fire-induced changes in nutrient quality and availability making carbon more accessible to saprobes. Increases in saprobic fungi with simultaneous increases in N-fixing bacteria seen here is as interesting result. The connection between saprobic capability and diazotroph abundances is not new but rarely addressed in successional ecology. Production of extracellular enzymes is a nitrogen expensive process that may favor syntrophic associations between nitrogen-fixers and saprobic fungi (Veal and Lynch, 1987; Hoppe et al., 2014; Johnston et al., 2016), and co-occurrence of wood-decaying fungi and diazotrophic bacteria have additive effects on decay rates (Kamei 2017). These data highlight that burn severity will not only select for specific functional guilds within weeks of an event, but also that such responses are location-dependent (Egidi et al., 2016). These responses may display differences in microbial community successional trajectories and, particularly in light of functional guilds, may translate into differential restoration of important ecosystem services, such as C sequestration.

5. Conclusions

We found that bacterial communities were more sensitive to fire disturbance than fungal communities: bacterial communities varied more across fire severities as demonstrated by a combination of community-wide (PERMANOVA) and OTU specific shifts with fire. Previous work has suggested that bacteria are more resilient to fire than fungi because of their faster growth rates and different life history strategies but our data indicates that this may be an over-simplification or generalization when studying extreme, spatially heterogeneous wildfires. Our findings demonstrate the need to better understand linkages between soil microbial community dynamics, disturbance ecology, and nutrient availability among functionally similar forest types. Although forest communities have similar land-use and disturbance histories, location was a strong secondary driver of microbial community divergence in response to fire severity. We suggest that drastic differences in initial nutrient pools and differential nutrient pool responses to fire between GRSM and NNF drives the context-dependency observed here. Additional insight will be gained by incorporating studies that span larger temporal scales, include functionality data, and encompass a larger spatial extent so that we can synthesize spatiotemporal variability in microbial responses to extreme fire events. This approach is imperative to predict ecosystem recovery and resiliency to wildfires, which are only likely to increase in frequency and severity with future climate change.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2019.117520.

References

Abarenkov, K., Tedersoo, L., Nilsson, R.H., Vellak, K., Saar, I., Veldre, V., Parmasto, E., Prous, M., Aan, A., Ots, M., Kurina, O., Ostonen, I., Jõgeva, J., Halapuu, S., Põldmaa, K., Toots, M., Truu, J., Larsson, K., Kõljalg, U., 2010. PlutoF-a web based workbench for ecological and taxonomic research, with an online implementation for fungal ITS sequences. Evol. Bioinform. 6, 189–196.
Abrams, M.D., 1992. Fire and the development of oak forests. Bioscience 45, 346–353.
Aburto, J., Moran, M., Galano, A., Torres-García, E., 2015. Non-isothermal pyrolysis of pectin: a thermochemical and kinetic approach. J. Anal. Appl. Pyrol. 112, 94–104.
Allison, S.D., Treseder, K.K., 2008. Warming and drying suppresses microbial activity and carbon cycling in boreal forest soils. Glob. Change Biol. 14, 2898–2909.
Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 26, 32–46.
Anderson, M.J., Walsh, D.C.I., 2013. PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: what null hypothesis are you testing? Ecol. Monogr. 83, 557–574.
Anderson, R.C., Menges, E.S., 1997. Effects of fire on sandhill herbs: nutrients, mycorrhizae, and biomass allocation. Am. J. Bot. 84, 938–948.
Bárcenas-Moreno, G., Bååth, E., 2009. Bacterial and fungal growth in soil heated at different temperatures to stimulate a range of fire intensities. Soil Biol. Biochem. 41,

2517–2526.
Baynes, M., Newcombe, G., Dixon, L., Castlebury, L., O'Donnell, K., 2012. A novel plant-fungal mutualism associated with fire. Fungal Biol. 116, 133–144.
Brown, S.P., Jumpponen, A., 2014. Contrasting primary successional trajectories of fungi and bacteria in retreating glacier soils. Mol. Ecol. 23, 481–497.
Brown, S.P., Veach, A.M., Ridgon-Huss, A.R., Grond, K., Lickteig, S.K., Lothamer, K., Oliver, A.K., Jumpponen, A., 2015. Scraping the bottom of the barrel: are rare high throughput sequences artifacts? Fungal Ecol. 13, 221–225.
Brown, S.P., Leopold, D.R., Busby, P.E., 2018. Protocols for investigating the leaf mycobiome using high throughput DNA sequencing. In: In: Ma, W., Wolpert, T. (Eds.), Plant Pathogenic Fungi and Oomycetes: Methods in Molecular Biology, vol. 1848. Humana Press, New York, pp. 39–51.
Buscardo, E., Rodríguez-Echeverría, S., Freitas, H., De Angelis, P., Pereira, J.S., Muller, L.A.H., 2015. Contrasting soil fungal communities in Mediterranean pine forests subjected to different wildfire frequencies. Fungal Divers. 70, 85–99.
Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. P. Natl. Acad. Sci. U.S.A. 108 (S1), 4516–4522.
Carballeira, J.D., Valmaseda, M., Alvarez, E., Sinisterra-Gago, J.V., 2004. *Gongronella butleri*, *Schizosaccharomyces octosporus* and *Diplogelasinospora grovesii*: novel microorganisms useful for the stereoselective reduction of ketones. Enzyme Microb. Tech. 34, 611–623.
Carpenter, S.E., Trappe, J.M., 1985. Phoenicoid fungi – a proposed term for fungi that fruit after heat-treatment of substrates. Mycotaxon 23, 203–206.
Carpenter, S.E., Trappe, J.M., Ammirati Jr., J., 1987. Observations of fungal succession in the Mount St. Helens devastation zone, 1980–1983. Can. J. Botany 56, 716–728.
Carson, C.M., Jumpponen, A., Blair, J.M., Zeglin, L.H., 2019. Soil fungal community changes in response to ling-term fire cessation and N fertilization in tallgrass prairie. Fungal Ecol. 41, 45–55.
Certini, G., 2005. Effects of fire on properties of forest soils: a review. Oecologia 143, 1–10.
Clarke, K.R., Somerfield, P.J., Chapman, M.G., 2006. On resemblance measured for ecological studies, including taxonomic dissimilarities and a zero-adjusted Bray-Curtis coefficient for denuded assemblages. J. Exp. Mar. Biol. Ecol. 330, 55–80.
de Alencar, S.A., Costa, F.S., Rodrigues, G.R., Barreto, C.C., 2016. Draft genome of a novel *Mucilaginibacter* member isolated from Brazilian Amazon soil. Genome Announc. 4, e01033–e1116.
Delgado-Baquerizo, M., Reich, P.B., Khachane, A.N., Campbell, C.D., Thomas, N., Freitag, T.E., Al-Soud, W.A., Sørensen, S., Bardgett, R.D., Singh, B.K., 2017. It is elemental: soil nutrient stoichiometry drives bacterial diversity. Environ. Microbiol. 19, 1176–1188.
DeLuca, T.H., MacKenzie, M.D., Gundale, M.J., Holben, W.E., 2006. Wildfire-produced charcoal directly influences nitrogen cycling in ponderosa time forests. Soil Sci. Soc. Am. J. 70, 448–453.
Dooley, S.R., Treseder, K.K., 2012. The effect of fire on microbial biomass: a meta-analysis of field studies. Biogeochemistry 109, 49–61.
Egidi, E., McMullan-Fisher, S., Morgan, J.W., May, T., Zeeman, B., Franks, A.E., 2016. Fire regime, not time-since-fire, affects soil fungal community diversity and composition in temperate grasslands. FEMS Microbiol. Lett. 363. https://doi.org/10.1093/femsle/fnw196.
Ferrenberg, S., O'Neill, S.P., Knelman, J.E., Todd, B., Duggan, S., Bradley, D., Robinson, T., Schmidt, S.K., Townsend, A.R., Williams, M.W., Cleveland, C.C., Meblourne, B.A., Jiang, L., Nemergut, D.R., 2013. Changes in assembly processes in soil bacterial communities following a wildfire disturbance. ISME J. 7, 1102–1111.
Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. P. Natl. Acad. Sci. U.S.A. 103, 626–631.
Fish, J.A., Chai, B., Wang, Q., Sun, Y., Brown, C.T., Tiedje, J.M., Cole, J.R., 2013. FunGene: the functional gene pipeline and repository. Frontiers Microb. 4, 291.
Fultz, L.M., Moore-Kucera, J., Dathe, J., Davinic, M., Perry, G., Wester, D., Schwilk, D.W., Rideout-Hanzak, S., 2016. Forest wildfire and grassland prescribed fire effects on soil biogeochemical processes and microbial communities: two case studies in the semi-arid Southwest. Appl. Soil Ecol. 99, 118–128.
Glassman, S.I., Levine, C.R., DiRocco, A.M., Battles, J.J., Bruns, T.D., 2016. Ectomycorrhizal fungal spore bank recovery after a severe forest fire: some like it hot. ISME J. 10, 1228–1239.
Glassman, S.I., Wang, I.J., Bruns, T.D., 2017. Environmental filtering by pH and soil nutrients drives community assembly in fungi at fine spatial scales. Mol. Ecol. 26, 6960–6973.
Goberna, M., García, C., Insam, H., Hernández, M.T., Verdú, M., 2012. Burning fire-prone Mediterranean shrublands: immediate changes in soil microbial community structure and ecosystem functions. Microb. Ecol. 64, 242–255.
González-Pérez, J.A., González-Vila, F.J., Almendros, G., Knicker, H., 2004. The effect of fire on soil organic matter – a review. Environ. Int. 30, 855–870.
Hamman, S.T., Burke, I.C., Knapp, E.E., 2008. Soil nutrients and microbial activity after early and late season prescribed burns in a Sierra Nevada mixed conifer forest. Forest Ecol. Manag. 256, 367–374.
Hebel, C.L., Smith, J.E., Cromack, K., 2009. Invasive plant species and soil microbial responses to wildfire burn severity in the Cascade Range of Oregon. App. Soil Ecol. 42, 150–159.
Hervé, M., 2017. RVAideMemoire: diverse basic statistical and graphical functions. R package. https://CRAN.R-project.org/package=RVAideMemoire.
Holden, S.R., Gutierrez, A., Treseder, K.K., 2013. Changes in soil fungal communities, extracellular enzyme activities, and litter decomposition across a fire chronosequence in Alaskan boreal forests. Ecosystems 16, 34–46.
Holden, S.R., Berhe, A.A., Treseder, K.K., 2015. Decreases in soil moisture and organic matter quality suppress microbial decomposition following a boreal forest fire. Soil Biol. Biochem. 87, 1–9.
Holden, S.R., Rogers, B.M., Treseder, K.K., Randerson, J.T., 2016. Fire severity influences the response of soil microbes to a boreal forest fire. Environ. Res. Lett. 11 035004.
Hoppe, B., Kahl, T., Karasch, P., Wubet, T., Bauhus, J., Buscot, F., Krüger, D., 2014. Network analysis reveals ecological links between N-fixing bacteria and wood-decaying fungi. PLoS ONE 9 e88141.
Huse, S.M., Welch, D.W., Morrison, H.G., Sogin, M.L., 2010. Ironing out the wrinkles in the rare biosphere through improves OTU clustering. Environ. Microb. 12, 1889–1898.
Ihrmark, K., Bödeker, I.T., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., Strid, Y., Stenlid, J., Brandström_Durling, M., Clemmensen, K.E., Lindahl, B.D., 2012. New primers to amplify the fungal ITS2 region – evaluation by 454-sequencing or artificial and natural communities. FEMS Microb. Ecol. 82, 666–677.
IPCC, 2014. Climate change 2014: Synthesis report. In: Pachauri, R.K., Meyer, L.A. (Eds.), Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, Switzerland, pp. 151.
Johnston, S.R., Boddy, L., Weightman, A.J., 2016. Bacteria in decomposing wood and their interactions with wood-decay fungi. FEMS Microb. Ecol. 92, fiw179.
Kamei, I., 2017. Co-culturing effects of coexisting bacteria on wood degradation by *Trametes versicolor*. Curr. Microbiol. 74, 125–131.
Keeley, J.E., 2009. Fire intensity, fire severity and burn severity: a brief review. Int. J. Wildland Fire 18, 116–126.
Key, C.H., Benson, N.C., 2006. Landscape assessment (LA): Sampling and analysis methods. USDA Forest Service Gen. Tech. Rep. RMRS-GTR-164-CD, 1-55.
Kõljalg, U., Nilsson, R.H., Abarenkov, K., Tedersoo, L., Taylor, A.F.S., Bahram, M., Bates, S.T., Bruns, T.D., Bengtsson-Palme, J., Callaghan, T.M., Douglas, B., Drenkhan, T., Eberhardt, U., Dueñas, M., Grebenc, T., Griffith, G.W., Hartmann, M., Kirk, P.M., Kohout, P., Larsson, E., Lindahl, B.D., Lücking, R., Martín, M.P., Matheny, P.B., Nguyen, N.H., Niskanen, T., Oja, J., Peay, K.G., Peintner, U., Peterson, M., Põldmaa, K., Saag, L., Saar, I., Schüßler, A., Scott, J.A., Senés, C., Smith, M.E., Suija, A., Taylor, D.L., Telleria, M.T., Weiss, M., Larsson, K.H., 2013. Toward a unified paradigm for sequence-based identification of fungi. Mol. Ecol. 22, 5271–5277.
Lambiase, A., 2014. The family *Sphingobacteriaceae*. In: Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., Thompson, F. (Eds.), The Prokaryotes. Springer, Berlin, pp. 907–914.
McMullan-Fisher, S.J.M., May, T.W., Robinson, R.M., Bell, T.L., Lebel, T., Catcheside, P., York, A., 2011. Fungi and fire in Australian ecosystems: a review of current knowledge, management implications and future directions. Aust. J. Bot. 59, 70–90.
Mitchell, K.J., Liu, Y., O'Brien, J.J., Elliott, K.J., Starr, G., Miniant, C.F., Hiers, J.K., 2014. Future climate and fire interactions in the southeastern region if the United States. For. Ecol. Manag. 327, 316–326.
Nave, L.E., Vance, E.D., Swanston, C.W., Curtis, P.S., 2011. Fire effects on temperate forest soil C and N storage. Ecol. Appl. 21, 1189–1201.
Neff, J.C., Harden, J.W., Gleixner, G., 2005. Fore effects on soil organic matter content, composition, and nutrients in boreal internal Alaska. Can. J. Forest Res. 35, 2178–2187.
Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., Schilling, J.S., Kennedy, P.G., 2016. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guilds. Fungal Ecol. 20, 241–248.
Nowaki, G.J., Abrams, M.D., 2008. The demise of fire and 'mesophication' of forests in the eastern United States. Bioscience 58, 123–138.
Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2017. Package vegan. R package. https://CRAN.R-project.org/package=vegan.
Oliver, A.K., Brown, S.P., Callaham, M.A., Jumpponen, A., 2015. Polymerase matters: non-proofreading enzymes inflate fungal community richness estimates by up to 15%. Fungal Ecol. 15, 86–89.
Paduano, C., Rodda, M., Ercole, E., Girlands, M., Perotto, S., 2011. Pectin localization in the Mediterranean orchid *Limodorum abortivum* reveals modulation of the plant interface in response to different mycorrhizal fungi. Mycorrhiza 21, 97–104.
Pankratov, T.A., Tindall, B.J., Liesack, W., Dedysh, S.N., 2007. *Mucilaginibacter paludism* gen. nov., s. nov. and *Mucilaginibacter gracilis* sp. nov., pectin- xylan- and laminarin-degrading members of the family Sphingobacteriaceae from acidic sphagnum peat bog. Int. J. Syst. Evol. Microb. 57, 2349–2354.
Pérez-Valera, E., Verdú, M., Navarro-Cano, J.A., Goberna, M., 2018. Resilience to fire of phylogenetic diversity across biological domains. Mol. Ecol. 27, 2896–2908.
Prendergast-Miller, M.T., de Menezes, A.B., MacDonald, L.M., Toscas, P., Bissett, A., Baker, G., Farrell, M., Richardson, A.E., Wark, T., Thrall, P.H., 2017. Wildfire impact: natural experiment reveals differential short-term changes in soil microbial communities. Soil Biol. Biochem. 109, 1–13.
R Core Team, 2017. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.
Reazin, C., Morris, S., Smith, J.E., Cowan, A.D., Jumpponen, A., 2016. Fires of differing intensities rapidly select distinct soil fungal communities in a Northwest US ponderosa pine forest ecosystem. For. Ecol. Manag. 377, 118–127.
Rodríguez, J., A.González-Pérez, J.A., Turmeroa, A., Hernández, M., Ball, A.S., González-Vila, F.J., Ariasa, M.E., 2018. Physio-chemical and microbial perturbations of Andalusian pine forest soils following a wildfire. Sci. Tot. Environ. 634, 650–660.
Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 4 e2584.
Rousk, J., Bååth, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., Knight, R., Fierer, N., 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME J. 4, 1340–1351.
Rutigliano, F.A., De Marco, A., D'Ascoli, R., Castaldi, S., Gentile, A., Virzo De Santo, A., 2007. Impact of fire on fungal abundance and microbial efficiency in C assimilation and mineralization in a Mediterranean maquis soil. Biol. Fertil. Soils 44, 377–381.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source, platform independent, community-supported software for describing and comparing microbial communities. App. Environ. Microb. 75, 7537–7541.

Santi, C., Bogusz, D., Franche, C., 2013. Biological nitrogen fixation in non-legume plants. Ann. Bot. 111, 743–767.

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., Huttenhower, C., 2011. Metagenomic biomarker discovery and explanation. Genome Biol. 12, R60.

Sweetingham, M.W., Cruickshank, R.H., Wong, D.H., 1986. Pectin zymograms and taxonomy and pathogenicity of the Ceratobasidiaceae. T. Brit. Mycol. Soc. 86, 305–311.

Tripathi, B.M., Stegen, J.C., Kim, M., Dong, K., Adams, J.M., Lee, Y.K., 2018. Soil pH mediates the balance between stochastic and deterministic assembly of bacteria. ISME J. 12, 1072–1083.

Veach, A.M., Stokes, C.E., Knoepp, J., Jumpponen, A., Baird, R., 2018. Fungal communities and functional guild shifts along an elevational gradient in the southern Appalachian mountains. Microb. Ecol. 76, 156–168.

Veal, D.V., Lynch, J.M., 1987. Associative cellulolysis and N_2 fixation by co-cultures of *Trichoderma harzianum* and *Clostridium butyricum*: the effects of ammonium-N on these processes. J. App. Bacteriol. 63, 245–253.

Wan, S., Hui, D., Lou, Y., 2001. Fire effects on nitrogen pools and dynamics in terrestrial ecosystems: a meta-analysis. Ecol. App. 11, 1349–1365.

Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. App. Environ. Microb. 73, 5261–5267.

Westcott, S.L., Schloss, P.D., 2017. OptiClust, an improves method for assigning amplicon-based sequence data to operational taxonomic units. mSphere 2, e00073–e117.

White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J., White, T.J. (Eds.), PCR Protocols: A Guide to Methods and Applications. Academic Press, London, pp. 315–322.

Williamson, T.B., Colombo, S.J., Duniker, P.N., Gray, P.A., Hennessey, R.J., Houle, D., Johnston, M.H., Ogden, A.E., Spittlehouse, D.L., 2009. Climate Change and Canada's Forests: From Impacts to Adaptation. Canadian Forest Service, Edmonton, AB, Canada, pp. 315–322.

Yagame, T., Yamato, M., Suzuki, A., Iwase, K., 2008. Ceratobasidiaceae mycorrhizal fungi isolated from nonphotosynthetic orchid *Chamaegastrodia sikokiana*. Mycorrhiza 18, 97–101.

Yeager, C.M., Northup, D.E., Grow, C.C., Barns, S.M., Kuske, C.R., 2005. Changes in nitrogen-fixing and ammonia-oxidizing bacterial communities in soil in a mixed conifer forest after wildfire. App. Environ. Microb. 71, 2713–2722.

Zavala, L.M., De Celis, R., Jordán, A., 2014. How wildfires affect soil properties. A brief review. Cuad. Invest. Geogr. 40, 311–331.